Clinical Microbiology in *action*

Cases from the Brigham & Women's Hospital

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Topics within this talk

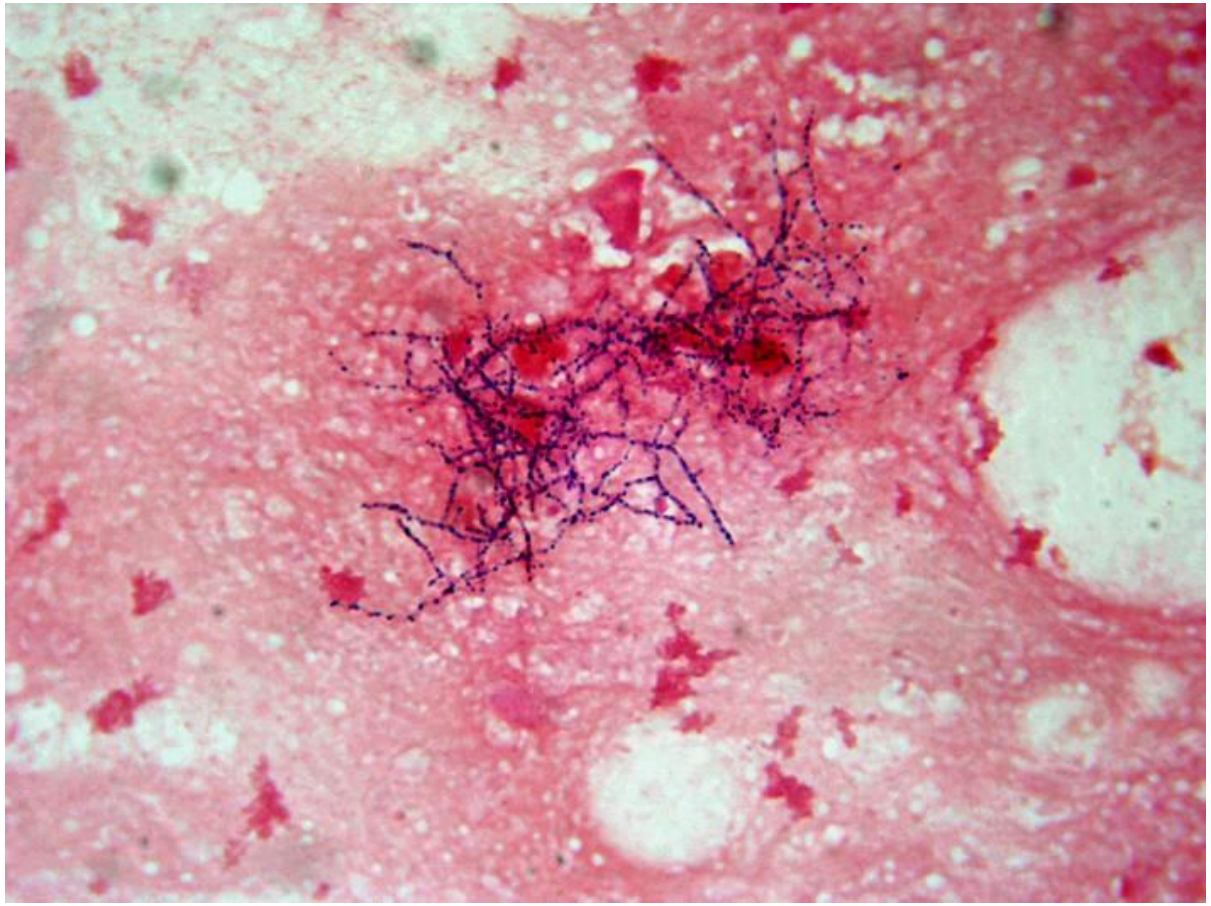
- The vital role of the technologist in patient care
- The integration of the clinical microbiology laboratory with multiple levels of healthcare decision makers
- The challenges of decision-making in the face of ever-increasing diagnostic complexity

A case of the coughs

- A 30 year old healthy female presents to her pulmonologist in June of 2016 with a history of bronchiectasis and recurrent pneumonia
- She had 3 episodes of pneumonia between January and April 2016 during which she coughed up sputum that started out as yellowish-green but eventually became tinged with blood
- Each episode seemed to respond to antibiotics

A case of the coughs

- A sputum culture is obtained at the time and is negative for mycobacteria, fungi and bacteria
- Gram stain shows...



A case of the coughs

- Because she appears well, no treatment is initiated at the time
- The following month (August 2016) has another episode of pneumonia for which she is treated with cefuroxime
- In November 2017 she delivers a healthy full term baby with no complications
- In March of 2018 she again presents to her pulmonologist with ‘rust-colored’ sputum, fever to 101F, and night sweats
- She receives another course of cefuroxime but her symptoms persist, therefore she is switched to a longer course of levofloxacin

A case of the coughs

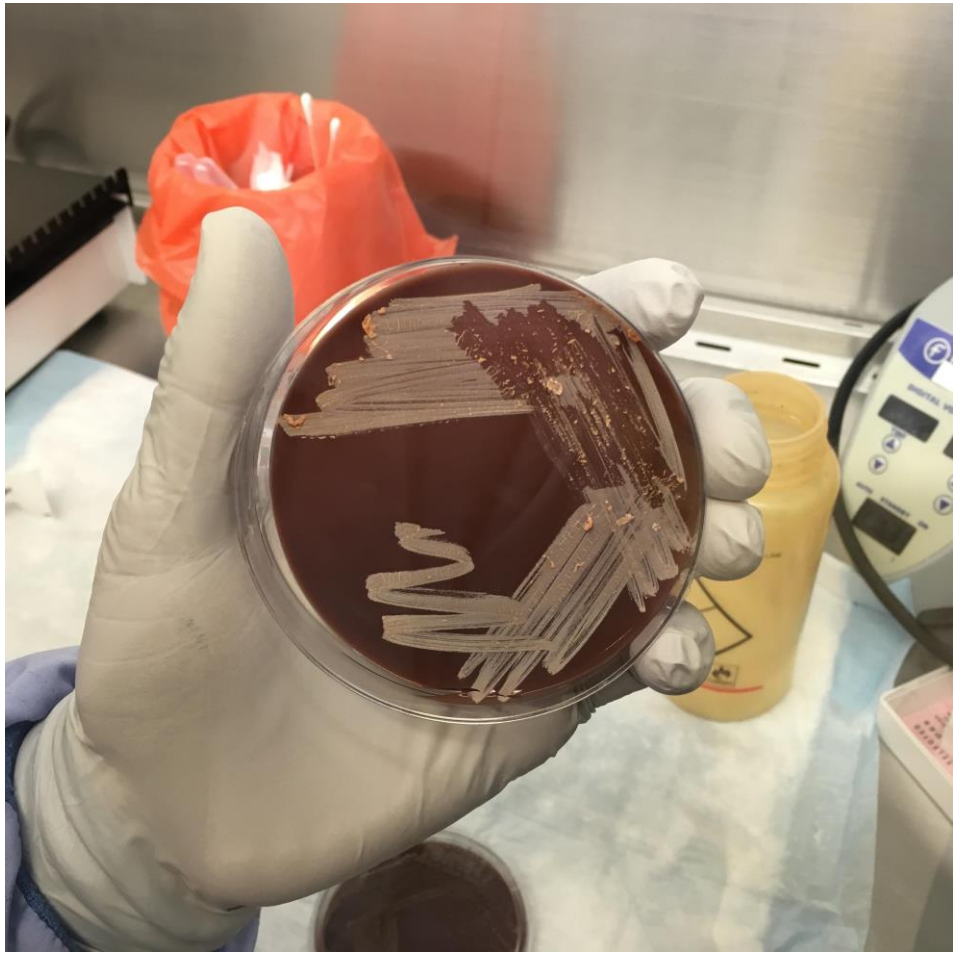
- Another sputum is obtained

Specimen Source/ Description	SPUTUM
SPECIAL REQUESTS	None
GRAM STAIN	4+ POLYS
GRAM STAIN	1+ EPITHELIAL CELLS
GRAM STAIN	**3+ BRANCHING GRAM POSITIVE RODS**
CULTURE / TEST	2+ ORAL FLORA

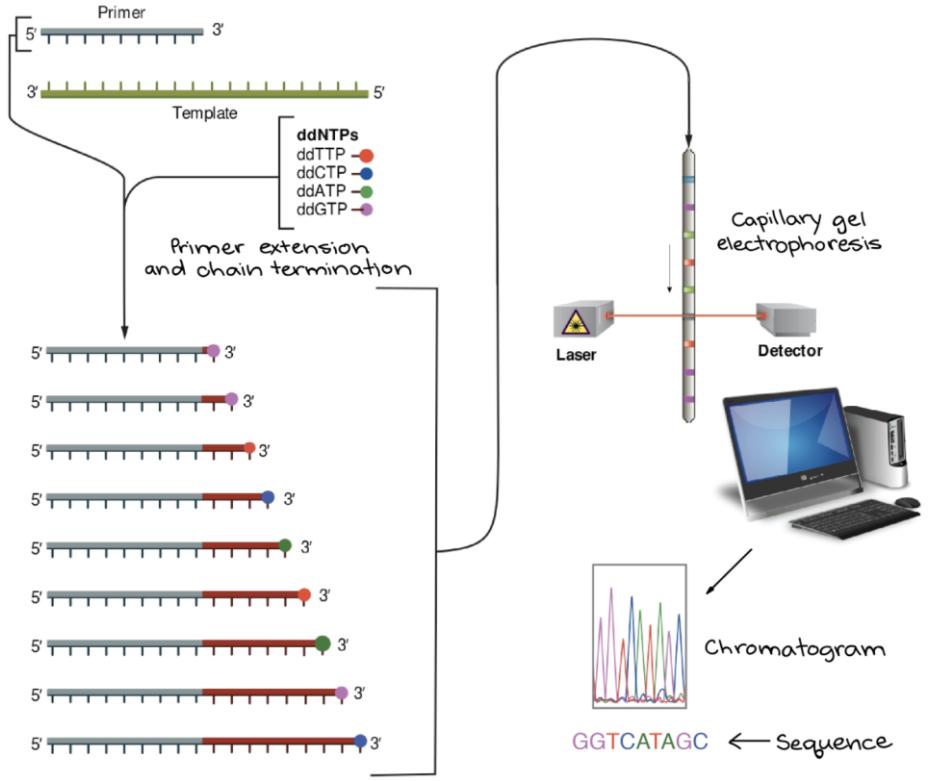
- What's going on here?

