Next-Generation Sequencing for Infectious Disease Diagnostics: The Next Paradigm Shift?

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Pathology Grand Rounds at the University of Utah/ARUP Laboratories

Disclosures

- Research Contracts:
  - BD Diagnostics, OpGen Inc., Affinity Biosensors, Qiagen Sciences Inc, T2 Diagnostics
- Speaker's Bureau
  - GenMark Dx, BD Diagnostics, OpGen Inc.,
- Research Collaborators:
  - Ares Genetics, CosmosID, IDbyDNA, Illumina
- Consulting:
  - OpGen Inc., BD Diagnostics, Shionogi Inc., GeneCapture, Qiagen Sciences Inc, Entasis

Objectives

1. List the applications of next-generation sequencing (NGS) for infectious disease diagnostics

2. Describe the performance, implementation and value of various NGS assays for patient management

3. Discuss the development of NGS assays to evaluate host response, the microbiome and antimicrobial resistance (AMR) detection

NGS Is Changing the Way We Practice Medicine

- Inherited diseases
- Constitutional disorders
- Oncology

Defining the genetic determinants of disease leads to improved diagnostic yield and allows for early or targeted therapeutic interventions

Yong et al, Ann Lab Med 2021; 41(1): 25-43

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What Is the Role of NGS for Infectious Disease Diagnostics?

Poor Diagnostic Yield

- 40-60% of meningoencephalitis cases
- 15-62% of pneumonia cases
- 20% of sepsis cases

<u>Despite all available diagnostics</u>

Piecemeal Tests

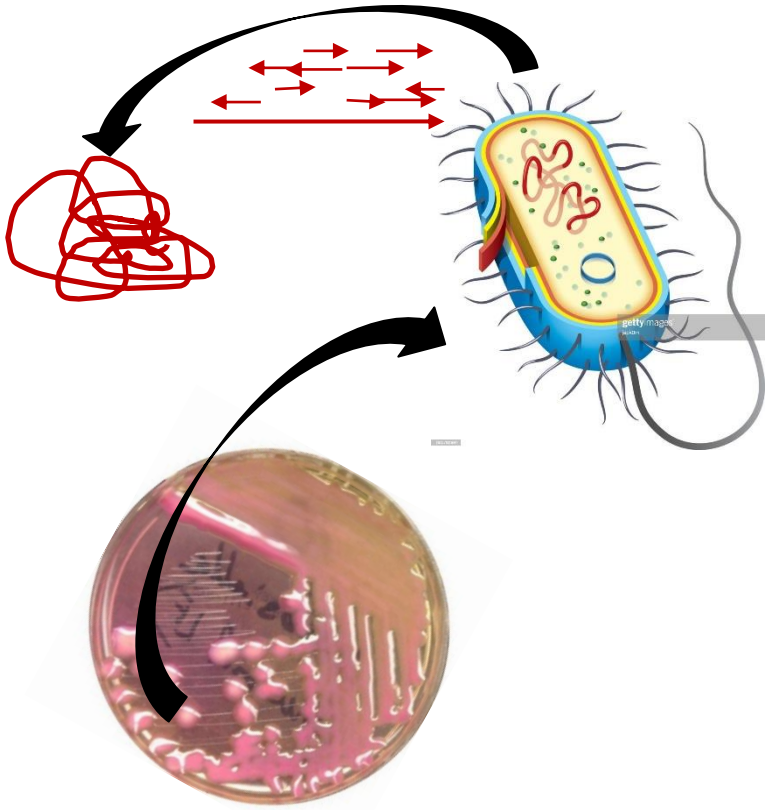
- Typical infectious disease patient undergoes a battery of tests
- Available tests have limited sensitivity & scope
- A prolonged diagnostic workup may lead to increased hospital stays, costs and unnecessary treatment

High Hospital Costs

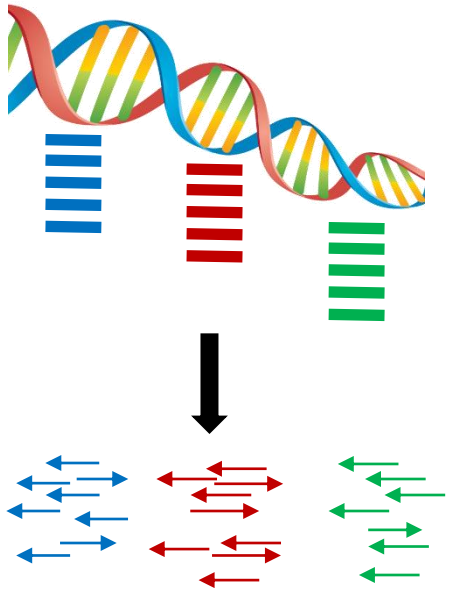
- Cost Center Incurred
- Pneumonia and sepsis incur $8.1 billion in costs each year
- Antimicrobial resistance (AMR) costs the global economy 1 trillion dollars

NGS Applications for Infectious Diseases

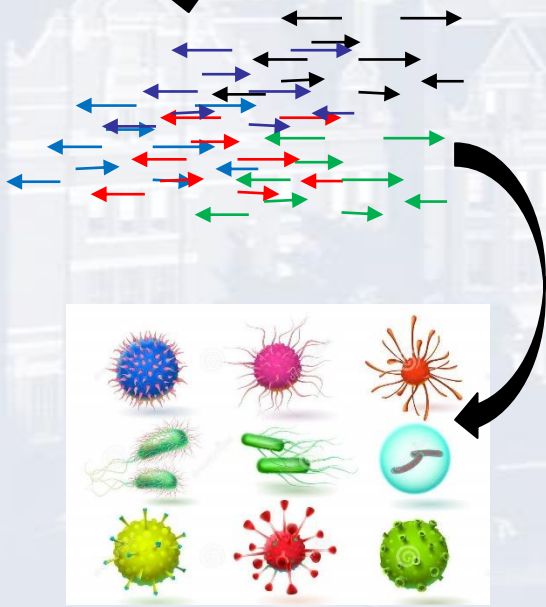
A. Whole Genome Sequencing



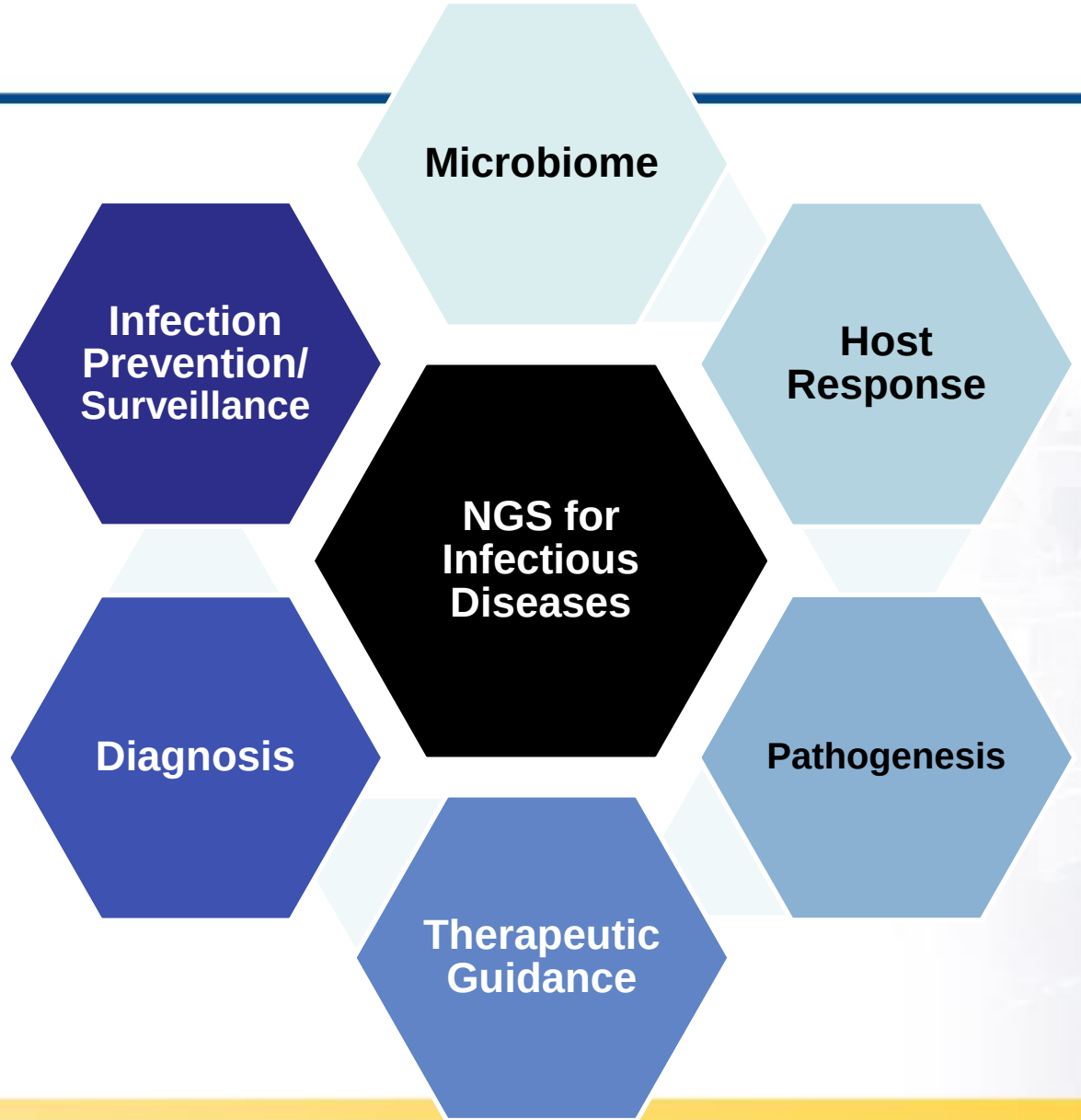
B. Targeted NGS (tNGS)



C. Metagenomic NGS (mNGS)

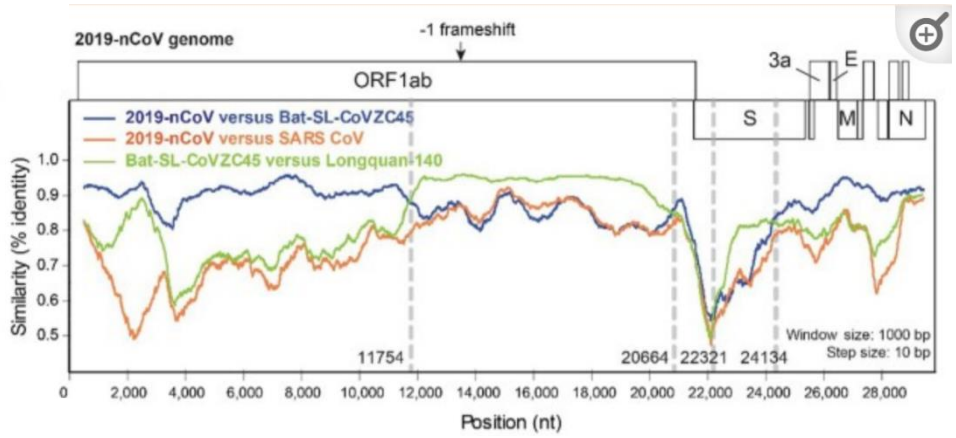


Holds the Potential To Be More!



SARS-CoV-2 Placed the Spotlight on NGS

- Discovery & diagnosis of SARS-CoV-2
  - Unknown until un-targeted, RNA based metagenomic NGS was able to identify a novel coronavirus



Chen et al, EID, 2020; PMID: 32020836

- Global Genomic Surveillance

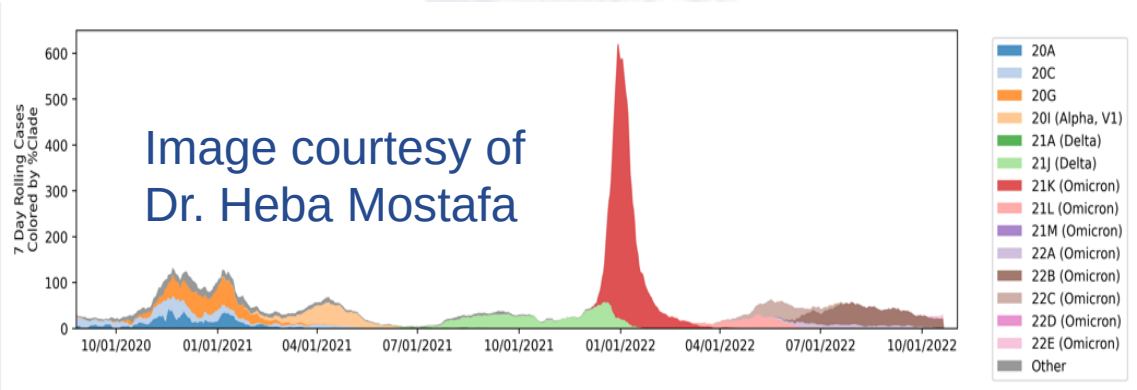
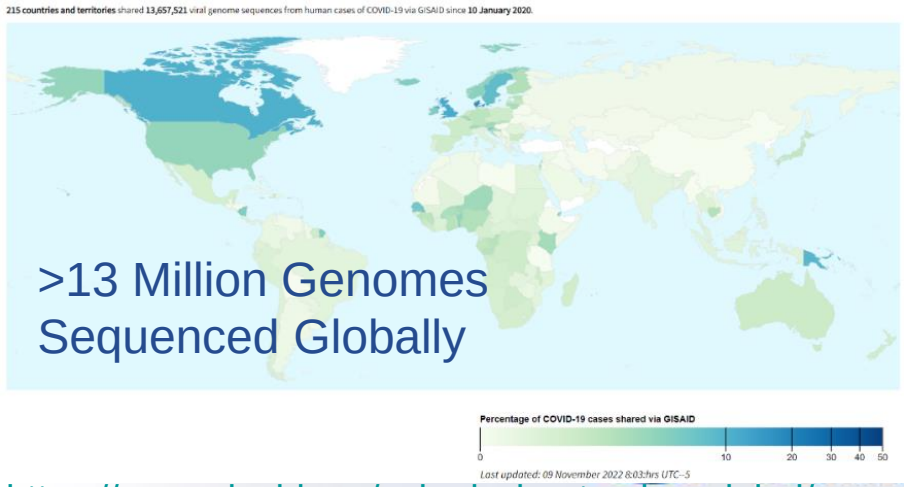