One in a million

- An astute technologist picked out a single small colony in the 2nd quadrant that appeared different than the surrounding oral flora
- The isolate failed to grown on Lowenstein-Jensen agar but did grow on chocolate and BCYE
- Gram stain matched sputum from 2016 and from 2018



When in doubt, sequence

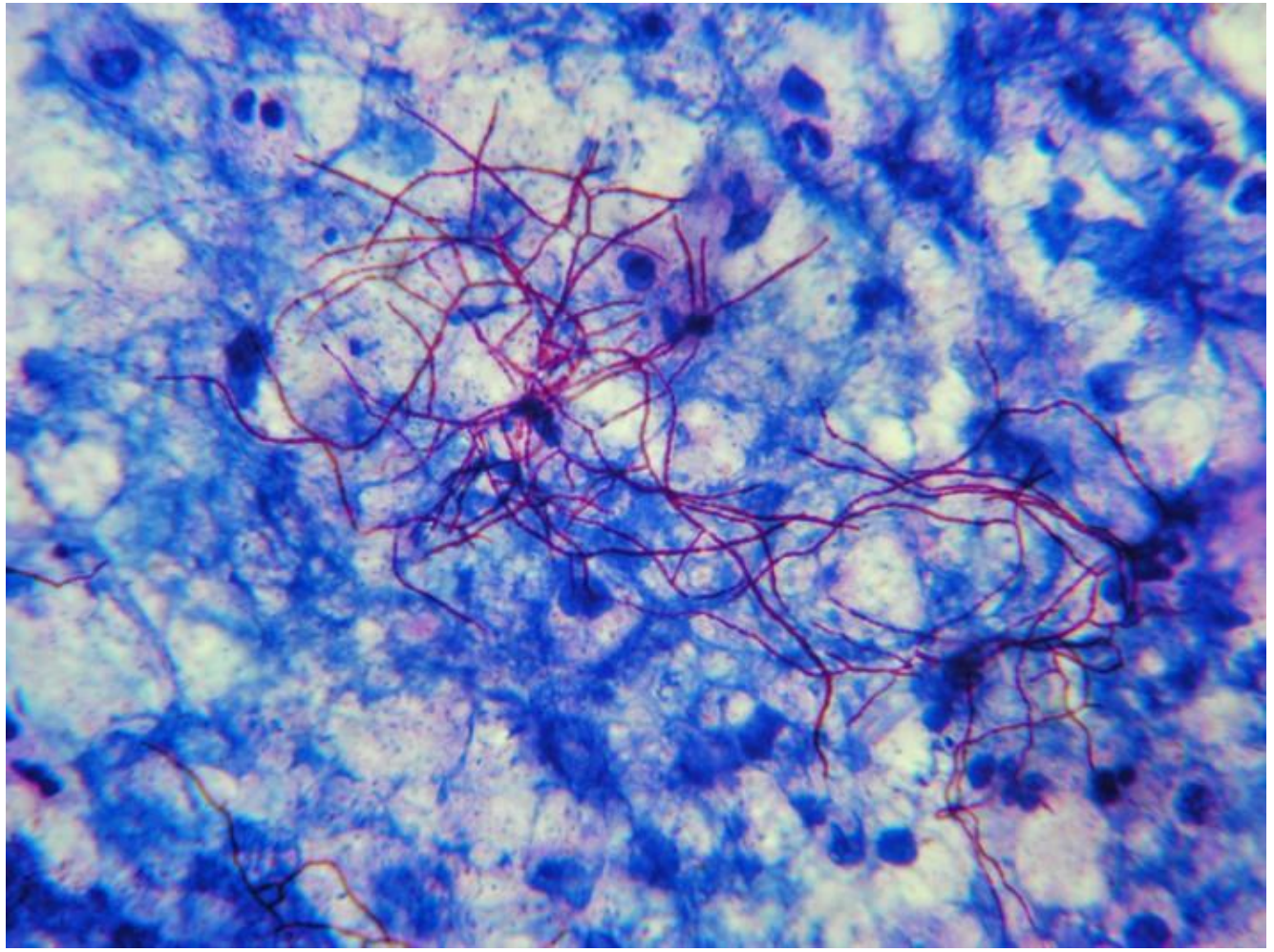


- 16S ribosomal gene sequencing was performed using a colony of pure growth
- 'Broad-based' bacterial primers successfully amplified the gene target
- Subsequent Sanger sequencing yielded a contig of ~1500 base pairs
- Using local sequence alignment software and a curated database of bacterial genomes, the genetic sequence had a 99.8% match to

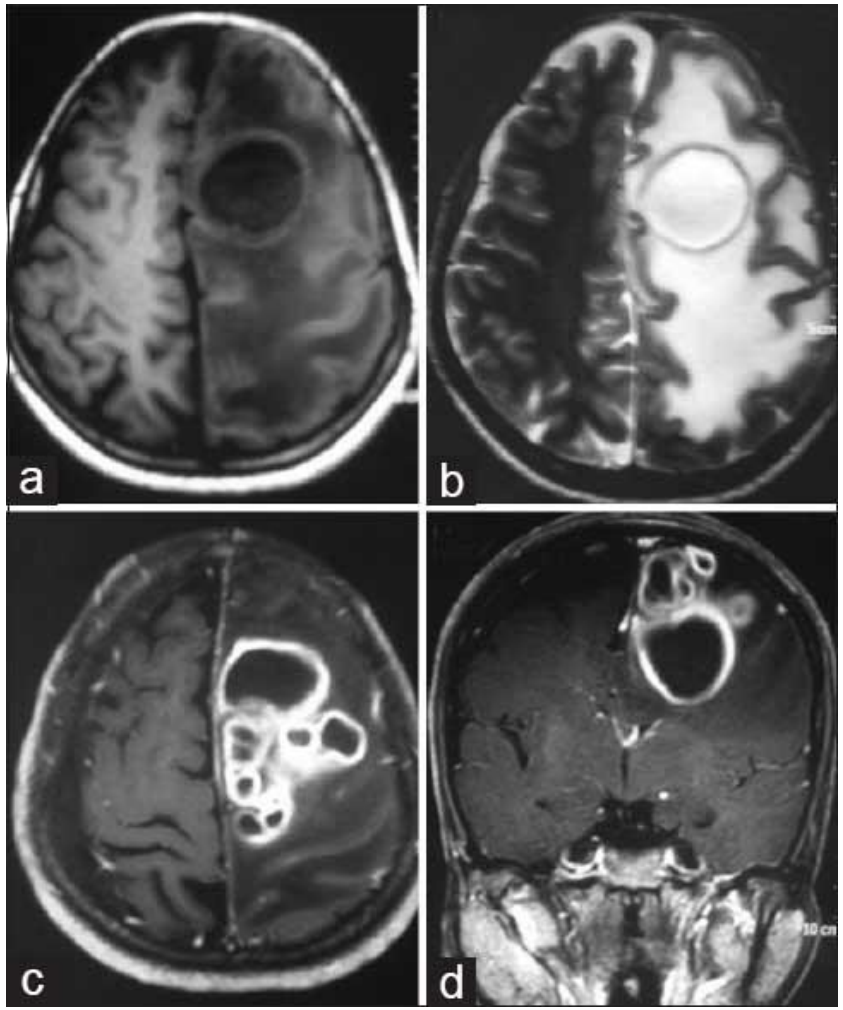
Nocardia asiatica

Nocardia : Everywhere you want to be

- Gram positive
- Filamentous
- Branching
- Modified acid-fast
- Soil saprophytes