Image courtesy of Dr. Heba Mostafa



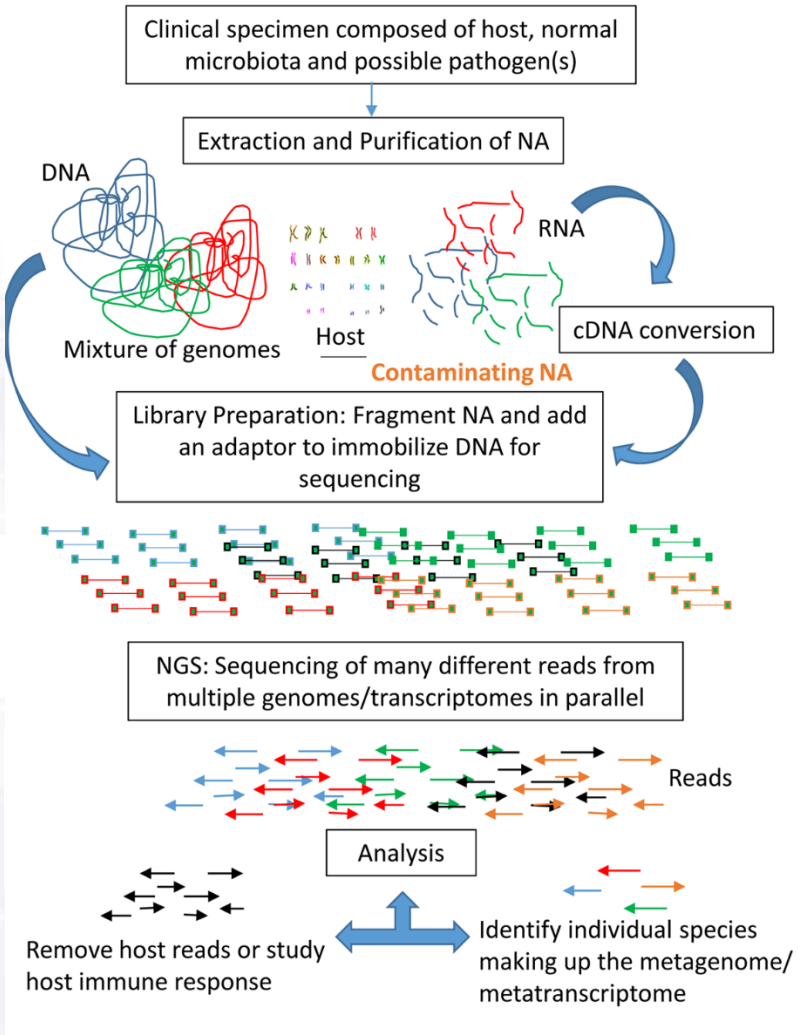
>13 Million Genomes Sequenced Globally

https://www.gisaid.org/submission-tracker-global/

PART 1: DIRECT FROM SPECIMEN SEQUENCING

Metagenomic Next-Generation Sequencing = mNGS

- Allows for pan-nucleic acid detection directly from patient specimens
- All nucleic acid within a specimen is extracted and sequenced in parallel, resulting in sequencing of both host and microbial reads
- ID diagnostics we ignore the host reads and focus on the microbial reads



Simner *et al*, Clin Infect Dis, 2018 (PMID: 29040428); Mitchell and Simner, Clin Lab Med, 2019 (PMID:31383265).

It All Started When…

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

Michael R. Wilson, M.D., Samia N. Naccache, Ph.D., Erik Samayoa, B.S., C.L.S., Mark Biagtan, M.D., Hiba Bashir, M.D., Guixia Yu, B.S., Shahriar M. Salamat, M.D., Ph.D., Sneha Somasekar, B.S., Scot Federman, B.A., Steve Miller, M.D., Ph.D., Robert Sokolic, M.D., Elizabeth Garabedian, R.N., M.S.L.S., Fabio Candotti, M.D., Teresa L. Meyer, R.N., M.D., Sheryl L. Henderson, M.D., a

Next-generation sequencing in neuropathologic diagnosis of infections of the nervous system

OPEN

Steven L. Salzberg, PhD
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ABSTRACT

Objective: To determine the feasibility of next-generation sequencing (NGS) microbiome approaches in the diagnosis of infectious disorders in brain or spinal cord biopsies in patients with suspected CNS infections.

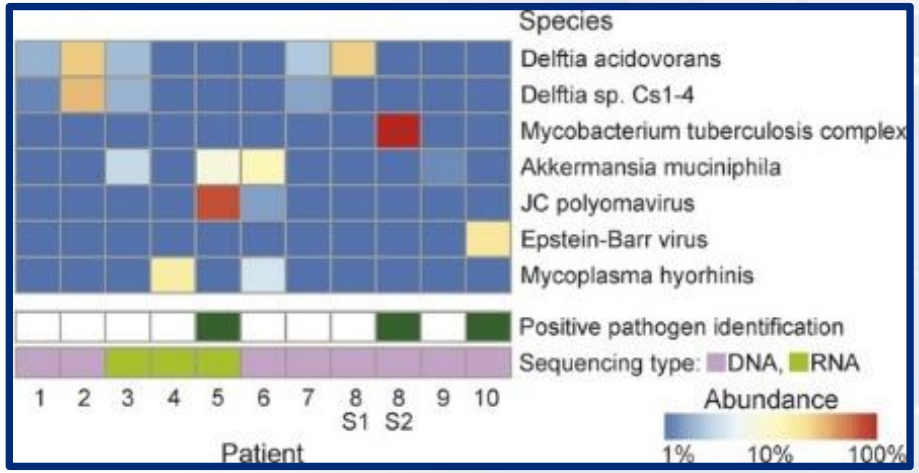
Methods: In a prospective pilot study, we applied NGS in combination with a new computational analysis pipeline to detect the presence of pathogenic microbes in brain or spinal cord biopsies from 10 patients with neurologic problems indicating possible infection but for whom conventional clinical and microbiology studies yielded negative or inconclusive results.

Results: Direct DNA and RNA sequencing of brain tissue biopsies generated 8.3 million to 29.1 million sequence reads per sample, which successfully identified with high confidence the infectious agent in 3 patients for whom validation techniques confirmed the pathogens identified by NGS. Although NGS was unable to identify with precision infectious agents in the remaining cases, it contributed to the understanding of neuropathologic processes in 5 others, demonstrating the power of large-scale unbiased sequencing as a novel diagnostic tool. Clinical outcomes were consistent with the findings yielded by NGS on the presence or absence of an infectious pathogenic process in 8 of 10 cases, and were noncontributory in the remaining 2.

Conclusions: NGS-guided metagenomic studies of brain, spinal cord, or meningeal biopsies offer the possibility for dramatic improvements in our ability to detect (or rule out) a wide range of CNS pathogens, with potential benefits in speed, sensitivity, and cost. NGS-based microbiome approaches present a major new opportunity to investigate the potential role of infectious patho-

Dr. Steven Salzberg

Dr. Carlos Pardo



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Optimization & Development of mNGS

Specimen Processing → Host Depletion → Extraction → DNA & RNA → Library Preparation → Sequencing → Analysis → Interpretation

BACTERIOLOGY

Check for updates

mization of Metagenomic Next-
Methods for Cerebrospinal Fluid

. Breitwieser,[b] Gabriel Pinilla Monsalve,[c] Carlos A. Pardo,[a,c]
L. Thomas,[d] Charles G. Eberhart,[a,f] Karen C. Carroll[a]

Heather Miller

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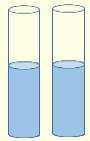
mNGS Method & Timeline

Day 1

IC: MS2 Phage → **RNA**

IC: T7 Phage → **DNA**



NEC CSF

Bead Beating

Total NA Extraction

WGA

RT-PCR/ WTA

Sequencing

Day 3

Quantification / Normalization/ Add PhiX

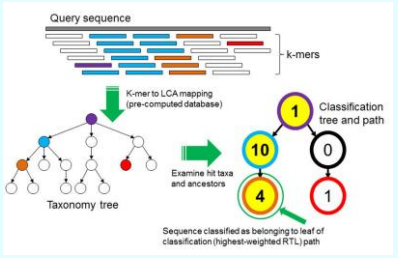
Library Preparation

Quantification

DNA& NTC

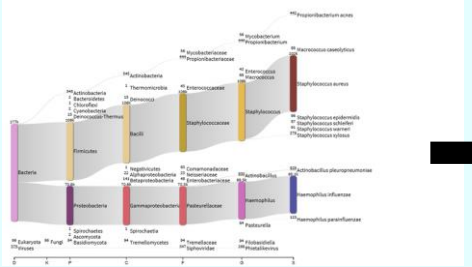
cDNA & NTC

Day 2



Day 4

Bioinformatics: Kraken & KrakenUnique



Interpretation: Pavian

Positive cutoff:
≥10X RPM of NTC
Unique kmer – Top 10
Compare RNA & DNA

+/- alignment

- Complex multiday process
- DNA & RNA Seq libraries
- Negative extraction control

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How Does mNGS Perform Compared to SOC?

All CSF		mNGS	
		Positive	Negative
SOC	Positive	45	**5**
	Negative	0	31

Agreement: 93.8%
PPA: 90.9%
PNA: 100%

Limits of detection:

- 1 CFU/ml for molds
- 1 CFU/ml for acid-fast bacilli
- 1 organism/ml for parasites
- 10 CFU/ml for yeast
- 10 CFU/ml for gram-negative bacteria
- 100 CFU/ml for gram-positive bacteria
- 100 genomes/ml for RNA viruses
- 10^4 genomes/ml for DNA viruses

Metagenomic NGS casts a broad net but targeted PCRs are often times more sensitive

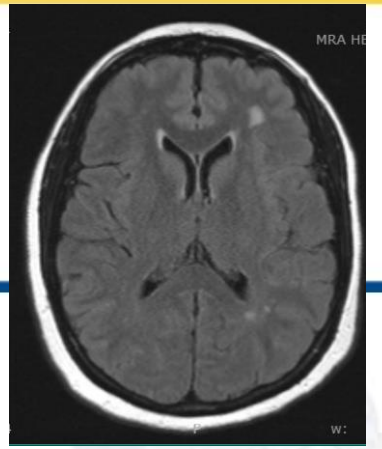
SOC: standard-of-care; PPA: positive percent agreement; NPA: negative percent agreement

Diagnostic Stewardship: Ordering Requirements

Requires Microbiology Faculty Approval – Reviewed case by case

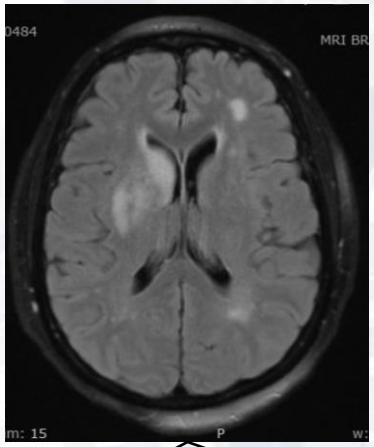
Differential	Pre-Testing Requirements (Variable depending on the specific case)	CSF Cell Count & Clinical Suspicion (Guideline)
Viral	Targeted PCRs for CMV, EBV, Enterovirus, HSV 1/2, JCV and VZV	Absolute cell count <100 Lymphocytic predominance Glucose Normal
Bacterial	Gram stain and bacterial culture	Absolute cell count >100; Neutrophil predominance Glucose Low
Fungal	Calcofluor white stain & fungal culture (incubating for at least 2 weeks) Antigen testing: Cryptococcal Ag and β-D-glucan	Lymphocytic predominance and elevated protein
AFB	Auramine/Rhodamine direct stain & AFB culture (incubating for at least 2 weeks) If MTB is on the differential, targeted MTB PCR	Lymphocytic predominance and elevated protein

The Power of mNGS



Initial MRI

- Women in her late 40's originally from Cameron who presented to neurology clinic due to prolonged history of headaches and fatigues
- During her workup she was found to have multiple abnormal autoimmune and infectious disease serologies (Lyme EIA & Western Blot IgM, Quantiferon & T-spot positive)
- Treated for Systemic Lupus Erythematosus and cryoglobulinemia with immunosuppressive drugs and Retuximab
- She continued to experience progressing symptoms, including hearing loss and the development of skin rashes



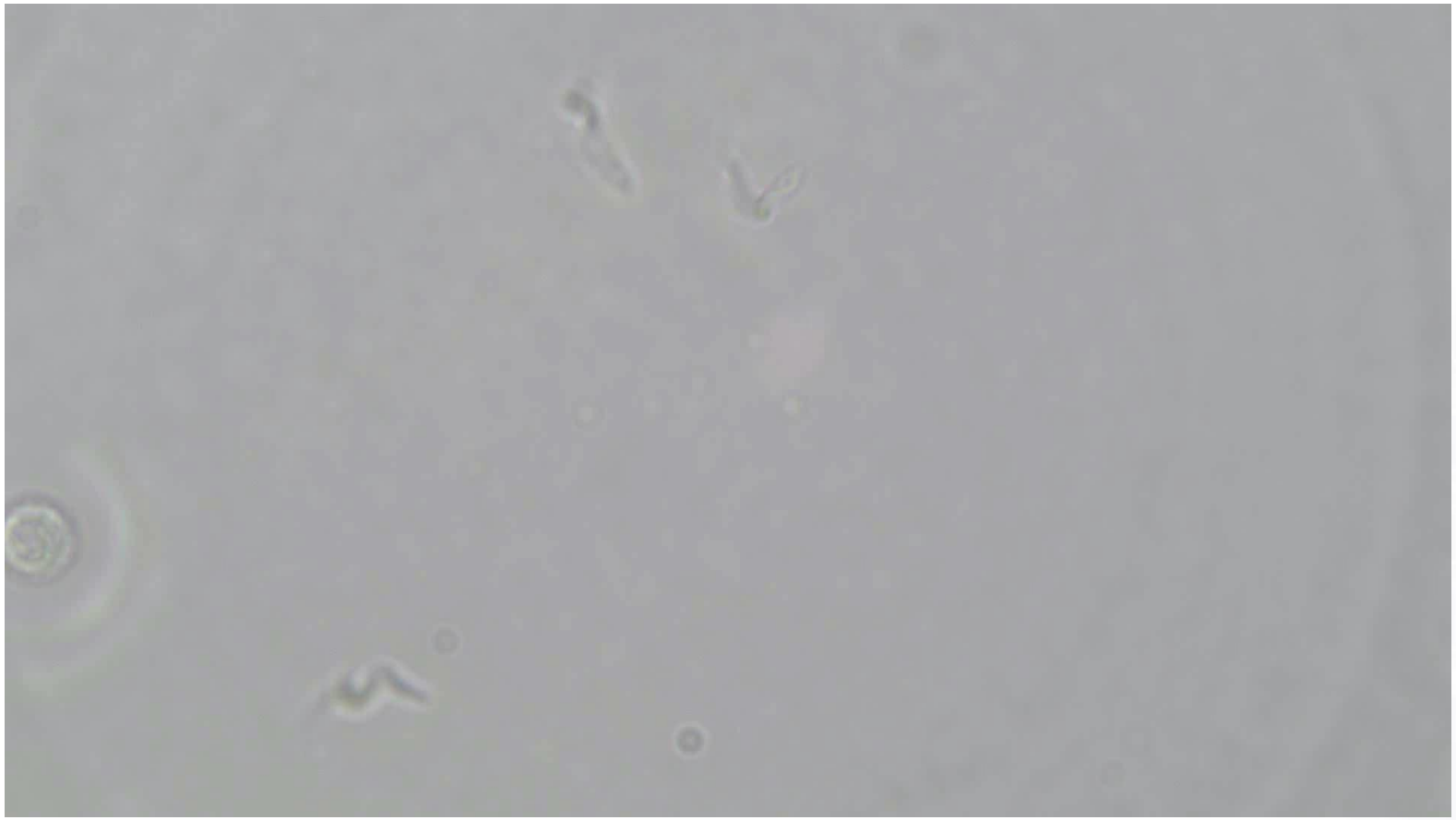
New T2 Flair hyperintensity of right anterior internal capsule & striatum

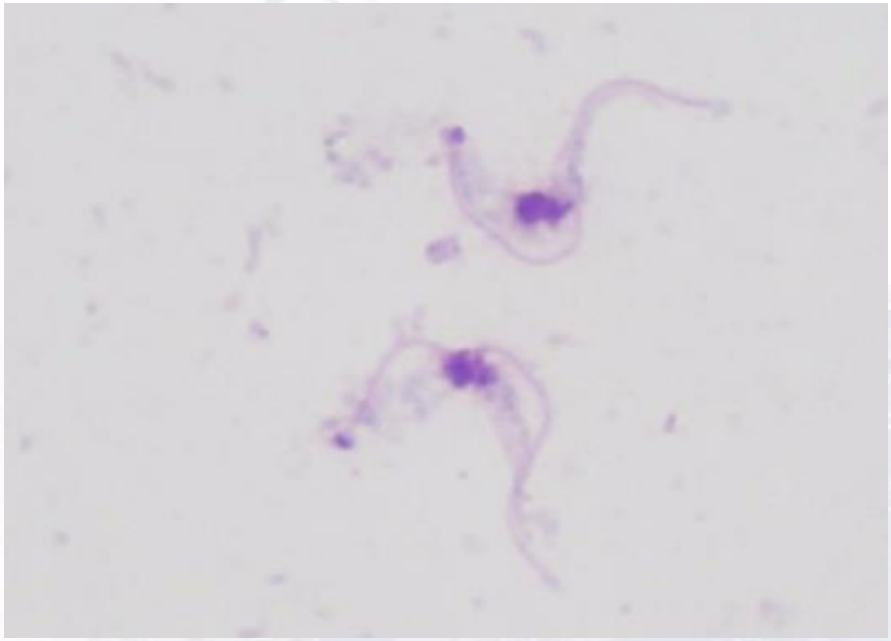
CSF #1
WBC: 48 cells/uL
Protein 58 mg/dL
Concerns for PML
JCV PCR negative

CSF # 2
WBC: 196/230 cells/uL
Protein 84 mg/dL
Microbiologic Workup (-)
CSF mNGS requested

Simner, Pardo *et al*, manuscript in preparation.

A High Volume CSF Sample Revealed…

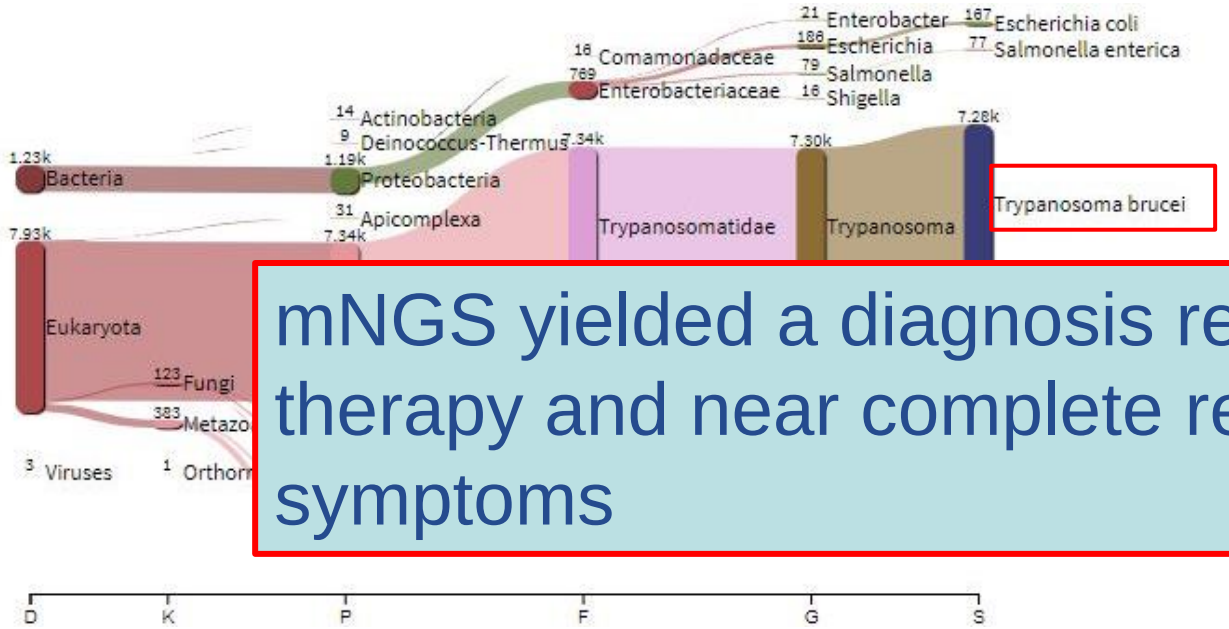




Giemsa stain; 1000x magnification with oil

Video: 1000x magnification with oil

mNGS Yielded a Diagnosis of Human African Trypanosomiasis (HAT)



Confirmed by targeted PCR

mNGS yielded a diagnosis resulting in appropriate therapy and near complete resolution of the patients symptoms

Trypanosome brucei genome coverage

Parasitic protozoan hemoflagellate

- Usually fatal if left untreated
- Treatment involves toxic agents & requires IND from the FDA (nifurtimox & efluornithine)

What is the Value of mNGS for CNS Infections?

- 13/48 (27%) yield
- Bacterial
  - Relapsing fever in a return patient from Portugal – *Borrelia* species, most closely related to *B. hispanica*
- Parasitic
  - *Trypansoma brucei*
- Viral
  - 3 EBV
  - 2 HIV
  - 2 Human Pegivirus
  - WNV
  - JCV
  - HBV
  - Parvovirus B19

mNGS is an adjunct test to standard-of-care methods – for rare, atypical or unsuspected cases

Can We Apply our Method to Other Sterile Fluids?

- Applied our method to pleural, peritoneal & synovial fluids
  - Poor sensitivity
    - Need for host depletion or targeted NGS assay
    - Requires further optimization/development
  - Focused on DNA Sequencing only

Synovial Fluid	SOC Gram Stain (GS) & culture	mNGS
1	GS: Mod PMN, L GPC Culture: Very light *Streptococcus pyogenes*	*Streptococcus pyogenes*
2	GS: L PMN, NOS Culture: MRSA	Not detected
3	GS: Mod PMN, L GPC Culture: Heavy Streptococcus Group C/G	*Streptococcus dysgalactiae*
4	GS: VL PMN, NOS Culture: Very Light *Corynebacterium striatum*	*Corynebacterium striatum (170)* TTV
5	GS: Mod PMN, NOS Culture: Very light *Enterobactear cloacae*	Not detected

GS: Gram Stain, VL: Very Light, L: Light, Mod: Moderate, H: Heavy

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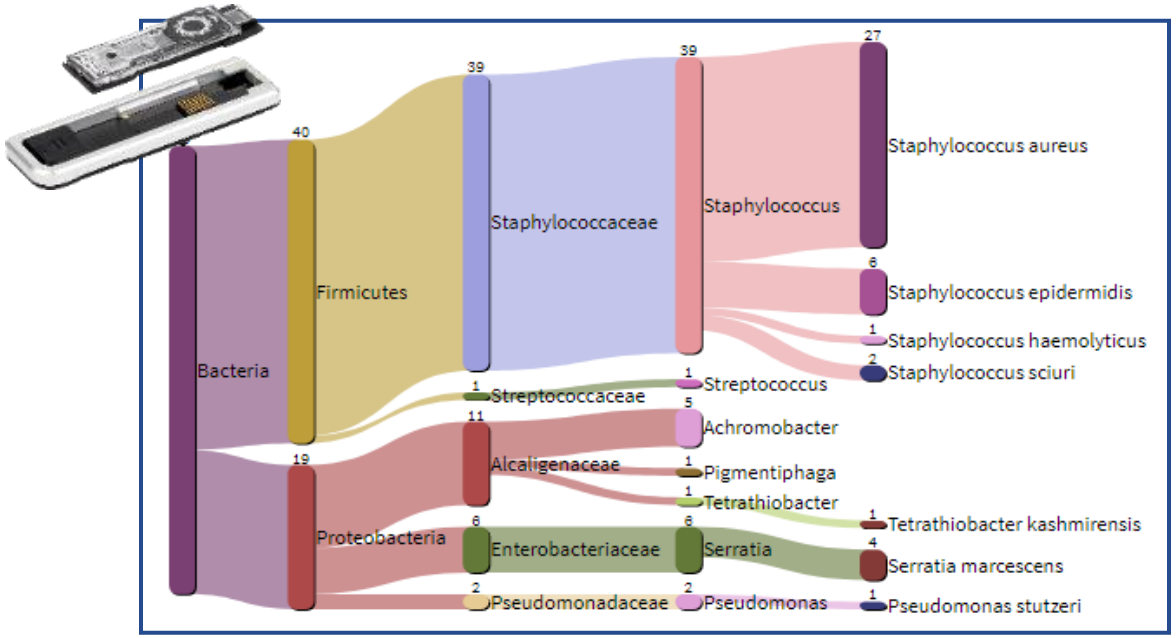
What About a Targeted Approaches?