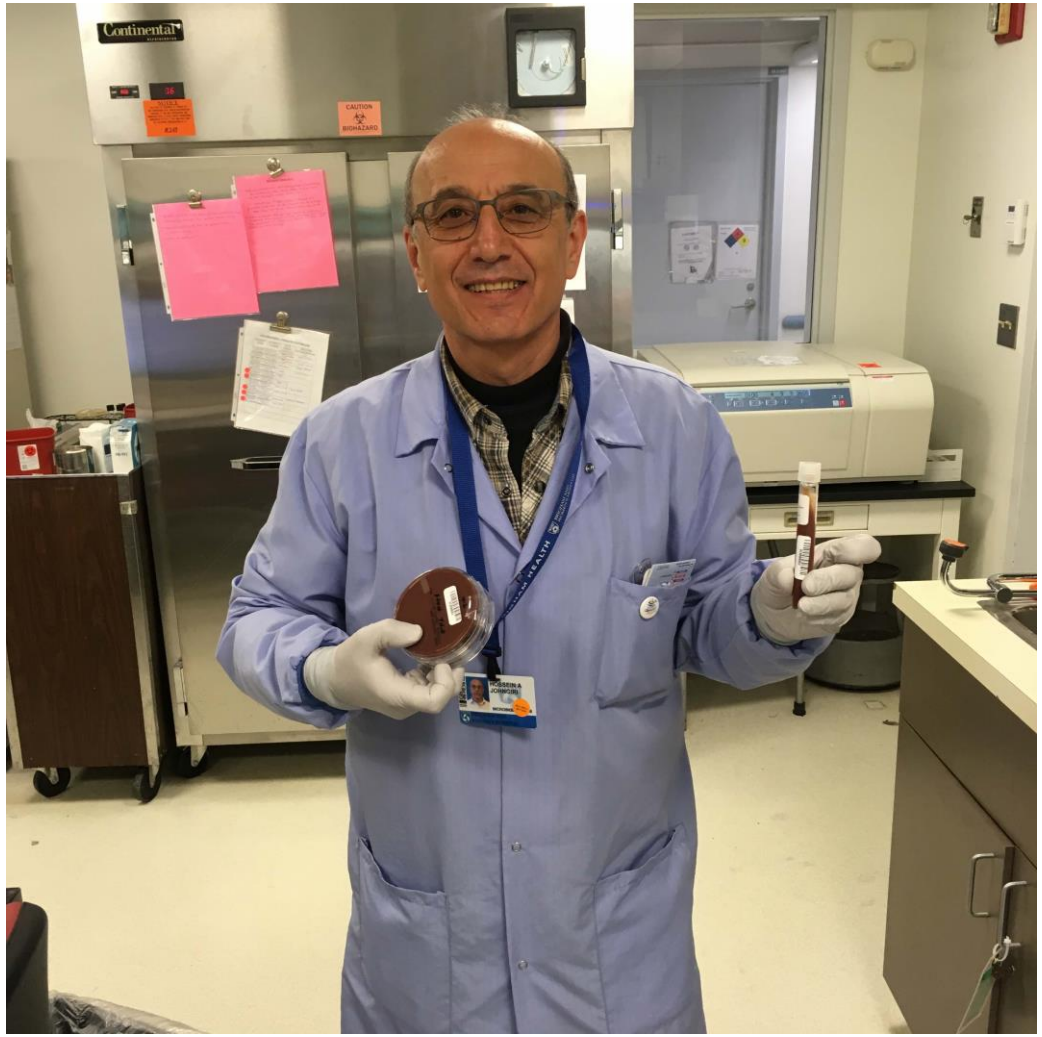
Nocardia : Everywhere you want to be



- Can cause disease in healthy and immunocompromised hosts
- 3 major clinical manifestations:
  - Cutaneous
  - Pulmonary
  - Disseminated (CNS in particular)

Micro saves the day (again)

- The patient was switched off levofloxacin and started on trimethoprim-sulfamethoxazole
- Her symptoms appear to have resolved and she is awaiting infectious diseases consultation for further management



Resistance is not futile

- 50 year old female with a history of uterine fibroids who presented to her general practitioner in India in August of 2017 with a complaint of R leg swelling for 2 weeks
- An ultrasound showed a 16x13cm mass attached to her uterus that was compressing her ureters
- She underwent a surgery to remove the tumor, which was identified as a leiomyosarcoma, and ureteral stents placed to maintain patency of her urinary tract
- She spent several days in the surgical intensive care unit in India

Resistance is not futile

- She comes to the Dana Farber Cancer Institute for further management of her cancer
- She is asymptomatic
- On routine pre-chemotherapy surveillance, she is noted on her urinalysis to have 50 WBCs
- A urine culture is obtained

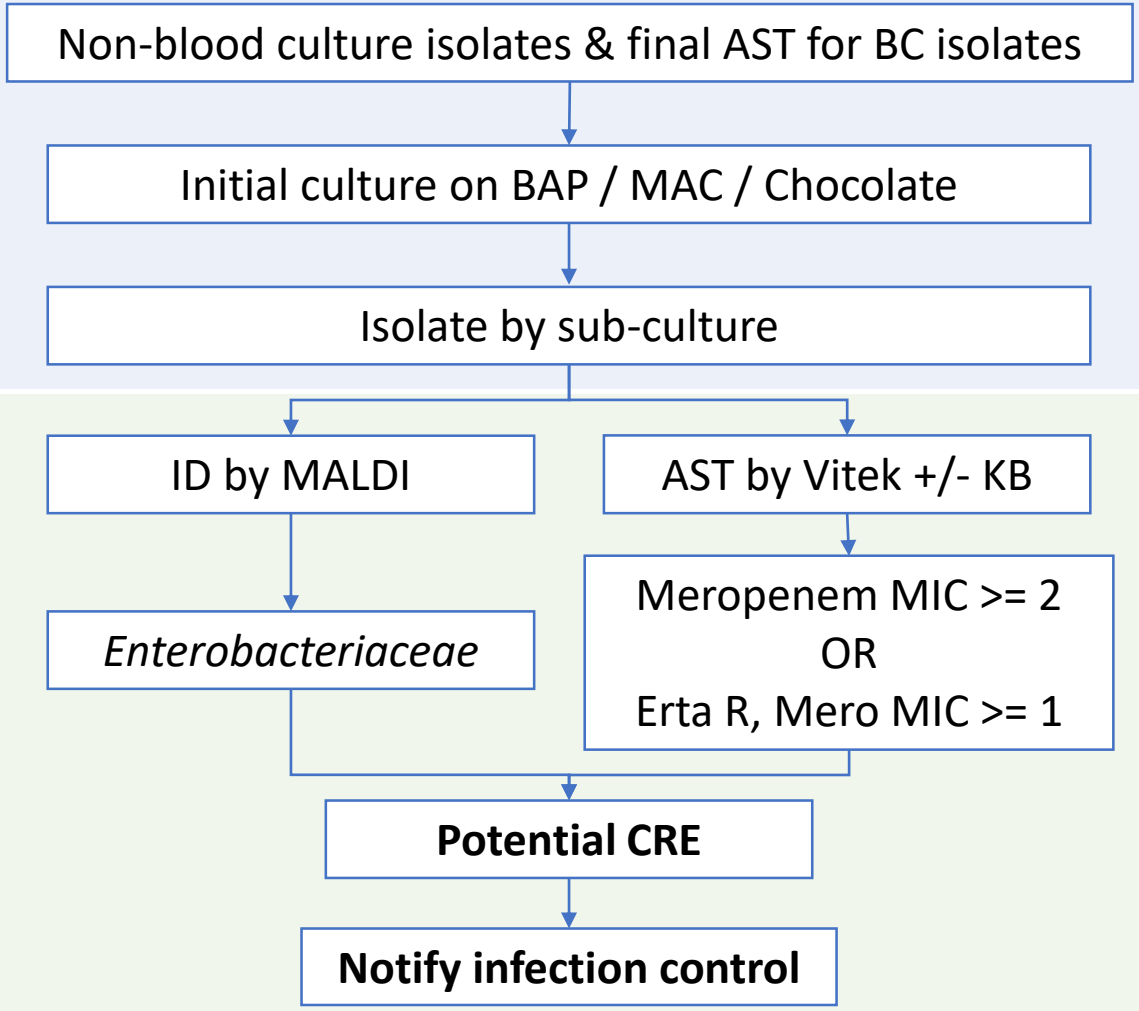
KLEBSIELLA PNEUMONIAE

Antibiotic	Interpretation	Value
Amikacin	Resistant	>=64
Amoxicillin-clavulanate	Resistant	>=32
Ampicillin	Resistant	>=32
Cefazolin	Resistant	>=64
Cefepime	Resistant	>=64
Cefoxitin	Resistant	>=64
Ceftazidime	Resistant	>=64
Ceftriaxone	Resistant	>=64
Ciprofloxacin	Resistant	>=4
Gentamicin	Resistant	>=16
Levofloxacin	Resistant	>=8
Meropenem	Resistant	>=16
Nitrofurantoin	Resistant	>=512
Piperacillin-tazobactam	Resistant	>=128
Tetracycline	Resistant	>=16
Tobramycin	Resistant	>=16
Trimethoprim/sulfamethoxazole	Resistant	80

Comments KLEBSIELLA PNEUMONIAE

100,000 colony forming units per ml KLEBSIELLA PNEUMONIAE

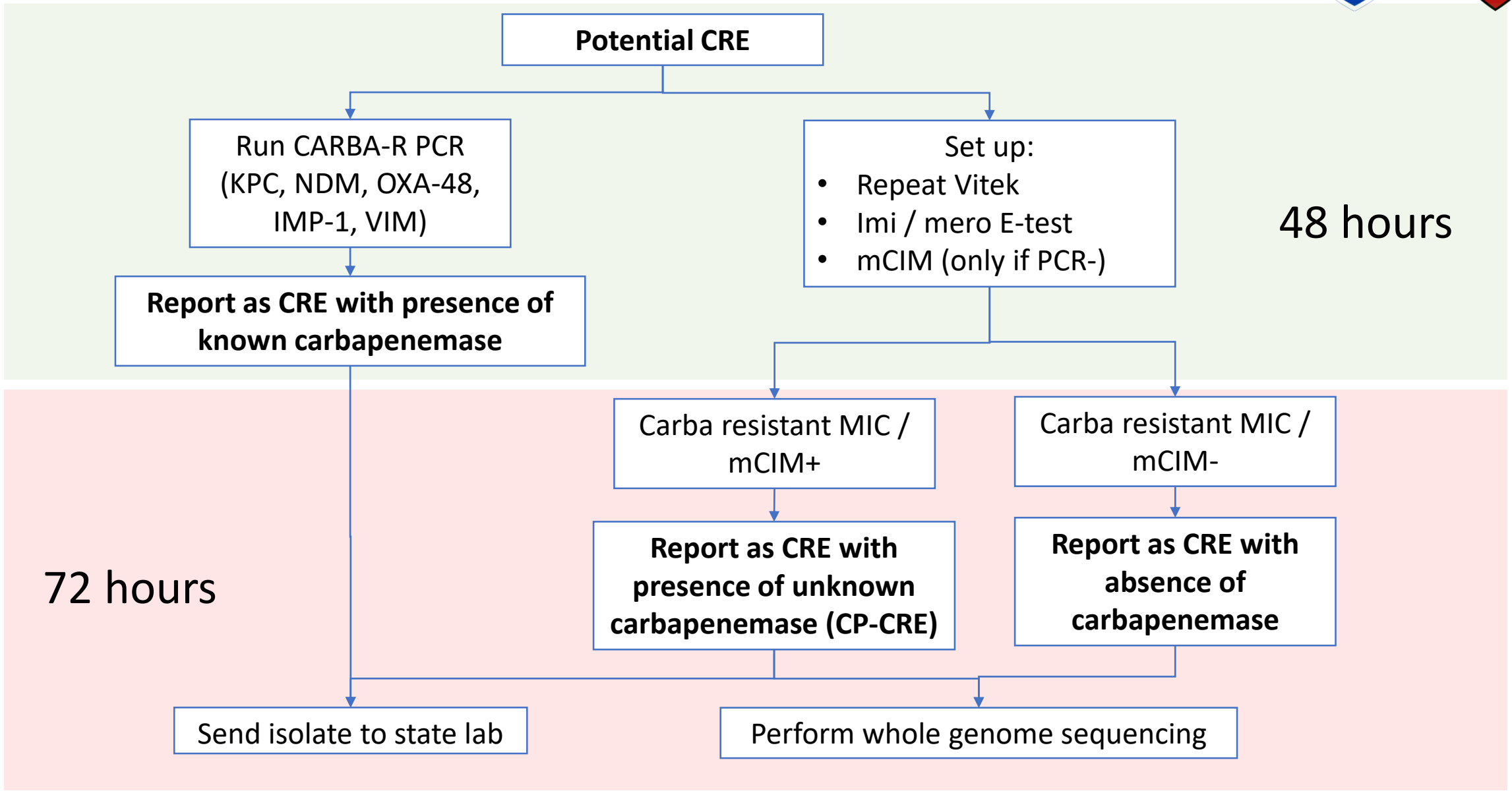
CRE protocol activated!