- 1.5 kb 16S rRNA bacterial profiling using Nanopore sequencing & the Flongle



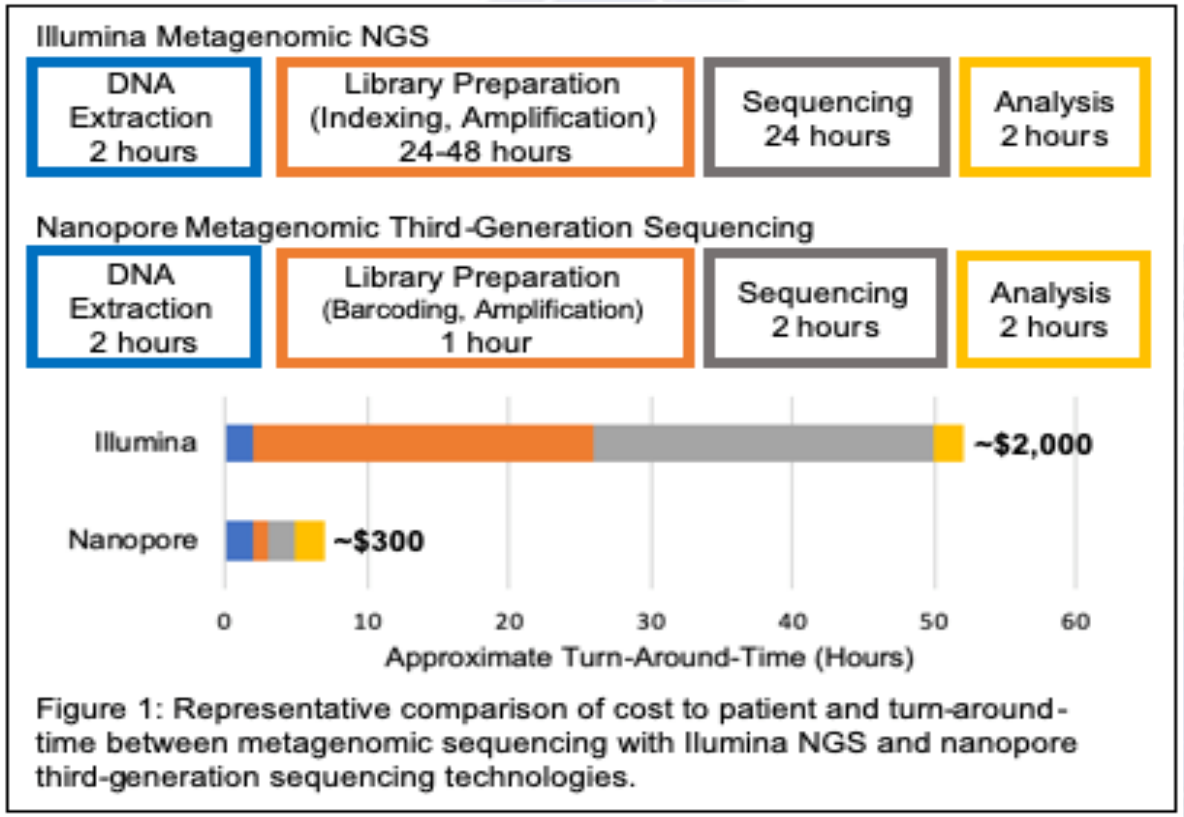


Figure 1: Representative comparison of cost to patient and turn-around-time between metagenomic sequencing with Ilumina NGS and nanopore third-generation sequencing technologies.

Gram stain: Heavy PMN, Light Gram-positive cocci in clusters

Culture: Moderate *Staphylococcus aureus*

Can we improve diagnosis of LRTIs by applying NGS approaches?

Dr. David Gaston

Dr. Karen Carroll

- Immunocompromised host BAL panel
  - Can we replace some of our SOC diagnostics?

- What additional value does tNGS versus a mNGS approach provide for patient management?

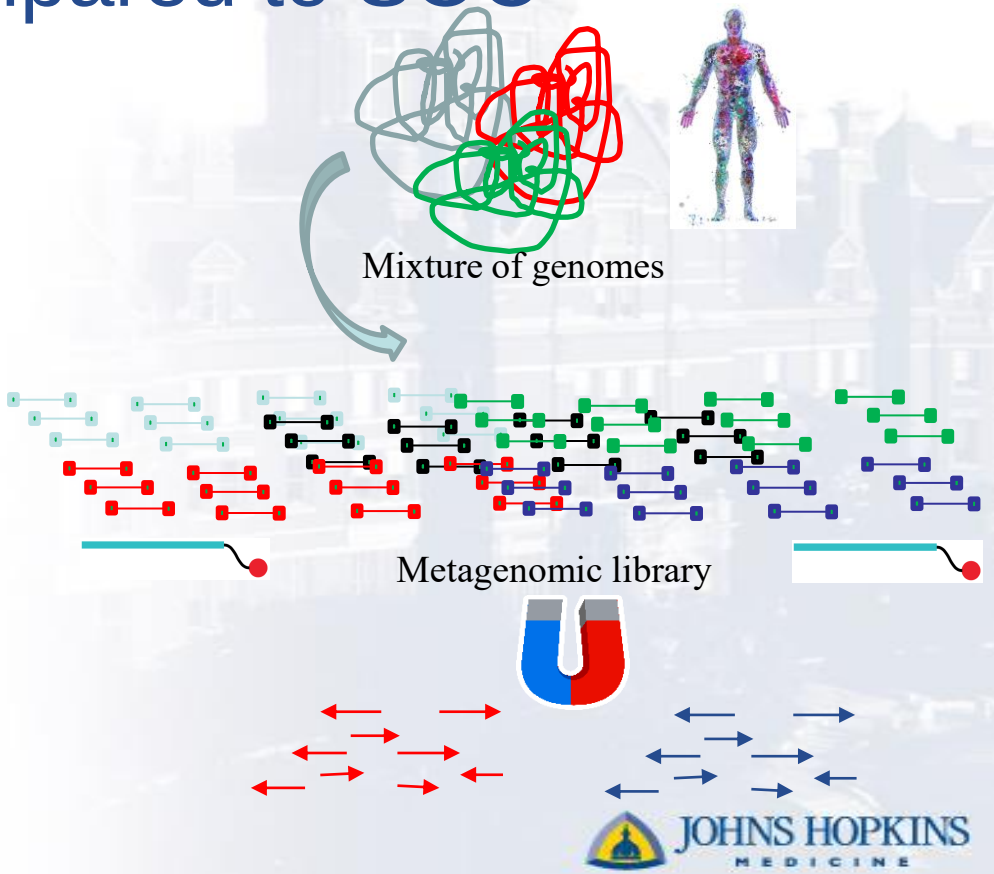
LRTI: lower respiratory tract infections; BAL: bronchoalveolar larvage fluid

tNGS versus mNGS

- Established performance characteristics & evaluated >200 BAL specimens by NGS methods compared to SOC

Viruses: 42 targets	
Bacteria: 187 targets	
Fungi: 53 targets	
AMR: 1218 markers	
Curated antibiotics	
Amoxicillin	Gentamicin
Amoxicillin-Clavulanate	Levofloxacin
Cefazolin	Meropenem
Cefepime	Oxacillin
Ceftriaxone	Sulfamethoxazole
Clindamycin	Tetracycline
Colistin	Trimethoprim
Erythromycin	Vanomycin
Curated bacterial pathogens	
Acinetobacter baumannii	*Mycobacterium abscessus*
Enterobacter cloacae	*Mycobacterium tuberculosis*
Enterococcus faecium	*Pseudomonas aeruginosa*
Enterococcus faecalis	*Staphylococcus aureus*
Escherichia coli	*Stenotrophomonas maltophilia*
Klebsiella pneumoniae	*Streptococcus pneumoniae*

Respiratory Pathogen ID/AMR Panel (RPIP) - Biotinylated capture probes



How Do We Interpret and Report NGS Based Results to Mimic Culture-Based Reporting?

Explify® Enrichment
▶ Respiratory ID/AMR Panel

SPECIMEN ID: **RPIPAcc27**
REPORT DATE: **31 March 2021**

DATE OF BIRTH: **Not Provided**
SEX: **Not Provided**
ID: **6063254743f7201b878b41ed**

ANALYSIS ID: **396104870**
SUBMITTER: **Path-NGSMicroLab@jhmi.edu**
ANALYSIS VERSION: **3.2.6**

SPECIMEN RECEIVED: **30 March 2021**
DATE OF COLLECTION: **Not Provided**
SPECIMEN TYPE: **Not Provided**

Analysis Performed: Explify® Respiratory Pathogen ID/AMR Panel (RPIP) - Data Analysis Solution
For Research Use Only. Not for use in diagnostic procedures.

RESULTS: **ONE OR MORE POTENTIAL PATHOGENS DETECTED**

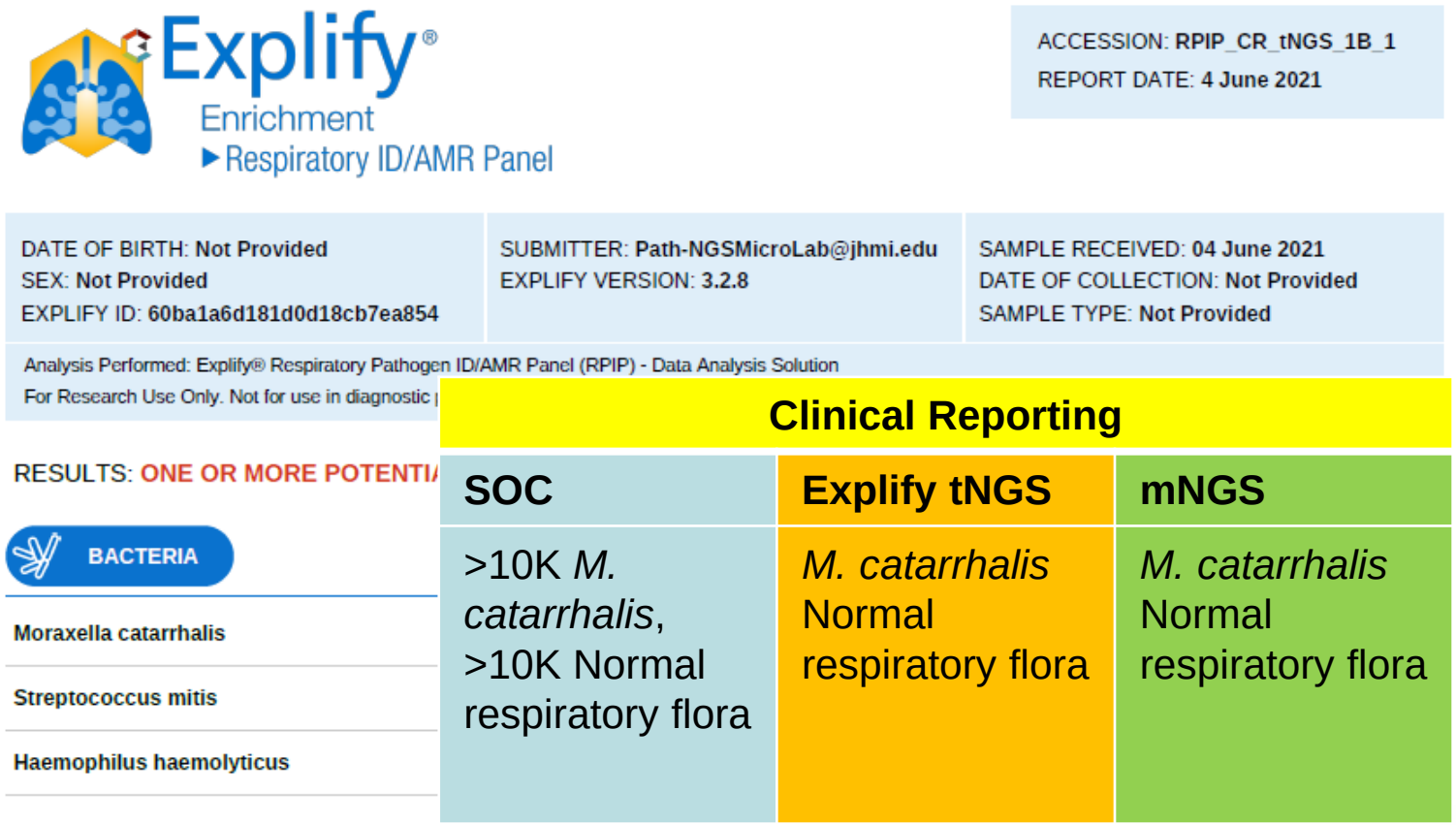
BACTERIA	QUANTITY (PROPORTION OF DETECTED BACTERIA)[1]	ASSOCIATED AMR MARKER DETECTED[2]	PHENOTYPIC GROUP[3]
Streptococcus mitis	2.3 x 10^7 copies/mL (72.0%)	n/a	2
Rothia mucilaginosa	4.4 x 10^6 copies/mL (13.6%)	n/a	1
Veillonella parvula	2.6 x 10^6 copies/mL (7.9%)	n/a	1
Streptococcus pneumoniae	2.1 x 10^6 copies/mL (6.3%)	Not Detected	2

mNGS Report

Name	Reads per Million
Rothia dentocariosa	414
Corynebacterium matruchotii	215
Lautropia mirabilis	198
Streptococcus mitis	118
Alloprevotella sp. E39	75
Gemella haemolysans	74
Rothia mucilaginosa	58
Veillonella parvula	50
Streptococcus oralis	46
Abiotrophia defectiva	39
Actinomyces sp. oral taxon 171	38
Ralstonia pickettii	37
Streptococcus gordonii	37
Streptococcus sanguinis	35
Ralstonia insidiosa	29
Haemophilus parainfluenzae	27
Streptococcus mutans	26
Actinomyces sp. oral taxon 169	25
Streptococcus pneumoniae	21

Conditional Reporting Criteria

Organism(s)/Group	SOC Interpretation	RPIP Targets	mNGS/tNGS reporting
Moraxella catarrhalis	Report if greater than or equal to normal microbiota	*Moraxella catarrhalis*	Report if in greater abundance than normal flora.
Streptococcus pneumoniae	Report if detected in **any amount**	*Streptococcus pneumoniae*	Report if in greater abundance than normal flora (do not report if less abundant than *S. mitis*)



Clinical Reporting

SOC	Explify tNGS	mNGS
>10K *M. catarrhalis*, >10K Normal respiratory flora	*M. catarrhalis* Normal respiratory flora	*M. catarrhalis* Normal respiratory flora

Organism	RPM
Morexella catarrhalis	848
Streptococcus mitis	96
Streptococcus pneumoniae	42
Moraxella osloensis	20
Streptococcus pseudopneumoniae	20
Veillonella atypica	17
Haemophilus haemolyticus	16
Salmonella enterica	15
Ralstonia pickettii	11
Streptococcus oralis	8

Accuracy of NGS Methods

	mNGS			tNGS		
	Accuracy	**PPA**	**NPA**	**Accuracy**	**PPA**	**NPA**
Bacterial	84%	46%	94%	85%	46%	96%
Mycobacterial	97%	38%	99%	96%	13%	100%
Fungal	90%	0%*	96%	93%	0%*	98%
Viral	88%	79%	92%	83%	60%	94%
Overall	**67%**	**57%**	**77%**	**66%**	**46%**	**86%**

*Neither workflow detected filamentous fungi recovered in culture and deemed clinically significant by the treating providers or a PCR positive *P. jirovecii*

Similar performance for both mNGS and tNGS methods

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Example of the Value of NGS for LRTI- Atypical Positive

An adult male status post bilateral orthotropic lung transplant (BOLT; one month prior) for pulmonary fibrosis with a complicated course requiring ECMO and tracheostomy tube. Treatment included cefepime/meropenem + vancomycin. His immunosuppressant agents included prednisone, mycophenolate and tacrolimus.