24 hours

48 hours

CRE protocol activated!



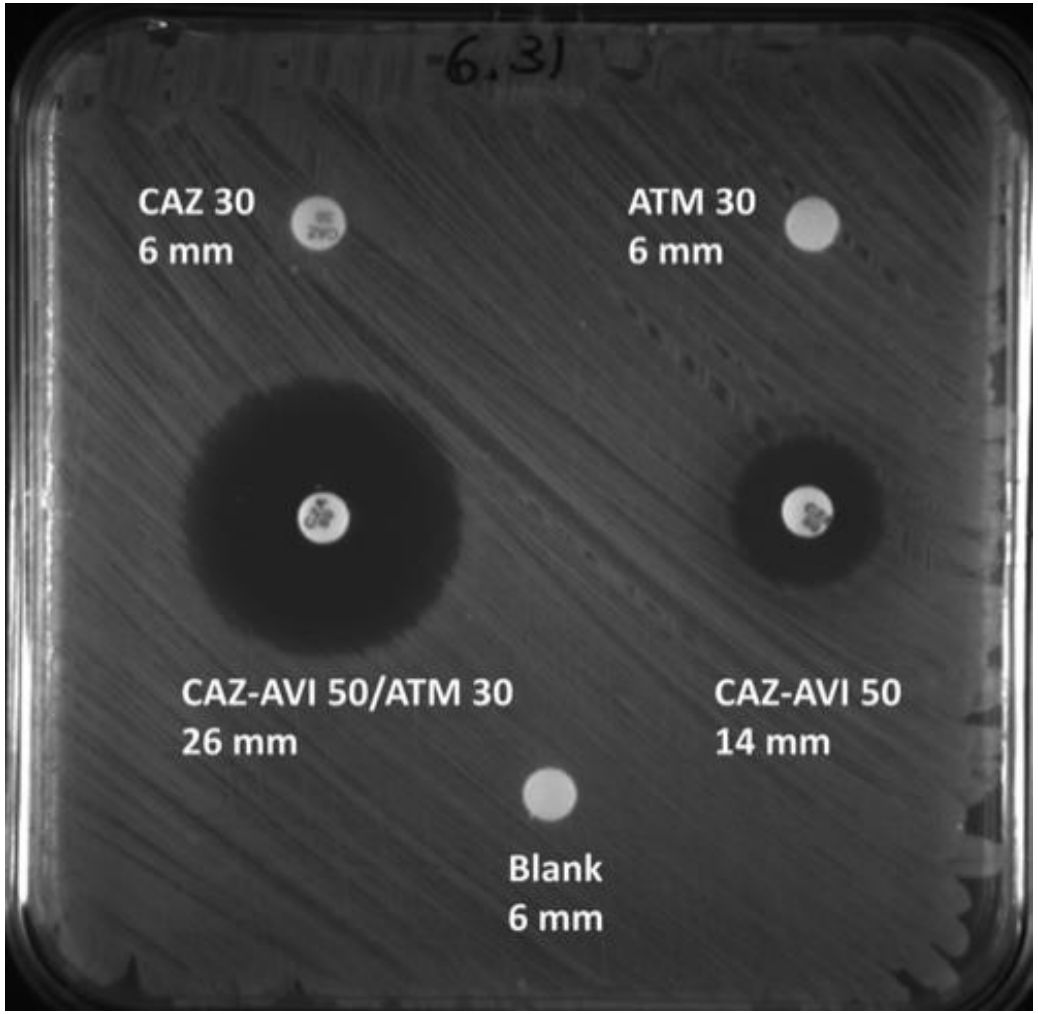
The Wild Wild East

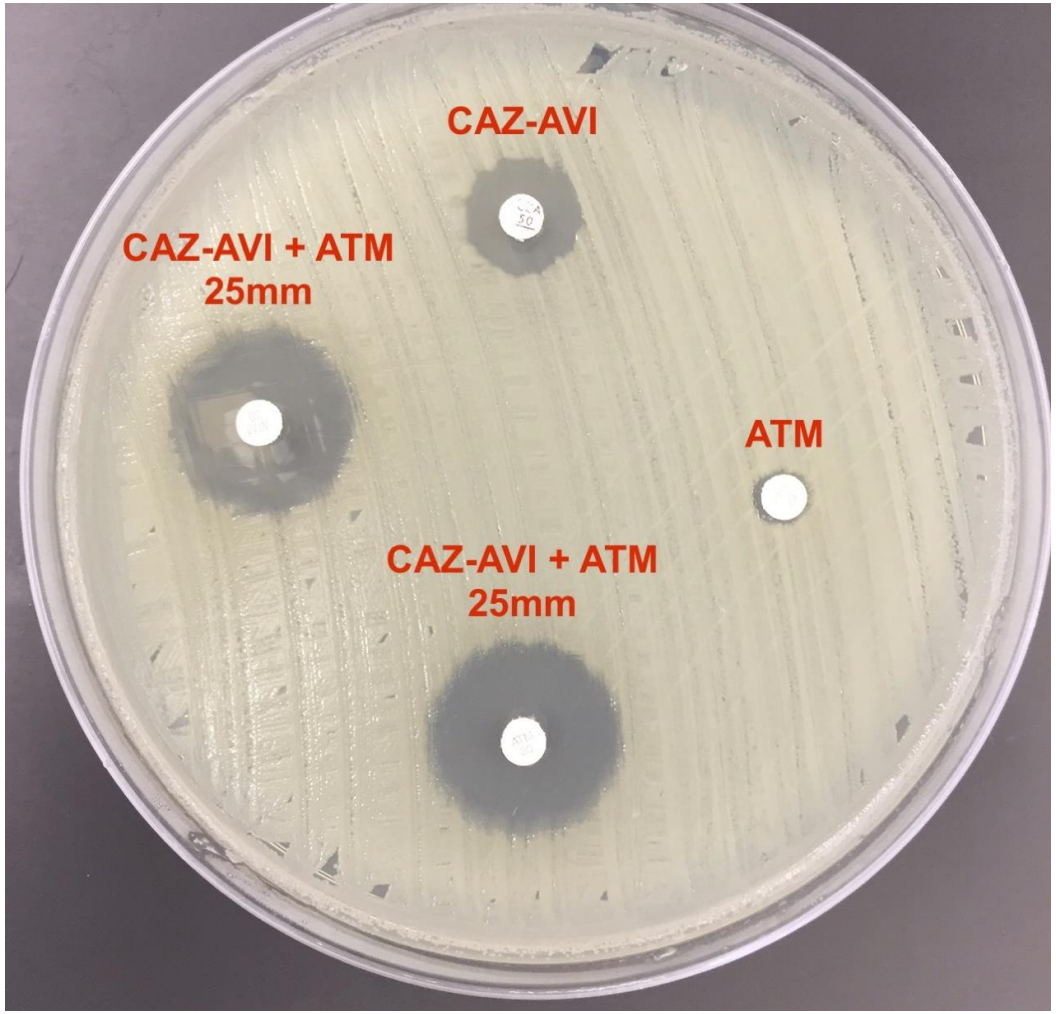
- An NDM-1 metalloproteinase was identified
- Special unvalidated antibiotic synergy testing was initiated

Can Ceftazidime-Avibactam and Aztreonam Overcome β-Lactam Resistance Conferred by Metallo-β-Lactamases in *Enterobacteriaceae*?

Steven Marshall,[a] Andrea M. Hujer,[a,b] Laura J. Rojas,[a,b,c]
Krisztina M. Papp-Wallace,[a] Romney M. Humphries,[d] Brad Spellberg,[e]
Kristine M. Hujer,[a,b] Emma K. Marshall,[a] Susan D. Rudin,[a,b] Federico Perez,[a,b]
Brigid M. Wilson,[a] Ronald B. Wasserman,[f] Linda Chikowski,[g] David L. Paterson,[h]
Alejandro J. Vila,[i] David van Duin,[j] Barry N. Kreiswirth,[k] Henry F. Chambers,[l]
Vance G. Fowler, Jr.,[m] Michael R. Jacobs,[n] Mark E. Pulse,[o] William J. Weiss,[o]
Robert A. Bonomo[a,b,c,p]

The Wild Wild East



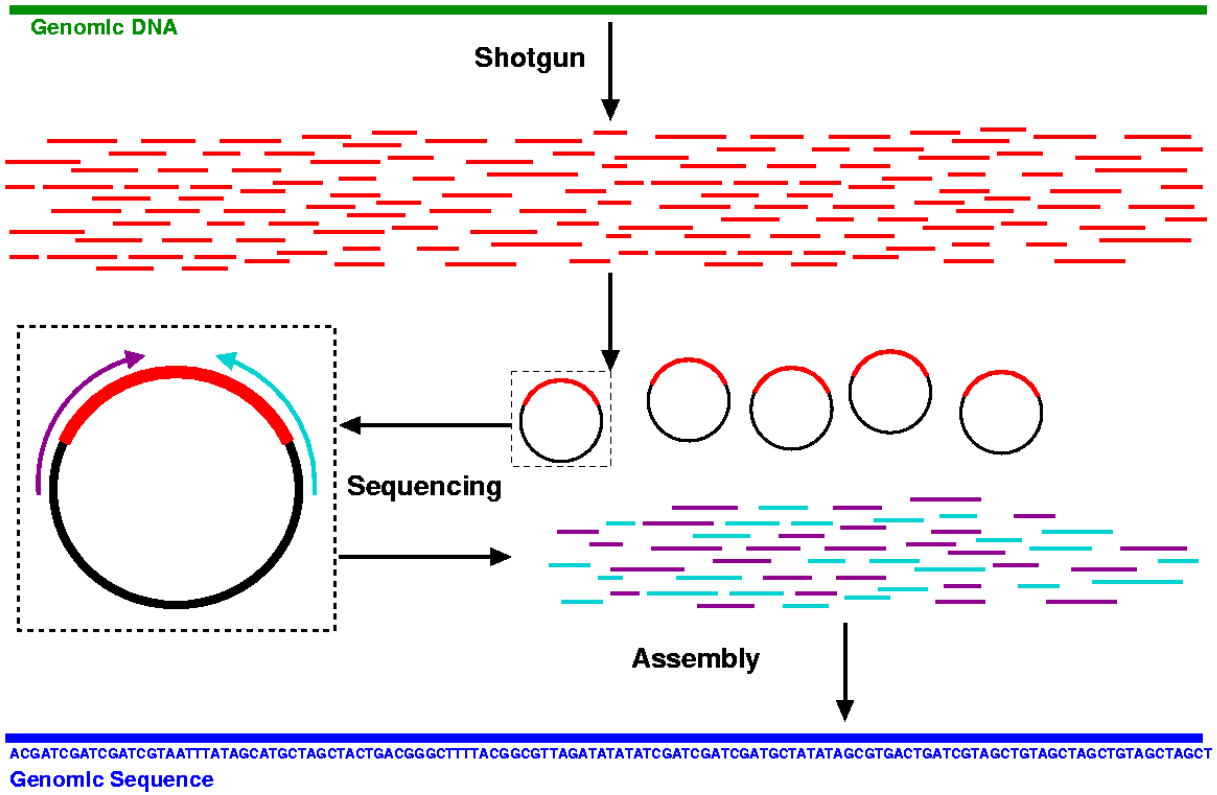


Micro saves the day (again)

- Patient had stents exchanged and was empirically started on meropenem and tigecycline but could not tolerate therapy
- She was switched to ceftazidime-avibactam + aztreonam and all subsequent urine cultures were negative for organisms
- She was able to start her chemotherapy

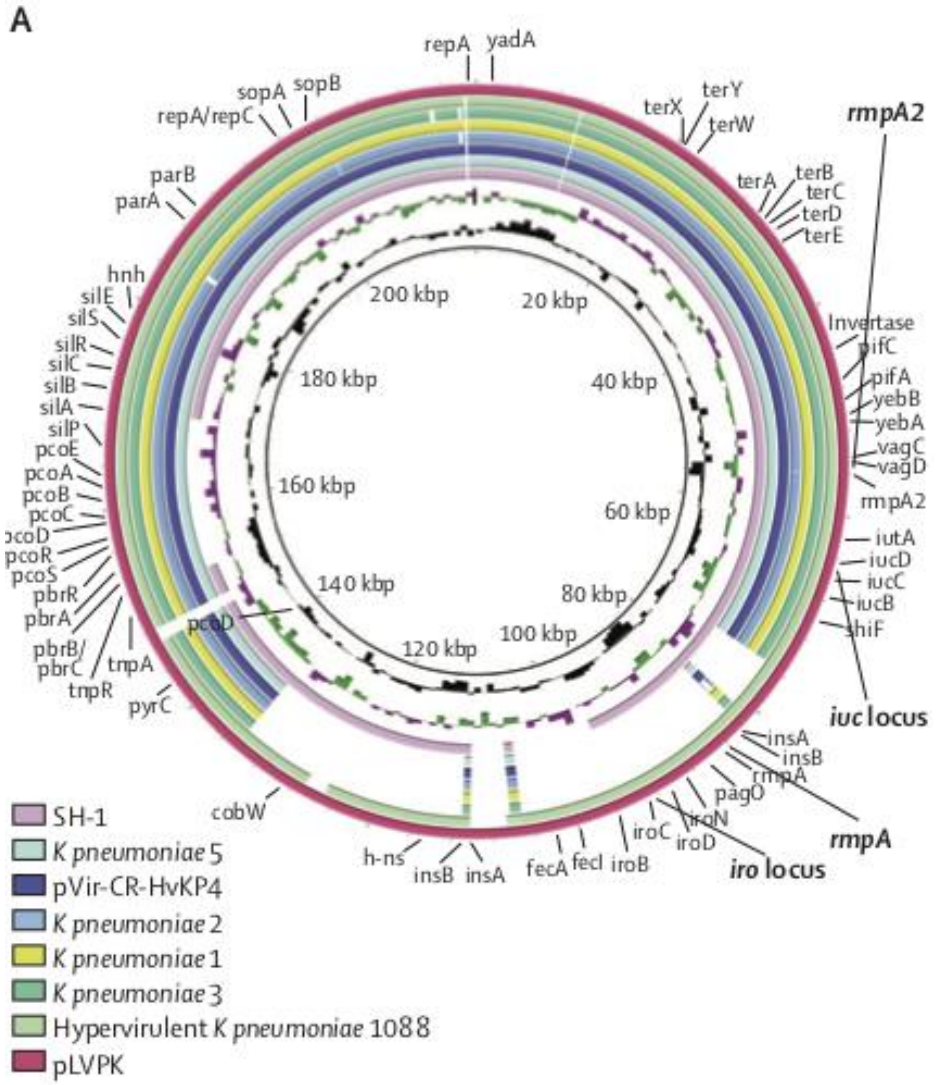
The future

- Whole genome sequencing is relatively slow but provides data that was rarely if ever utilized in clinical practice
- Provides a new way of thinking about clinical management that promotes
  - Better estimation of patient treatment response
  - Improved insight into the inter-connectedness of patients

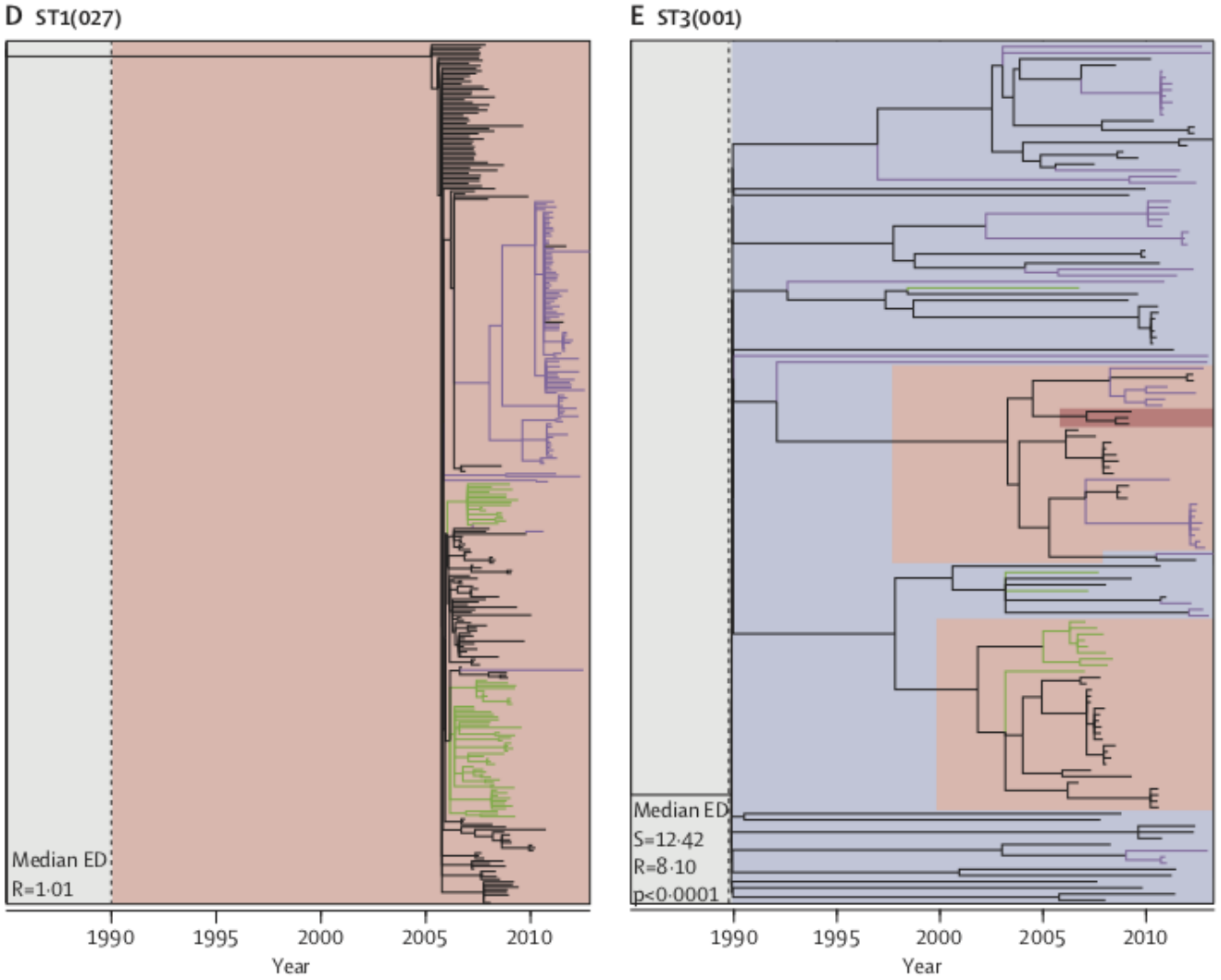


From one test, many answers (and even more questions)

- From a single sequence
  - Genetic basis of antibiotic susceptibility
  - Genetic basis of virulence
  - Presence of mobile genetic elements
  - Identification of new mechanisms of resistance



From one test, many answers (and even more questions)