SOC:

- Immunocompromised host BAL panel was unrevealing

mNGS & tNGS:

- *Ureaplasma urealyticum*

Patient had altered mental status changes and elevated ammonia level following BOLT consistent with the possibility of donor-derived *Ureaplasma* syndrome

Ureaplasma infection in untreated donor lungs can approach 30% (Chiteru et al, Transplantation, 2021)

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What about AMR Marker Detection to Inform Treatment?

- tNGS AMR markers were associated with 13 pathogens detected by SOC
- Full or partial agreement between AMR and phenotypic AST was found in 7 of 13 (54%) of pathogens
  - Extended-spectrum β-lactamase (ESBL)-producing *E. coli*, VRE, MRSA and *M. tuberculosis* to 1st & 2nd line agents
- mNGS being evaluated for AMR
  - Abundance of organism, composition of specimen

AMR[5]	REPRESENTATIVE ANTIMICROBIAL[6]	ASSOCIATED MICROORGANISMS DETECTED[7]
ANT(3'') (Best Match: aadA5)	Spectinomycin Streptomycin	Escherichia coli
CTX-M (Best Match: CTX-M-27) ESBL	Amoxicillin Ampicillin Cefalexin Cefazolin Cefepime Cefixime Cefotaxime Ceftazidime Ceftriaxone Penicillin	Escherichia coli Pseudomonas aeruginosa
Dfr (Best Match: dfrA17)	Trimethoprim	Escherichia coli Pseudomonas aeruginosa
MPH (Best Match: mphA)	Azithromycin Clarithromycin Erythromycin	Escherichia coli

Unable to link AMR to the organism

AMR: antimicrobial resistance

Microbiome

Infection Prevention/ Surveillance

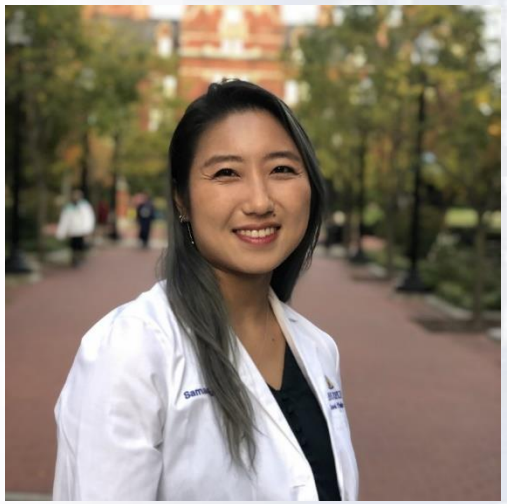
Host Response

NGS for Infectious Diseases

Diagnosis

Pathogenesis

Therapeutic Guidance

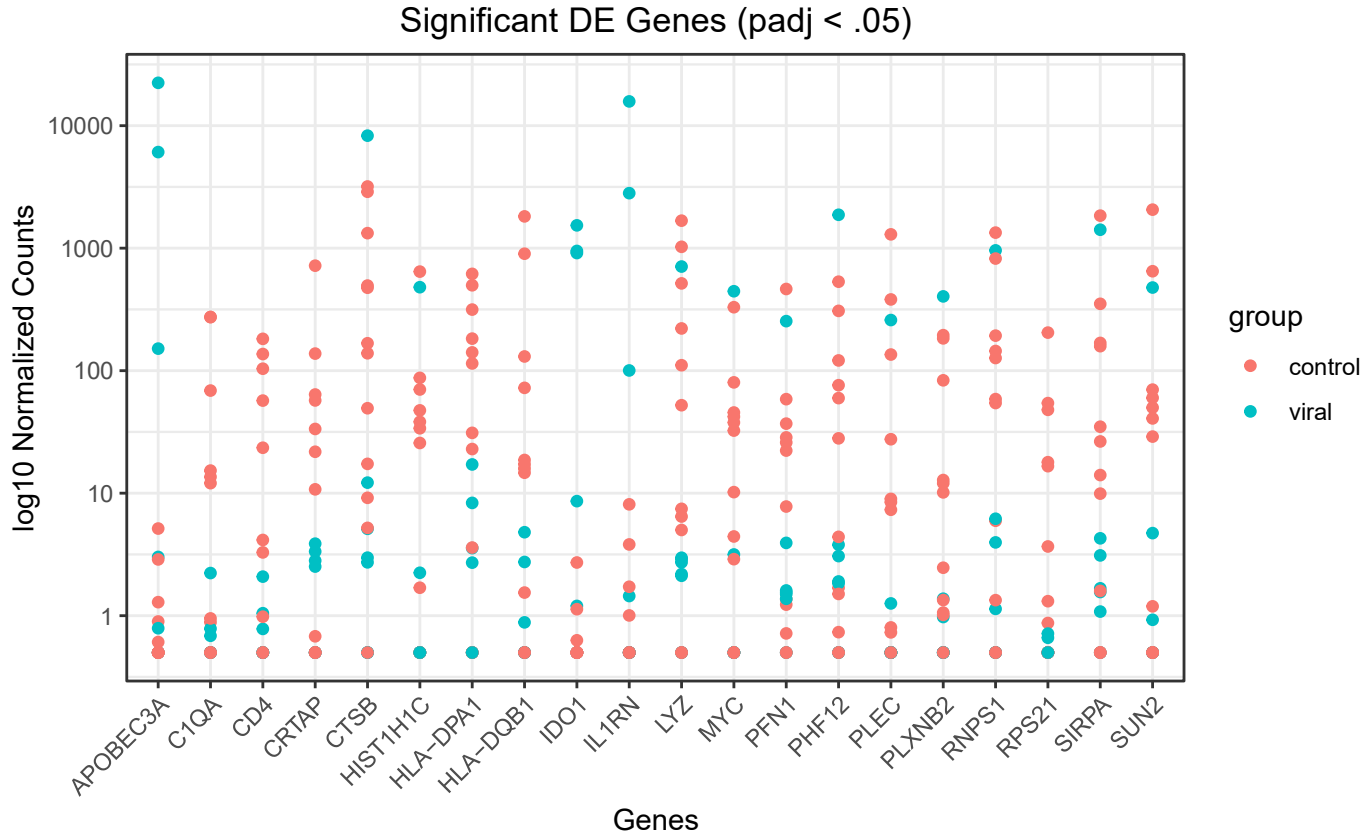


Samantha Hao

JOHNS HOPKINS MEDICINE

Noninfectious versus Viral

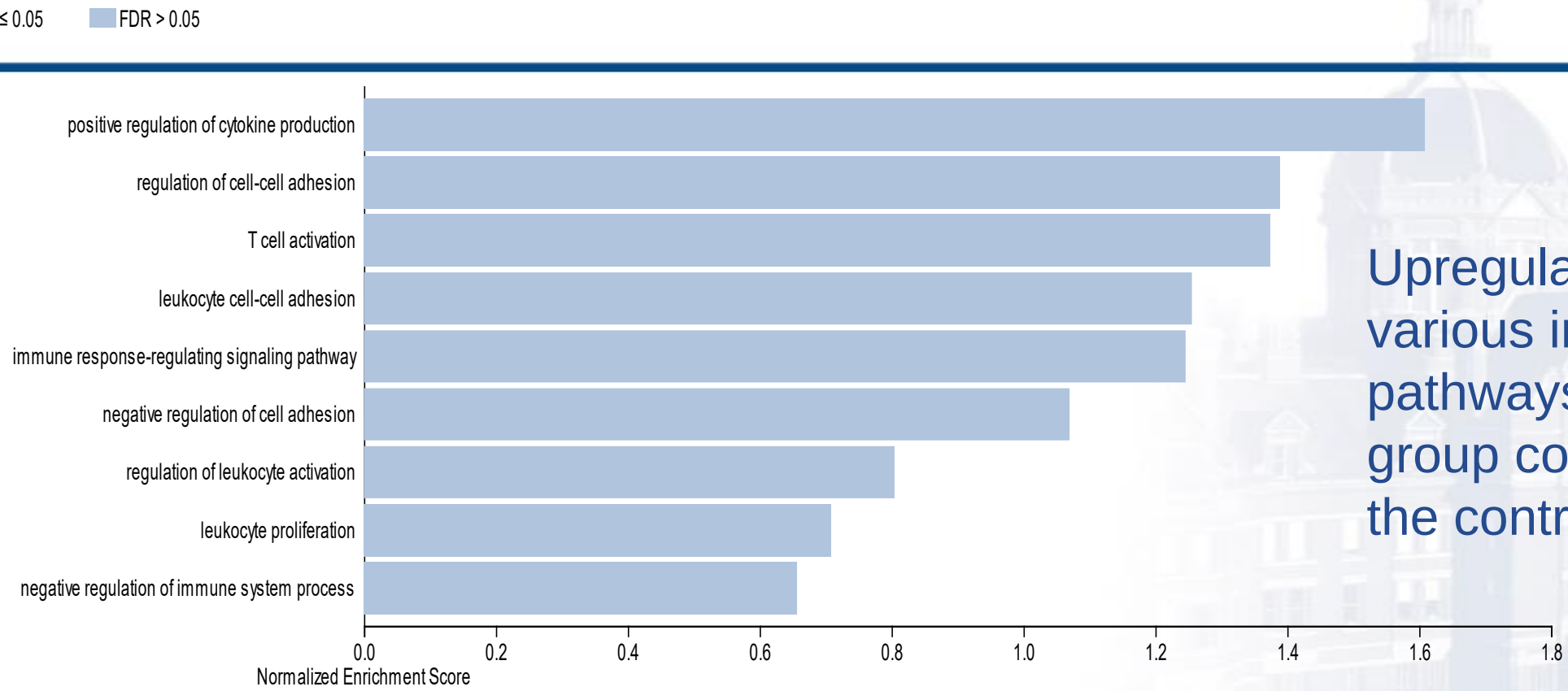
- 20 noninfectious vs 8 viral infections
  - RNASeq ->removed rRNA → performed differential gene expression (DE)



37 DE genes

- APOBEC3
  - Restricts viral replication by converting C to U via deaminase activity
- IDO1
  - Elevated IDO1 expression is a hallmark of some viral infection

Gene Set Enrichment Analysis



Upregulation in various immune pathways in the viral group compared to the control group

Differential gene expression analysis on human RNAseq data from the mNGS assay is consistent with known clinical diagnoses and correlated with mechanism of disease