- From comparison of multiple sequences
  - Identification of high-risk clones for infection control, state / national labs
  - Identification of nosocomial transmission events
  - Prediction of treatment response
  - Evolutionary dynamics of antibiotic resistance

Back to our patient

Class	Subclass	Gene(s)
Beta-lactams	Penicillins Beta-lactam combinations Cephalosporins	*SHV-11* *CTX-M-15* *OXA-11* *TEM-199*
	Carbapenems	*NDM-1*
Macrolides		*msr(E)*
Aminoglycosides		*APH(3')* *AAC(6')*
Rifamycins		*arr-6*
DHFR		*sul1*
Fosfomycin		*fosA5*
Multidrug		*emrD*

- A second *K. pneumoniae* isolate obtained at the same time was identical except it lacked the NDM-1 gene
  - Dynamic acquisition and loss?
- 2 subsequent NDM-1+ isolates have been identified since October
  - No travel history for both

We serve multiple masters

Direct patient care

Inpatient / outpatient populations

Financial imperatives

Policy makers

Providers

Antimicrobial stewardship

Infection control

State / national public health agencies

Academic researchers

Clinical microbiology lab

Our deliverables are deceptively simple

- Organism identification and quantification
- Susceptibilities to anti-infectives
- Assaying indirect biomarkers of infection
  - β-D-glucan
  - Antigen / antibody tests

Our true deliverables span a much wider range

- For the provider
  - Communication of critical results
  - Interpretation of test results
  - Offline / unvalidated testing for challenging cases
  - Prompt send out to reference laboratories or initiation of additional testing
- For ASPs
  - Calculation of cumulative antimicrobial susceptibility reports
- For infection control
  - Surveillance of high-risk organisms
  - Identification of new resistant phenotypes
  - Identification of newly emerging or highly dangerous pathogens
- For all
  - Diagnostic stewardship

A major headache

- 27 year old female presents in February 2018 with a 1 day history of severe headache and vomiting.
- She denies fever, chills and has no sick contacts
- The week before her presentation she had travelled to Mexico for vacation and stayed at a resort
  - While there, she ate some soft cheeses

A major headache

- A lumbar puncture was performed

CSF CHEMISTRIES	
Lactate, CSF	2.6 *
Glucose, CSF	63 *
Total Protein, CSF	70.5 *

CSF COUNTS AND DIFF				
Color, CSF	COLORLESS		COLORLESS	
Turbidity/Appearan...	SL HAZY	!	SL HAZY	!
RBC, CSF	183	!	8	!
Nucleated cells, CSF	263 *	!!	463 *	!!
Blasts, CSF (%)	0		0	
Bands, CSF (%)	0		0	
Neutrophils, CSF (%)	74		58	
Lymphs, CSF (%)	12		21	
Monos, CSF (%)	13		19	
Eos, CSF (%)	0		0	
Basos, CSF (%)	0		1	
Plasma cells, CSF			1	
NRBC#, CSF	0		0	
Xanthochromia, CSF	NOT PRESENT		NOT PRESENT	

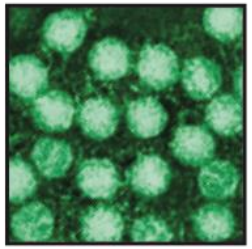
- She is started on broad-spectrum antibiotics and antivirals
- A specimen was run on the Biofire meningitis/encephalitis platform

What is the BIOFIRE?

- The first FDA-approved syndromic panel for diagnosis of meningitis / encephalitis

Bacteria

Escherichia coli K1
Haemophilus influenzae
Listeria monocytogenes
Neisseria meningitidis
Streptococcus agalactiae
Streptococcus pneumoniae



Viruses

Cytomegalovirus (CMV)
Enterovirus
Herpes simplex virus 1 (HSV-1)
Herpes simplex virus 2 (HSV-2)
Human herpesvirus 6 (HHV-6)
Human parechovirus
Varicella zoster virus (VZV)

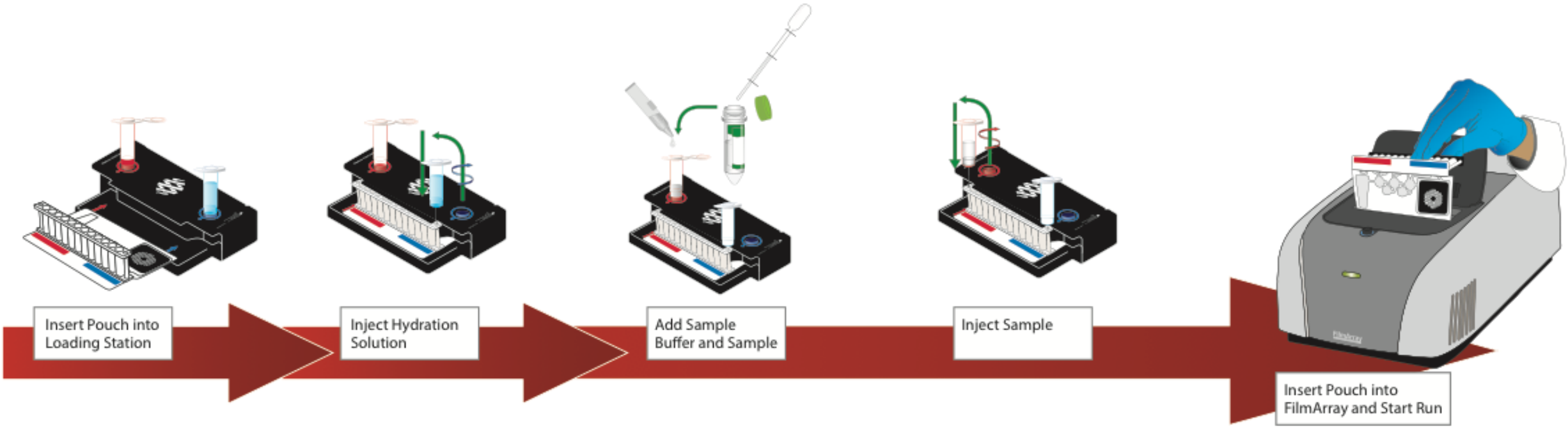
Fungi

Cryptococcus neoformans/gattii

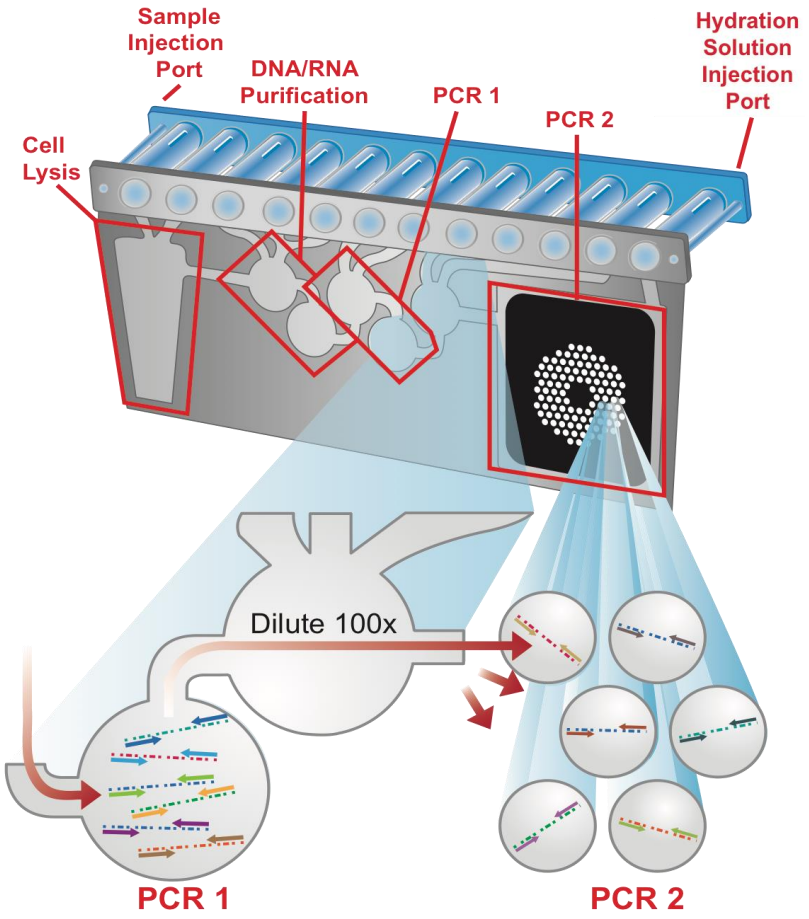
Why use the BIOFIRE?

- Diagnosis of meningitis and encephalitis is really hard
  - Non-specific symptoms
  - Deadly for some but not all organisms
  - Sensitivity of traditional diagnostics is poor
- Minimal hands-on (5’) and rapid turnaround (60’) times
- Detects most common pathogens
- High sensitivity

The FilmArray

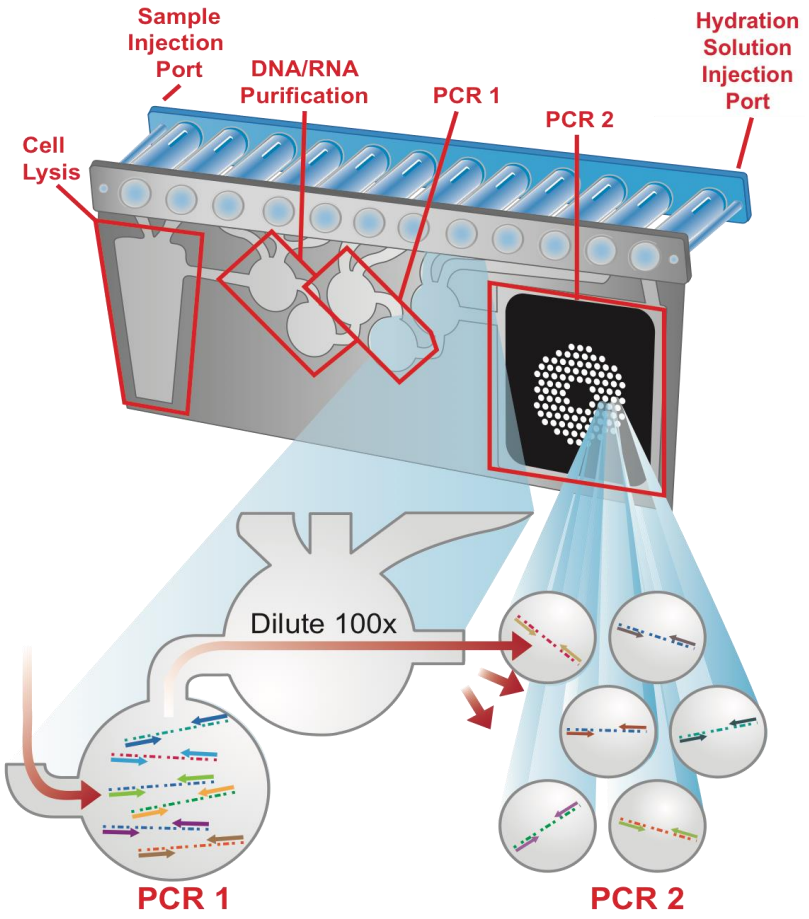


The FilmArray



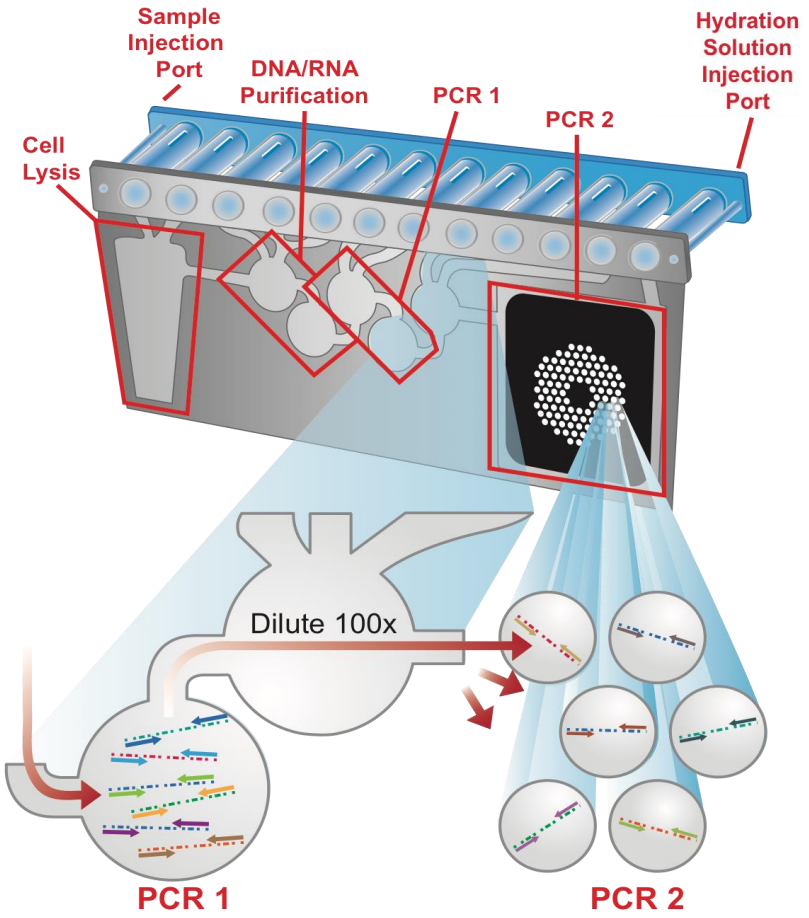
- Step 1: Cell lysis
  - Ceramic beads break up human and bacterial cells and virions
  - Free RNA/DNA bound by magnetic beads and moved to purification chamber

The FilmArray



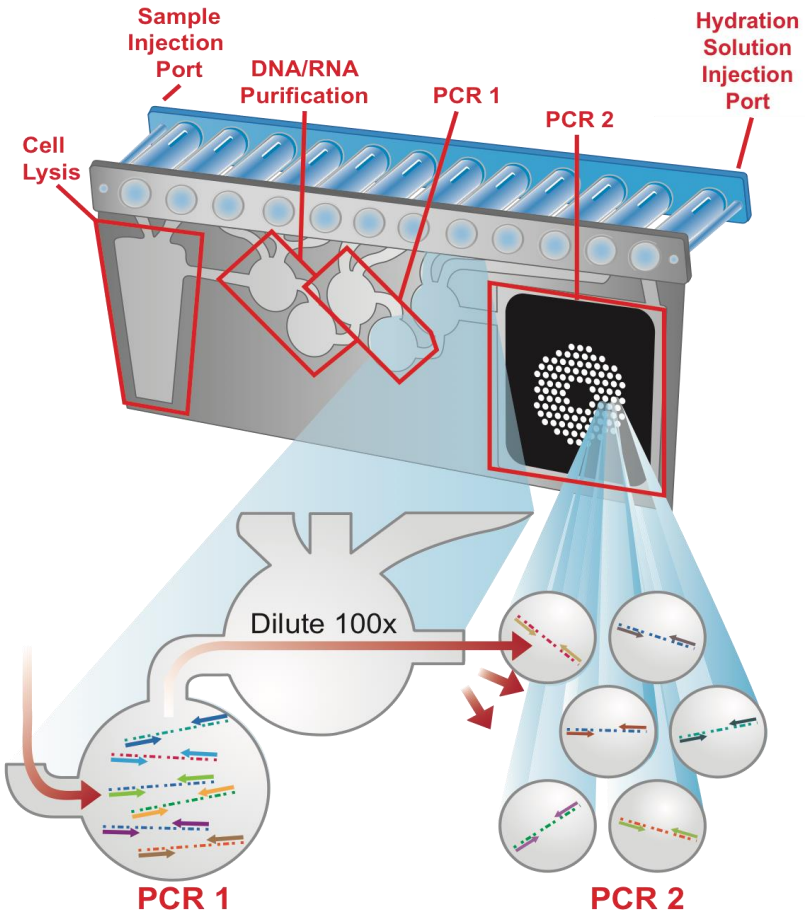
- Step 2: Purification
  - Beads held stationary by a magnet outside of the pouch while remaining cellular debris is washed away
  - Beads released and elution buffer washes the nucleic acid off of them
  - Beads are again held by magnets as eluted nucleic acids move to next chamber

The FilmArray



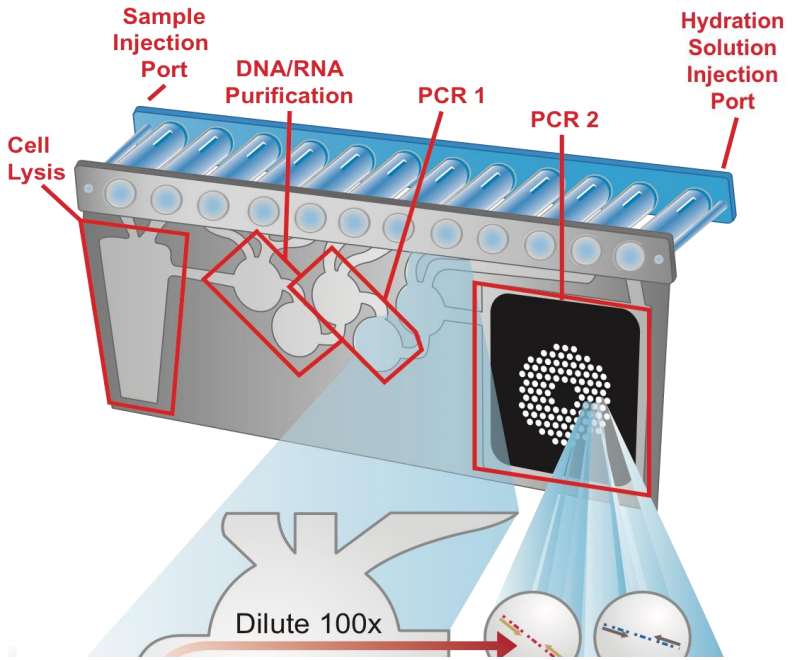
- Step 3: PCR stage 1
  - RNA converted to DNA
  - Multiplex PCR amplification
  - Buffer added to dilute remaining unbound PCR primers
  - Fresh master mix added and solution added to each cell of microarray

The FilmArray



- Step 4: PCR stage 2
  - Wells spotted with 2nd stage primers highly specific to amplicons made in the first stage
  - Detected through addition of a fluorescent dye that binds ds-DNA
- Step 5: DNA melting
  - Compares the measured T_m to known T_m of ds-DNA increases specificity

The FilmArray



- Advantages
  - Closed system
  - Required volume: 200ul
  - Hands on time: ~5 minutes
  - Analysis time: 60 minutes
- Disadvantages
  - Closed system

Clin Chem Lab Med. 2017 Aug 1. pii: /j/cclm.ahead-of-print/cclm-2017-0518/cclm-2017-0518.xml. doi: 10.1515/cclm-2017-0518. [Epub ahead of print]

False negative results caused by erroneous automated result interpretation algorithm on the FilmArray 2.0 instrument.

Lee CK[1], Chiu L[1], Yan G[1], Chew KL[1], Yan B[1], Jureen R[1], Loh TP[1].

Back to our patient

- Antibiotics were discontinued and the patient's symptoms resolved in 48 hours
- Infection was presumed acquired in Mexico

CRYPTOCOCCUS NEO/GAT	Not Detected
CYTOMEGALOVIRUS	Not Detected
ENTEROVIRUS	DETECTION !
ESCHERICHIA COLI K1	Not Detected
H. INFLUENZAE	Not Detected
HSV 1	Not Detected
HSV 2	Not Detected
HUMAN HERPESVIRUS 6	Not Detected
HUMAN PARECHOVIRUS	Not Detected
L. MONOCYTOGENES	Not Detected
N. MENINGITIDIS	Not Detected
S. AGALACTIAE(GRP B)	Not Detected
STREP. PNEUMO	Not Detected
VZV	Not Detected

Challenges

BE THOROUGH

High penalty for incorrect identification or AST results

BE FAST

Intense pressure to report results as fast as possible

- The availability of effective empiric antibiotic therapy provides the lab a safety window within which they can perform a workup with relatively low harm to the patient

A false sense of security

BE THOROUGH

High penalty for incorrect identification or AST results

BE FAST

Intense pressure to report results as fast as possible

- Empiric antibiotic therapy is not without significant problems
  - Inadequate therapy associated with poor treatment outcomes
  - Overly broad therapy associated with selection of drug resistant organisms

A false sense of security

BE THOROUGH

High penalty for incorrect identification or AST results

BE FAST

Intense pressure to report results as fast as possible

- The philosophy of clinical microbiology labs has been to favor patient-level outcomes over population-level outcomes
- Delays in reporting make de-escalation difficult

The future?