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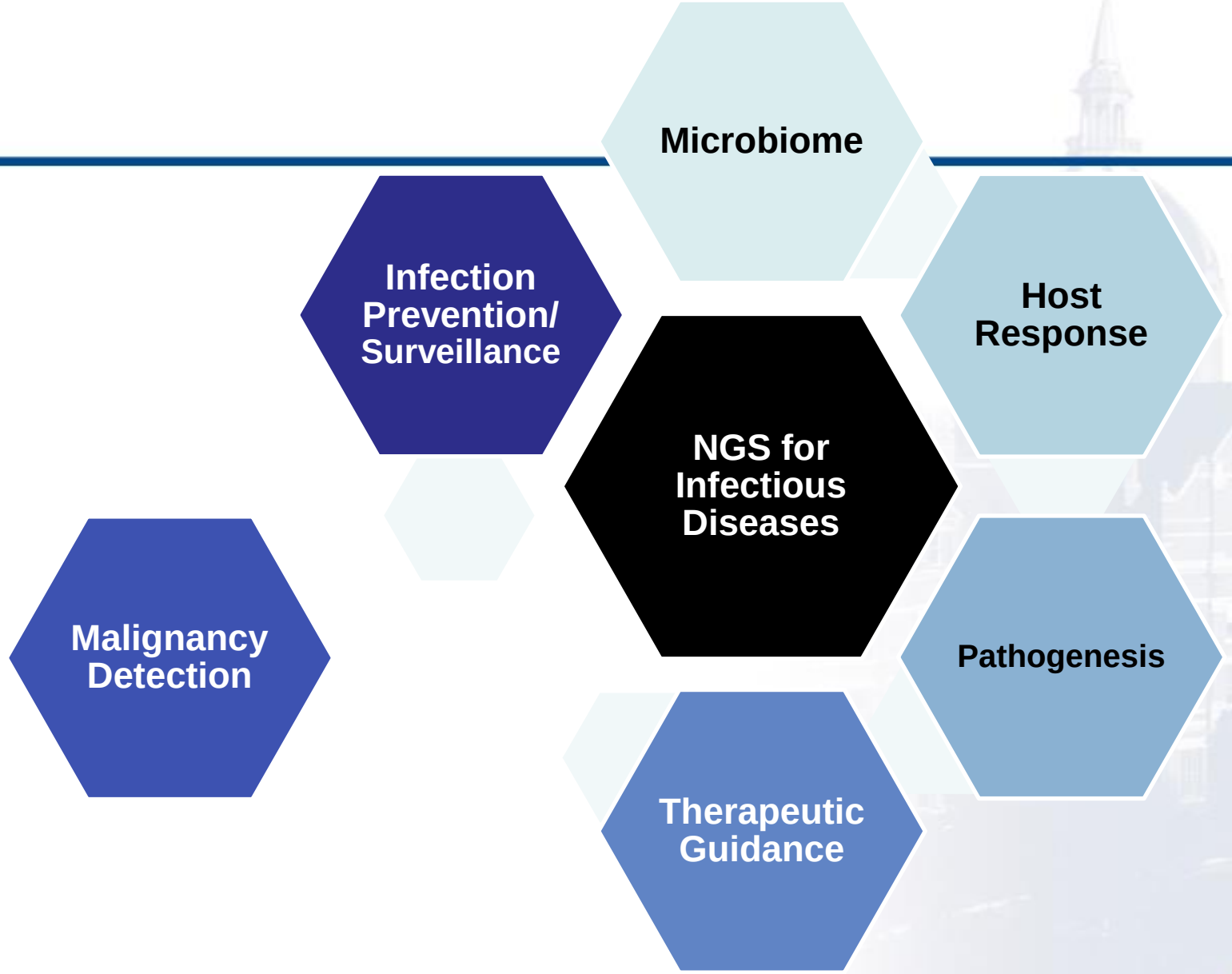


JOHNS HOPKINS MEDICINE

Detection of Cryptogenic Malignancy

- Using the same data for ID diagnostics
- Focusing on the DNA based host reads
- Screen for malignancies using copy number variation
- Illustrating the ability to detect undiagnosed acute illness due to cancer or infection using the same specimen & method

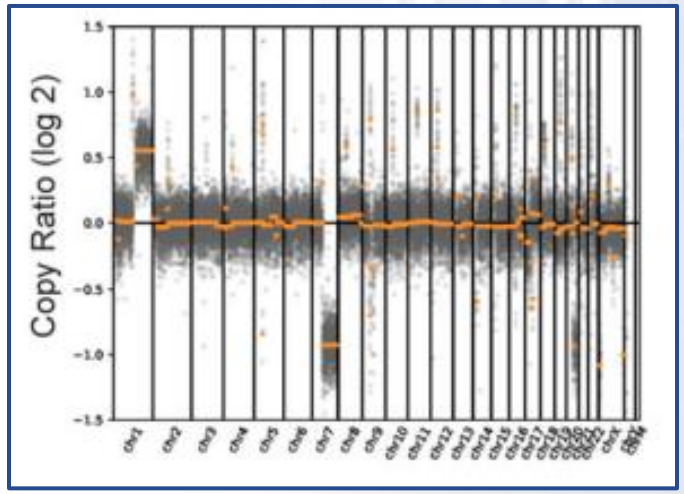
Gu *et al. Genome Medicine* (2021) 13:98
https://doi.org/10.1186/s13073-021-00912-z

Genome Medicine

RESEARCH Open Access

Detection of cryptogenic malignancies from metagenomic whole genome sequencing of body fluids

Wei Gu[1,2,3,4*], Eric Talevich[5], Elaine Hsu[1], Zhongxia Qi[1], Anatoly Urisman[6], Scot Federman[1,2], Allan Gopez[1,2], Shaun Arevalo[1,2], Marc Gottschall[1], Linda Liao[4], Jack Tung[3], Lei Chen[4], Harumi Lim[4], Chandler Ho[4], Maya Kasowski[3], Jean Oak[3,4], Brittany J. Holmes[3,4], Iwei Yeh[6], Jingwei Yu[1], Linlin Wang[1], Steve Miller[1,2], Joseph L. DeRisi[7,8], Sonam Prakash[1], Jeff Simko[6†] and Charles Y. Chiu[1,2,9*†]



Sensitivity: 87%
Specificity: 100%

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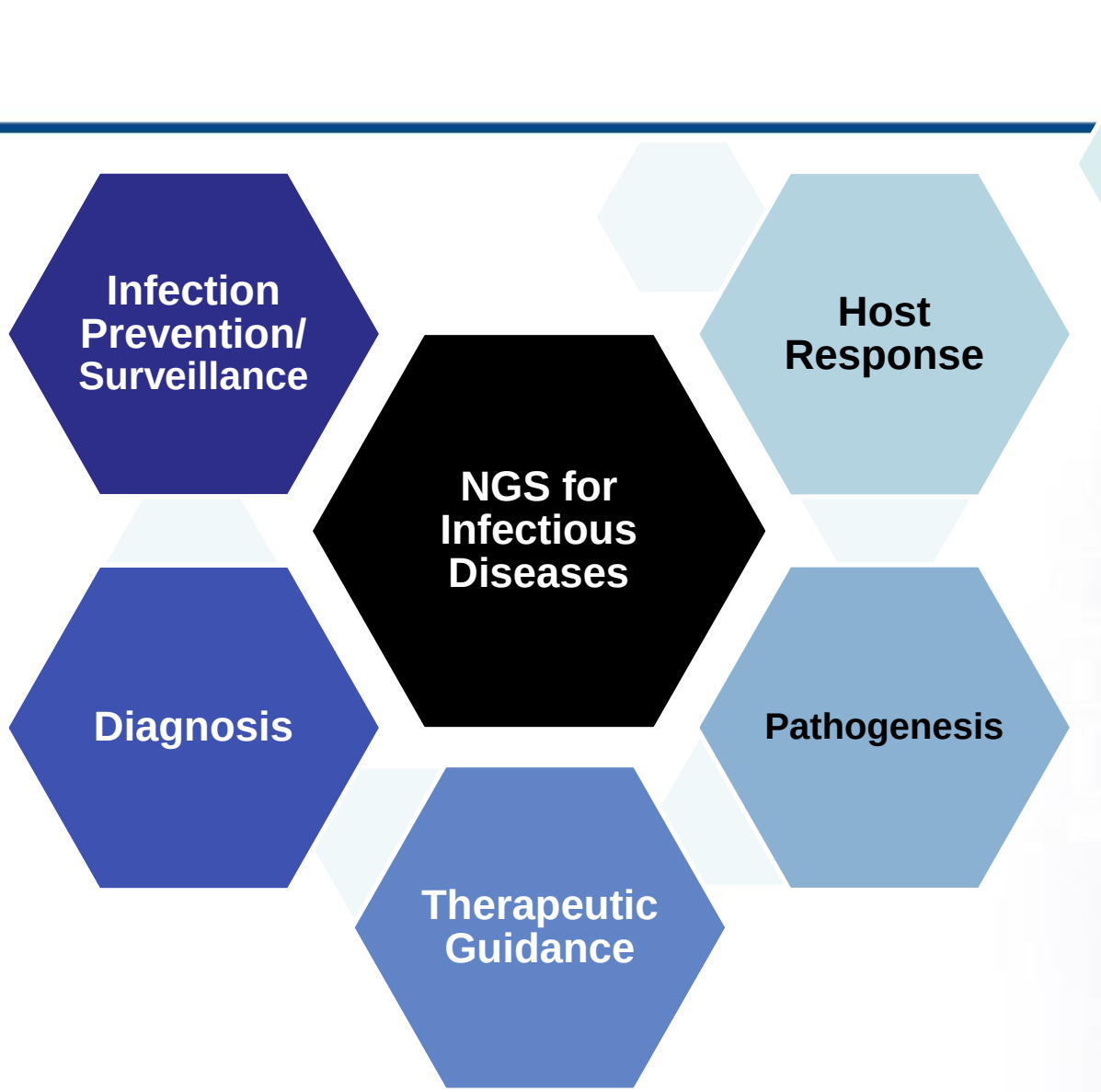
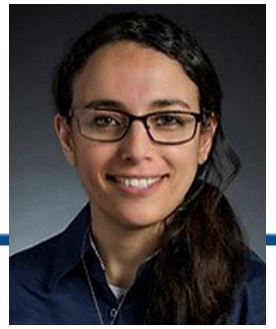


JOHNS HOPKINS MEDICINE

mNGS to Study SARS-CoV-2 Co-Infections & Microbiome



Dr. Heba Mostafa

Dr. John Fissel

Dr. Karen Carroll

- 50 nasopharyngeal swabs sequenced by mNGS (DNA & RNA libraries) using Nanopore with Cosmos ID for analysis
- 31/40: 78% correlation with RT-PCR
  - Correlated with lower Ct values & fewer days from symptom onset
  - Time to detection: 1 min & up to 15 hrs
- Co-infections:12.5% of SARS-CoV-2 positive specimens
  - *Haemophilus influenzae* (n:2), *Moraxella catarrhalis* (n:1), hMPV (n:1) & HSV1 (n:1)

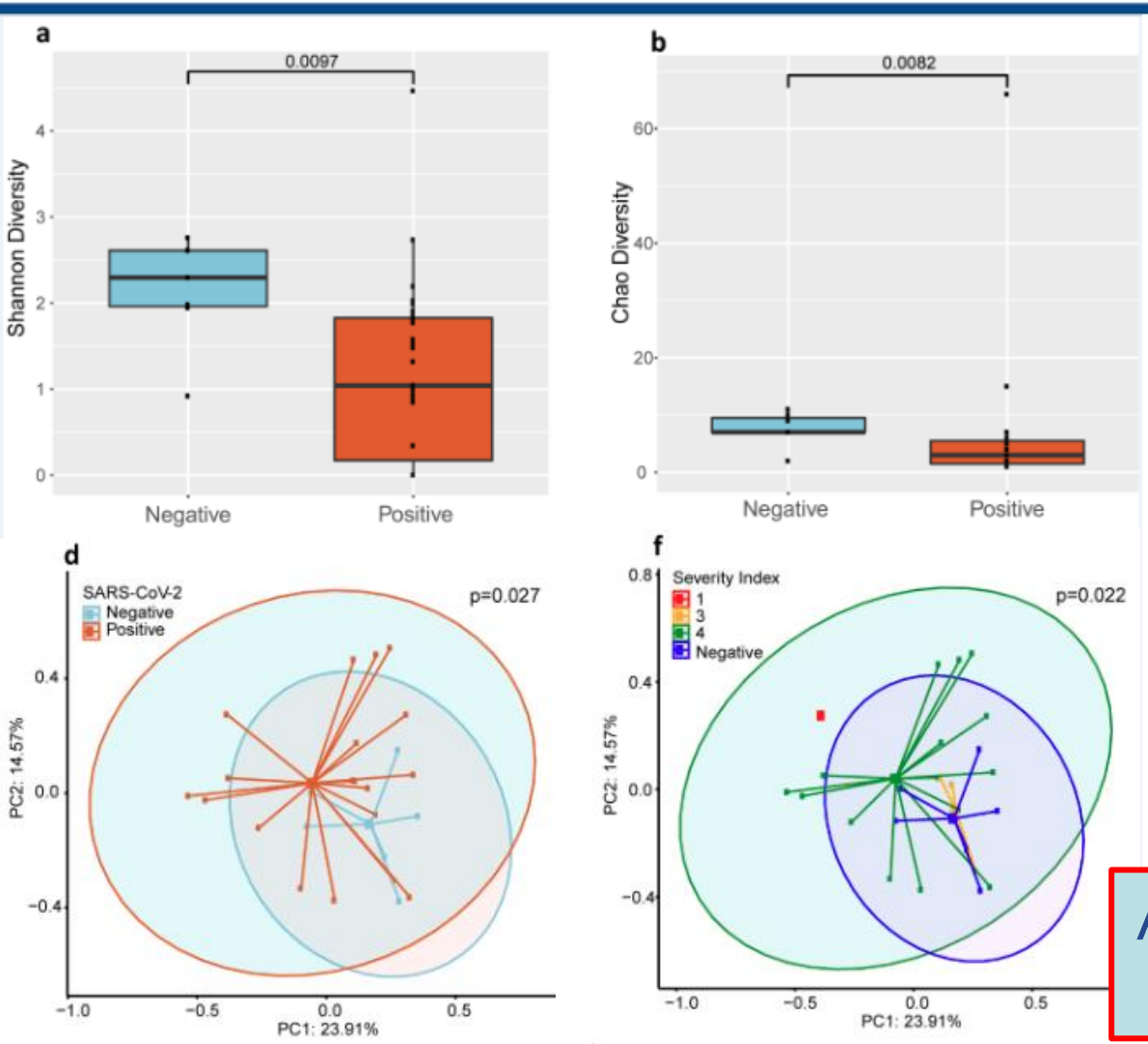
AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

RESEARCH ARTICLE
Clinical Science and Epidemiology

Metagenomic Next-Generation Sequencing of Nasopharyngeal Specimens Collected from Confirmed and Suspect COVID-19 Patients