BE THOROUGH

High penalty for incorrect identification or AST results

BE FAST

Intense pressure to report results as fast as possible

- Advances in the sensitivity, specificity and throughput of molecular platforms has engendered intense academic and commercial interest
- The hope is for these technologies to resolve the tension between accuracy and turnaround time

Syndromic panels are IN

Syndrome	Manufacturer	Model	Market entry
Bloodstream infection	Biofire	FilmArray	2013
	Luminex	Verigene GP	2012
		Verigene GN	2014
	Accelerate	Pheno	2017
Respiratory infection	Biofire	FilmArray	2011
	GenMark	eSensor RVP	2013
		ePlex	2017
	Luminex	xTAG RVP v1	2008
		RVP Fast	2011
		Verigene Respiratory Pathogen Flex	2015
		NxTAG	2015
Meningitis / encephalitis	Biofire	FilmArray	2015

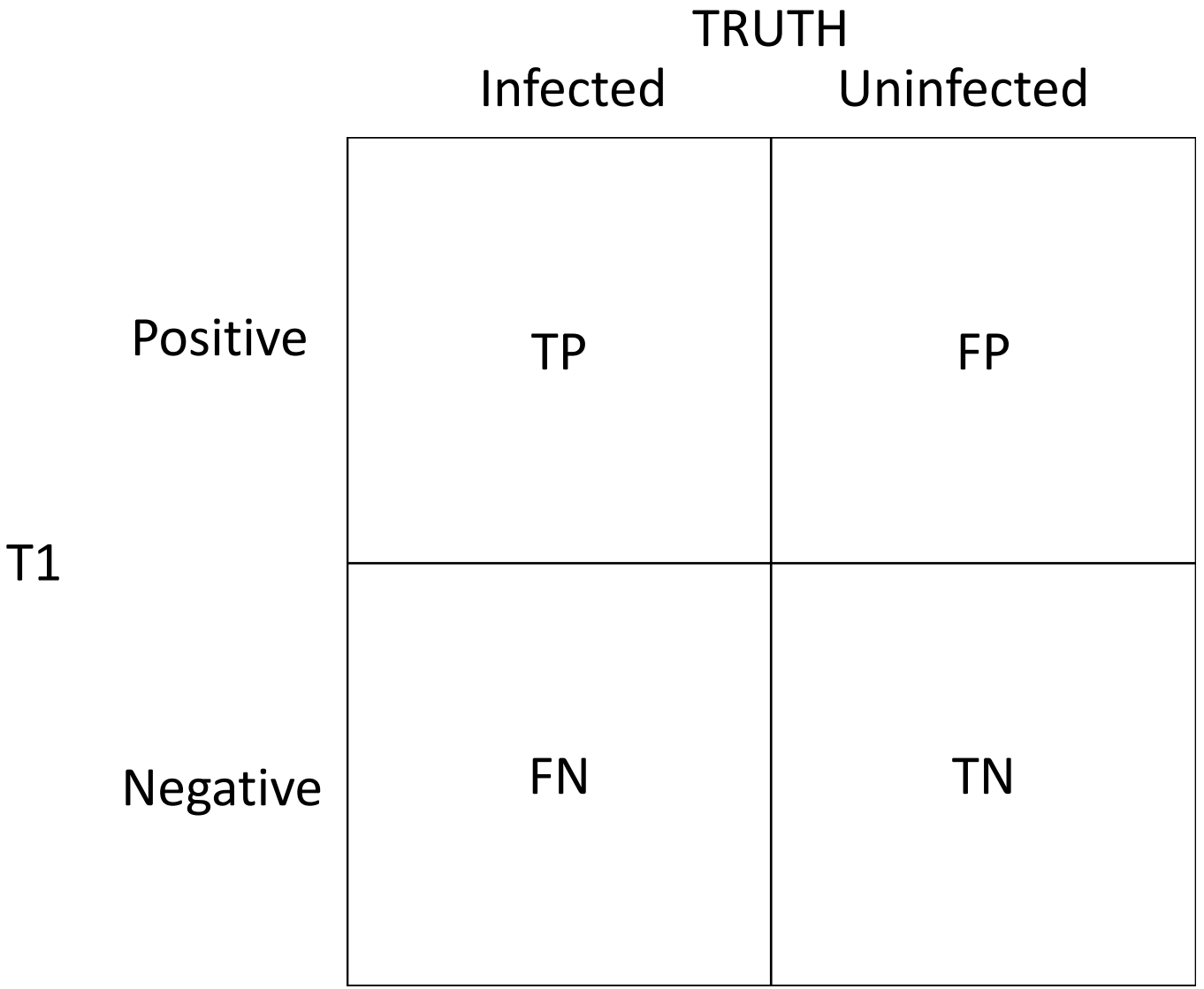
Why newer platforms will not replace traditional diagnostics (yet)

- Do not perform comprehensive AST*
- Finite range of targets
- Poor performance in polymicrobial infections
- Slightly decreased sensitivity

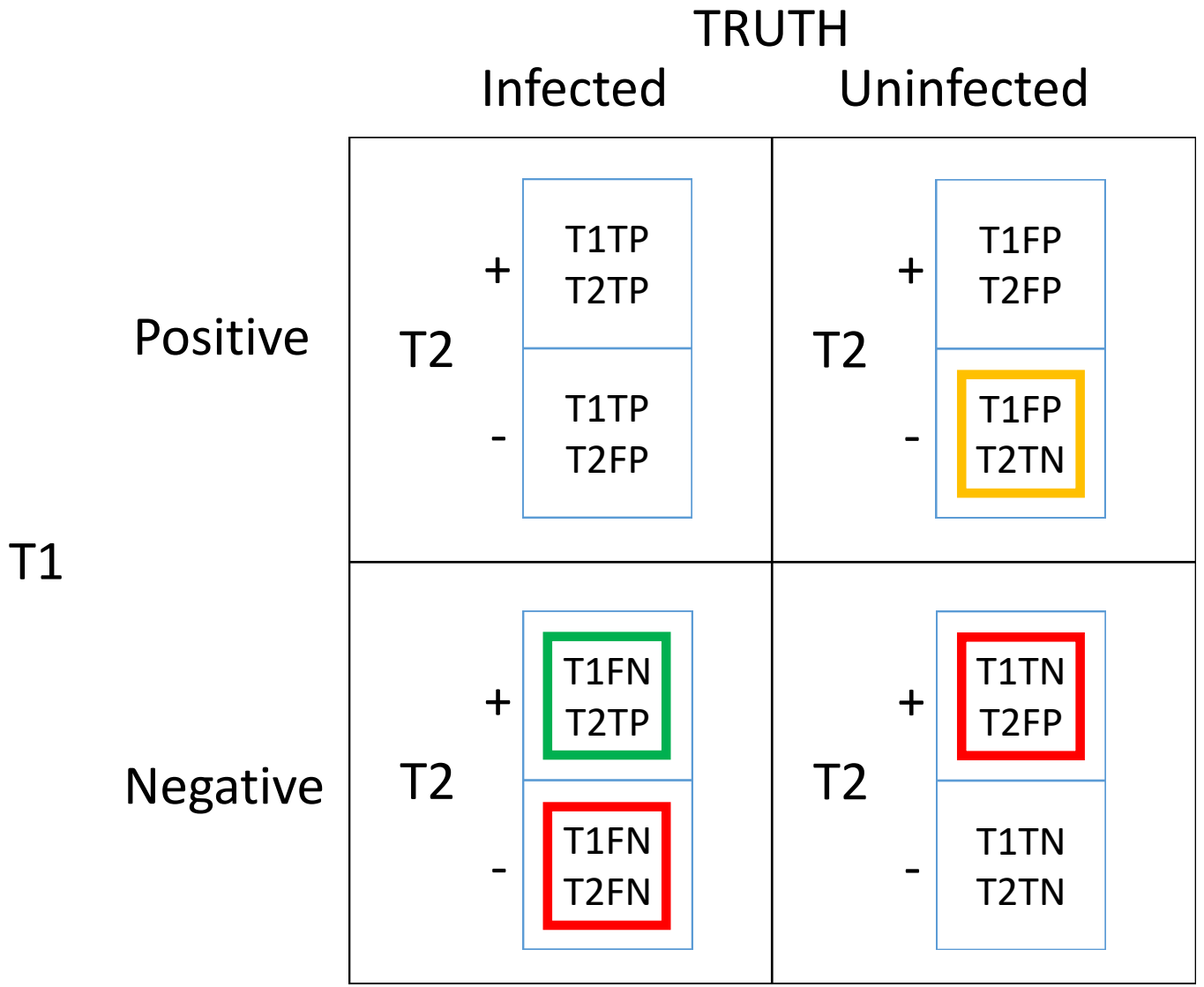
Why newer platforms will not replace traditional diagnostics (yet)

- Results from such platforms will arrive within a few hours and should be viewed as ‘preliminary’
  - The understanding that additional confirmation and/or information will arrive in the following 24 – 48 hours through older algorithms
  - Would not change initial empiric management
- Cost and hands-on time prohibits running on every patient sample

With more data comes more complexity



With more data comes more complexity



Take home points

- The clinical micro lab is a dynamic and exciting environment
- Much of what we do depends heavily on the skills of the lab staff
- Our work connects not only to direct patient care but to systems of public health
- The field is undergoing a sea change in terms of our approaches and requires a new set of critical thinking skills

Thank you!

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