Heba H. Mostafa,[a] John A. Fissel,[a] Brian Fanelli,[b] Yehudit Bergman,[a] Victoria Gniazdowski,[a] Manoj Dadlani,[b] Karen C. Carroll,[a] Rita R. Colwell,[b,c] Patricia J. Simner[a]

SARS-CoV-2 Associated with Changes in the Respiratory Microbiome

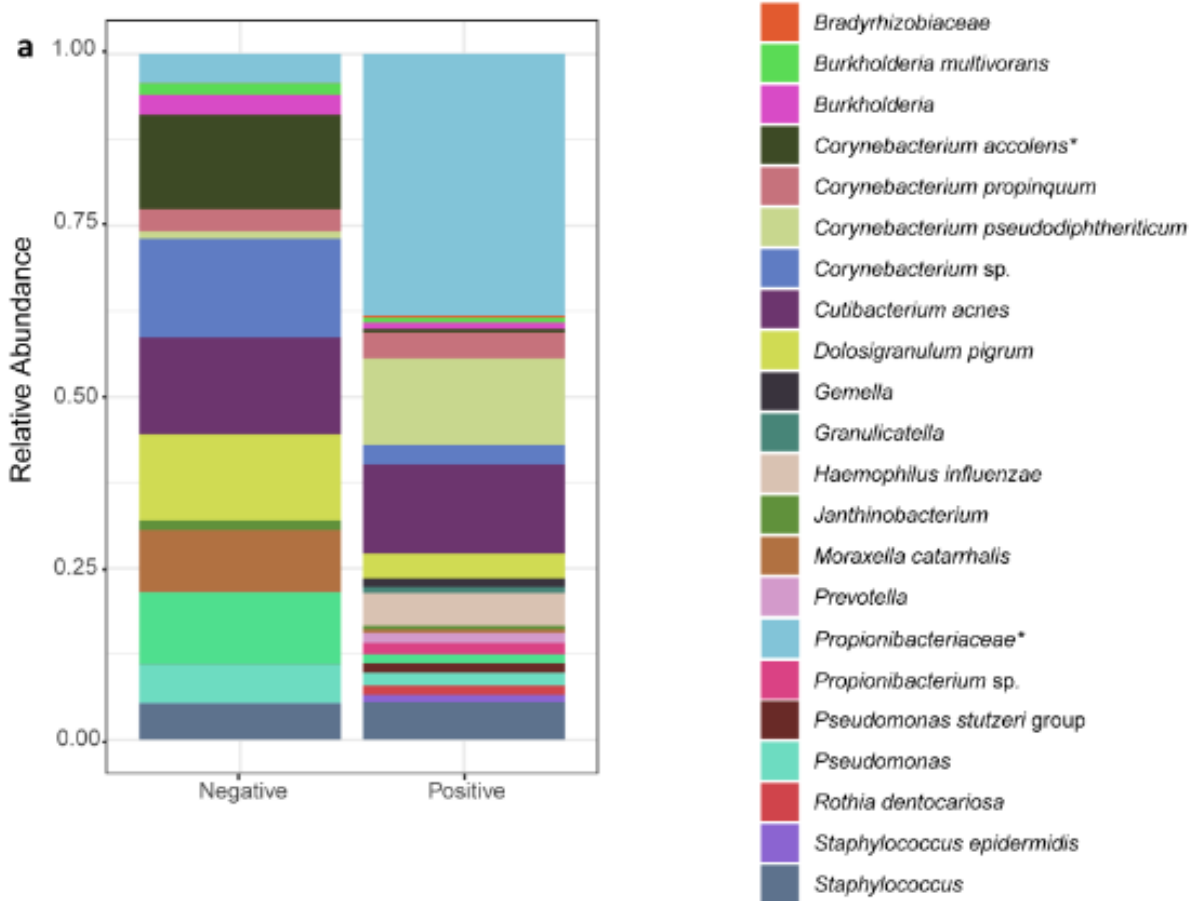


- α-diversity: SARS-CoV-2 positive specimens had a significant reduction in the diversity of their bacterial communities

- β-diversity: Differences were observed between communities in patients with or without SARS-CoV-2 and at the species level when comparing severity index

A decrease in microbial diversity was observed among COVID_19 confirmed patients

Are There “Signatures” in the Microbiota?

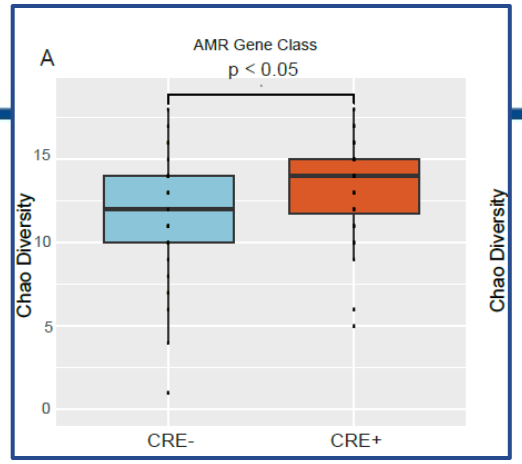


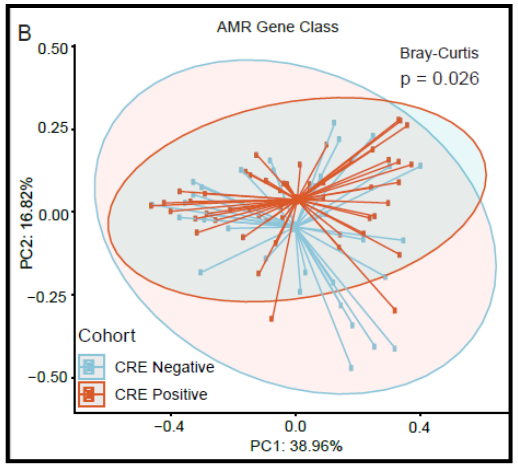
Statistically significant shifts in the microbiome were identified among SARS-CoV-2 positive and negative patients

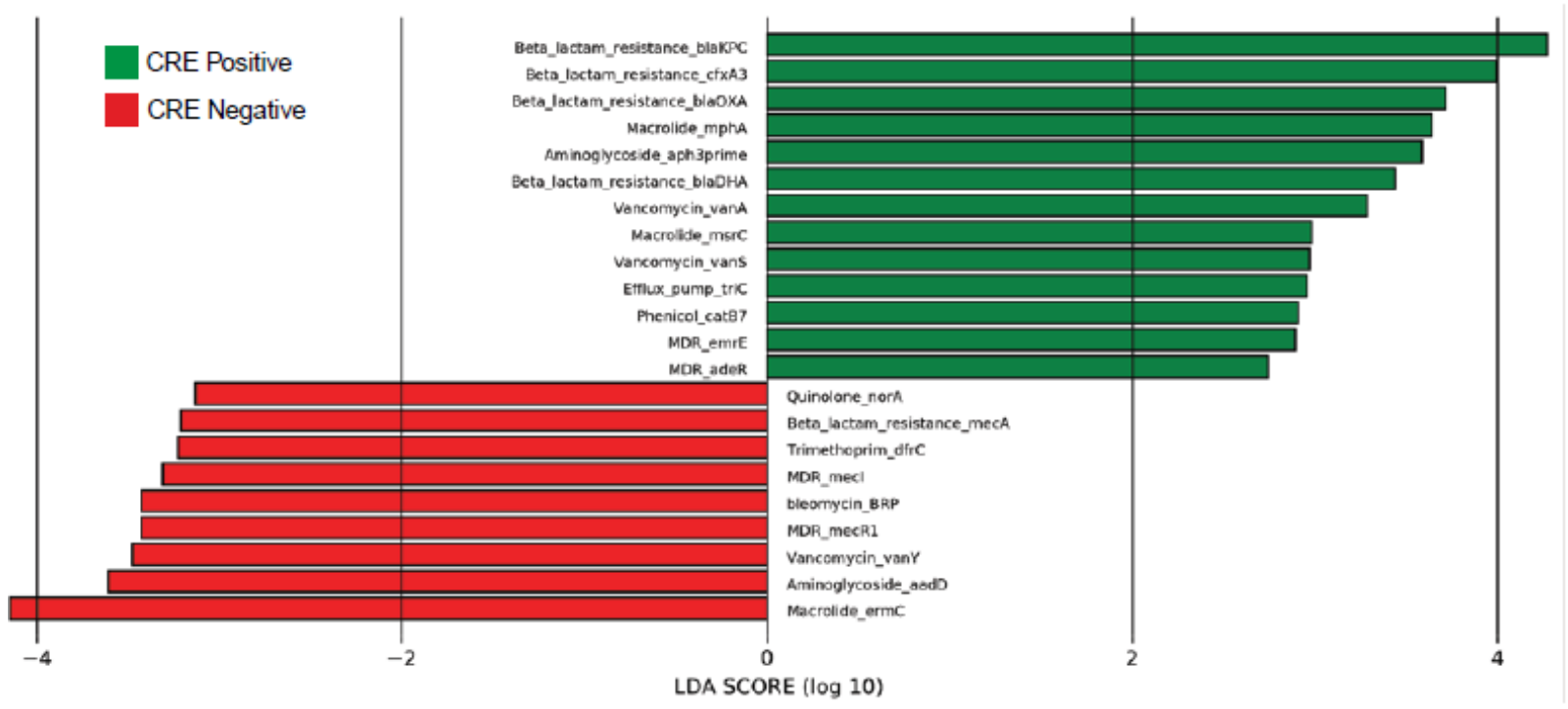
SARS-CoV-2 patients: higher abundance of *Propionibacteriaceae* (*P* 0.028) & a reduction in the abundance of *Corynebacterium accolens* (*P* 0.025)

JOHNS HOPKINS MEDICINE

GI Microbiome & Resistome of High Risk Patients Colonized with CRE





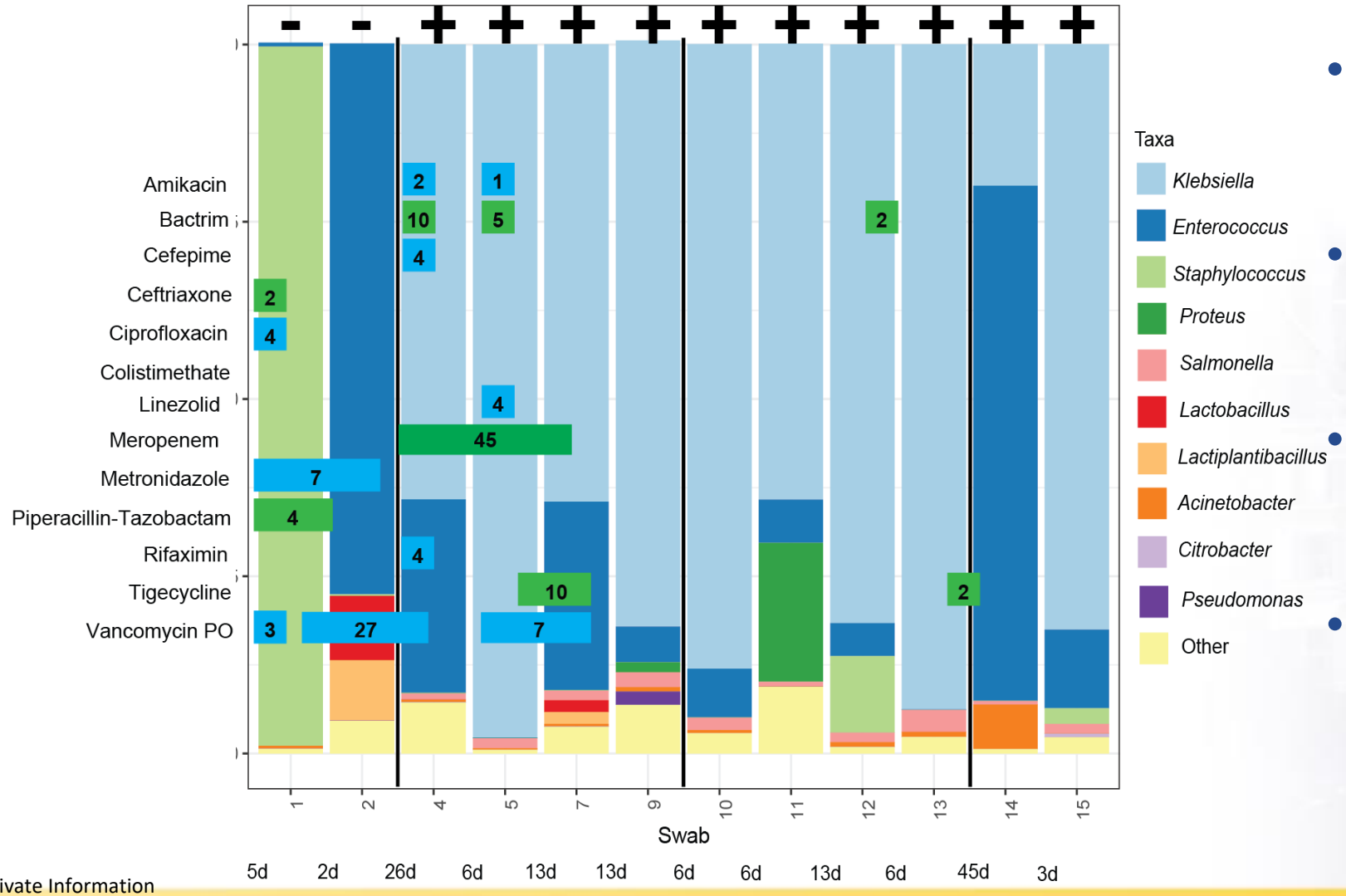


No change in the microbiome but significant changes in the resistome

Beta-lactamase genes were enriched among CRE positive specimens including genes associated with anaerobes.

Fissel *et al*, manuscript in preparation.

Longitudinal Changes Among Individual Patients



- Initially tested negative for CRE colonization but acquired CRE
- Shift from Gram-positives to the predominance of *Klebsiella*
- Shifts in the microbiome & resistome correlating with therapy
- Patient became bacteremic with a carbapenemase-producing *K. pneumoniae*

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Part 1: Summary

- Direct from specimen NGS assays have potential to improve clinical detection of pathogens from various specimen types
- NGS based approaches should be considered an adjunct to standard methods for the diagnosis of infectious diseases
  - Value: detection of rare, atypical, or unsuspected or previously treated pathogens
  - Still learning: Correct time to perform testing, patient populations & syndromes
- mNGS has the potential to become a precision medicine based diagnostic

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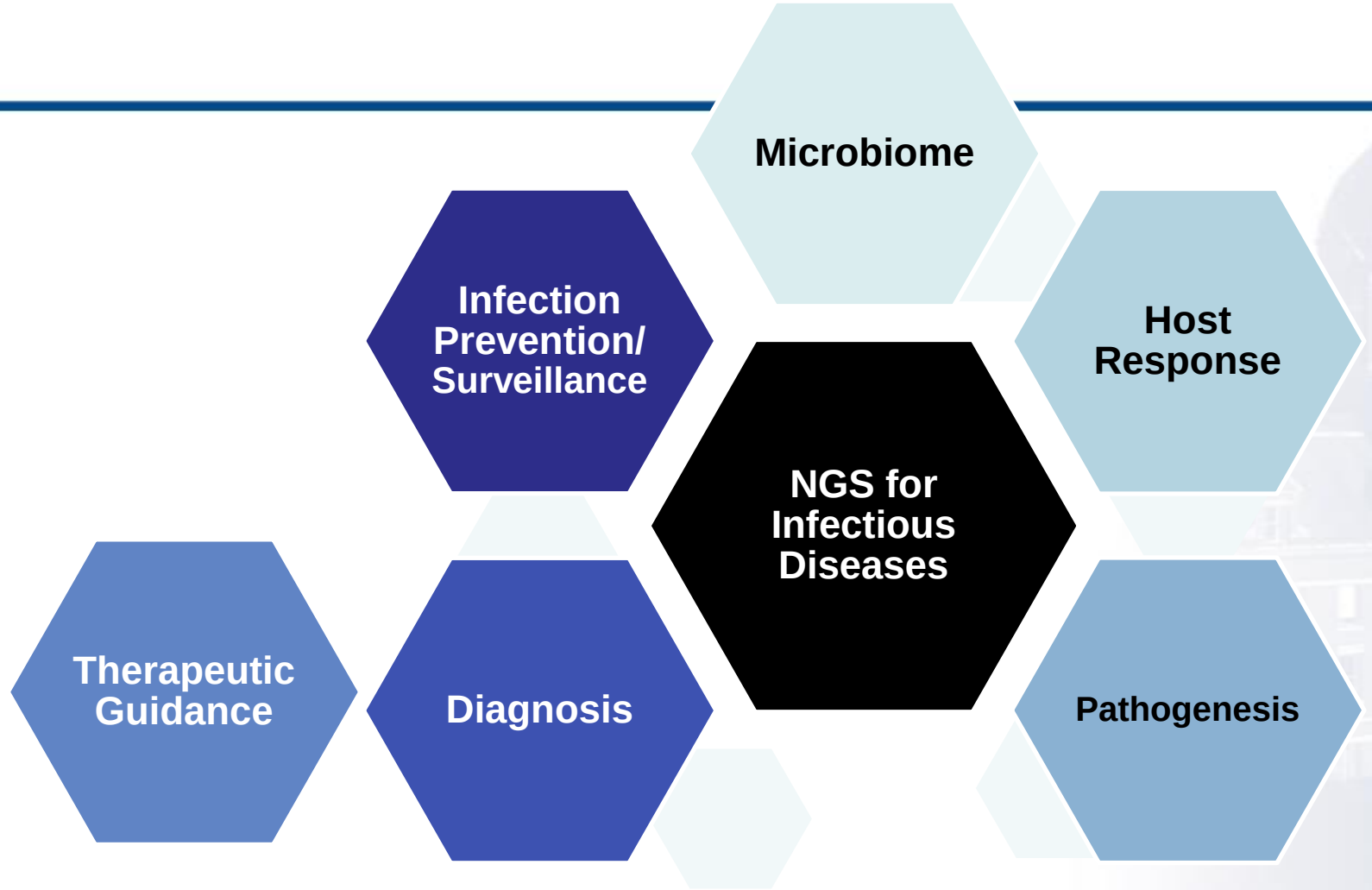
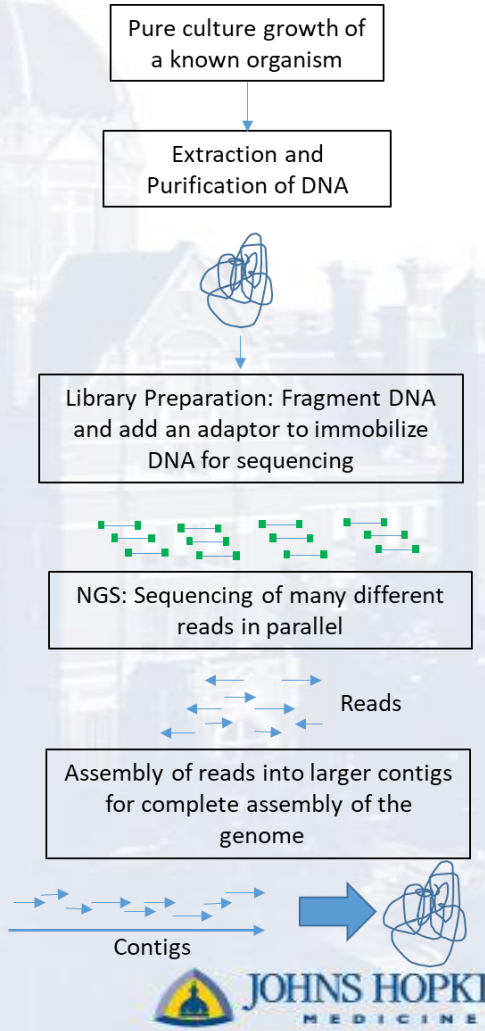


PART 2: WGS TO DETECT AMR & PREDICT PHENOTYPIC AST

Whole Genome Sequencing (WGS): A Powerful Tool to Study the Resistome

- Next-generation sequencing of the genome of the pathogen of interest with detection of antimicrobial resistance (AMR) genes
- Resistome: all antimicrobial resistance genes in a given organism or microbiome
- Use the resistome or the entire genome to define mechanisms of resistance or to predict antimicrobial susceptibility and resistance



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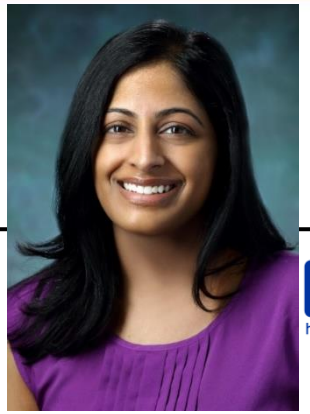
Retrospective Assessments to Guide Therapeutic & Diagnostic Approaches



Dr. Pranita Tamma

MICROBIAL DRUG RESISTANCE
Volume 28, Number 2, 2022

https://doi.org/10.1089/mdr.2021.0095

Mary Ann Liebert, Inc. publishers

Mechanisms

Defining Baseline Mechanisms of Cefiderocol Resistance in the Enterobacterales

Patricia J. Simner [1], Stephan Beisken[2], Yehudit Bergman[1], Michael Ante[2], Andreas E. Posch[2], and Pranita D. Tamma[3]

- Devise empiric treatment strategies

Clinical Infectious Diseases

MAJOR ARTICLE

hivma hiv medicine association

OXFORD

Progressive Development of Cefiderocol Resistance in *Escherichia coli* During Therapy is Associated With an Increase in bla_{NDM-5} Copy Number and Gene Expression

Patricia J. Simner,[1] Heba H. Mostafa,[1] Yehudit Bergman,[1] Michael Ante,[2] Tsigereda Tekle,[1] Ayomikun Adebayo,[1] Stephan Beisken,[2] Kathryn Dzintars,[3] and Pranita D. Tamma[4]

- Address diagnostics for detection of AMR

Clinical Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America

hivma hiv medicine association

OXFORD

Modifiable Risk Factors for the Emergence of Ceftolozane-tazobactam Resistance

Pranita D. Tamma,[1] Stephan Beisken,[2] Yehudit Bergman,[3] Andreas E. Posch,[4] Edina Avdic,[5] Sima L. Sharara,[6] Sara E. Cosgrove,[7] and Patricia J. Simner[8]

- Identify modifiable risk factors & cross-resistance to other agents

JAC Antimicrob Resist
https://doi.org/10.1093/jacamr/dlac046

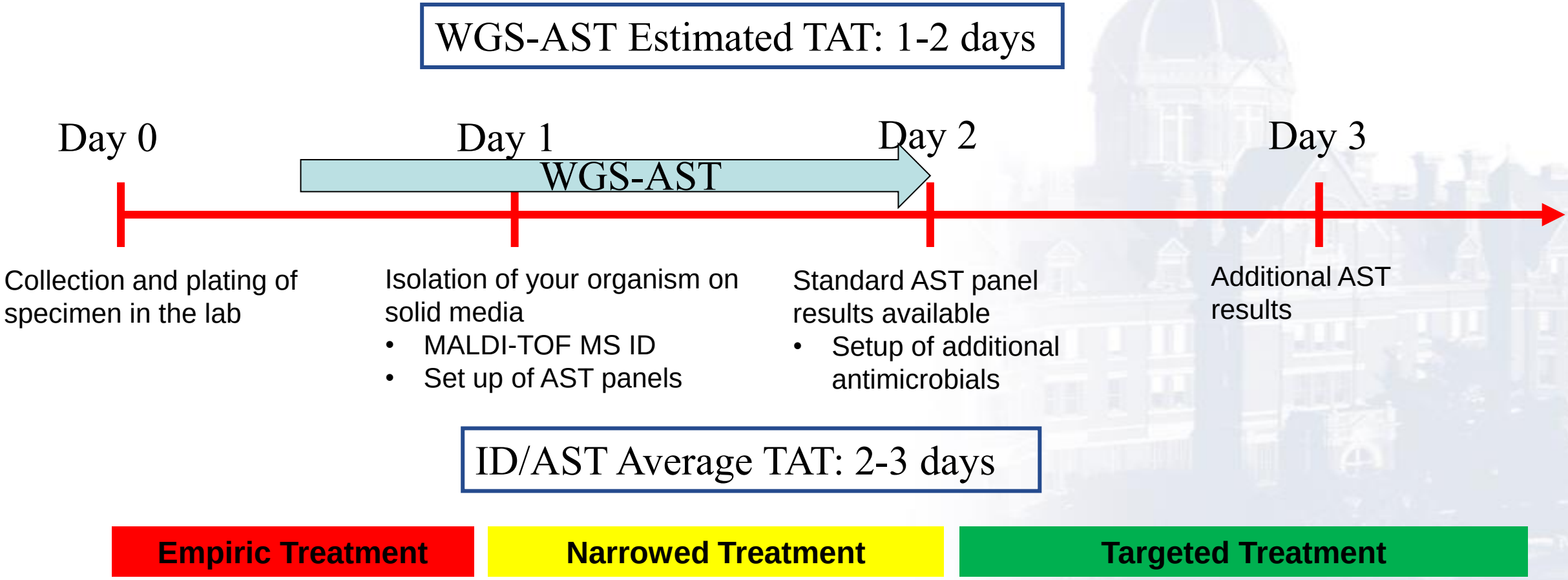
JAC-Antimicrobial Resistance

Combination of phage therapy and cefiderocol to successfully treat *Pseudomonas aeruginosa* cranial osteomyelitis

Patricia J. Simner[1], Jerald Cherian[1], Gina A. Suh[2], Yehudit Bergman[1], Stephan Beisken[3], Joseph Fackler[4], Martin Lee[4], Robert J. Hopkins[1] and Pranita D. Tamma [1]*

- Identify novel approaches to therapy

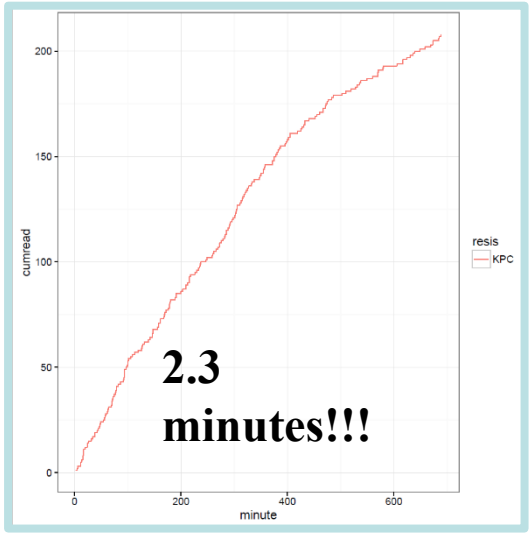
What About Using WGS to Predict Phenotypic AST Profiles to Guide Care?

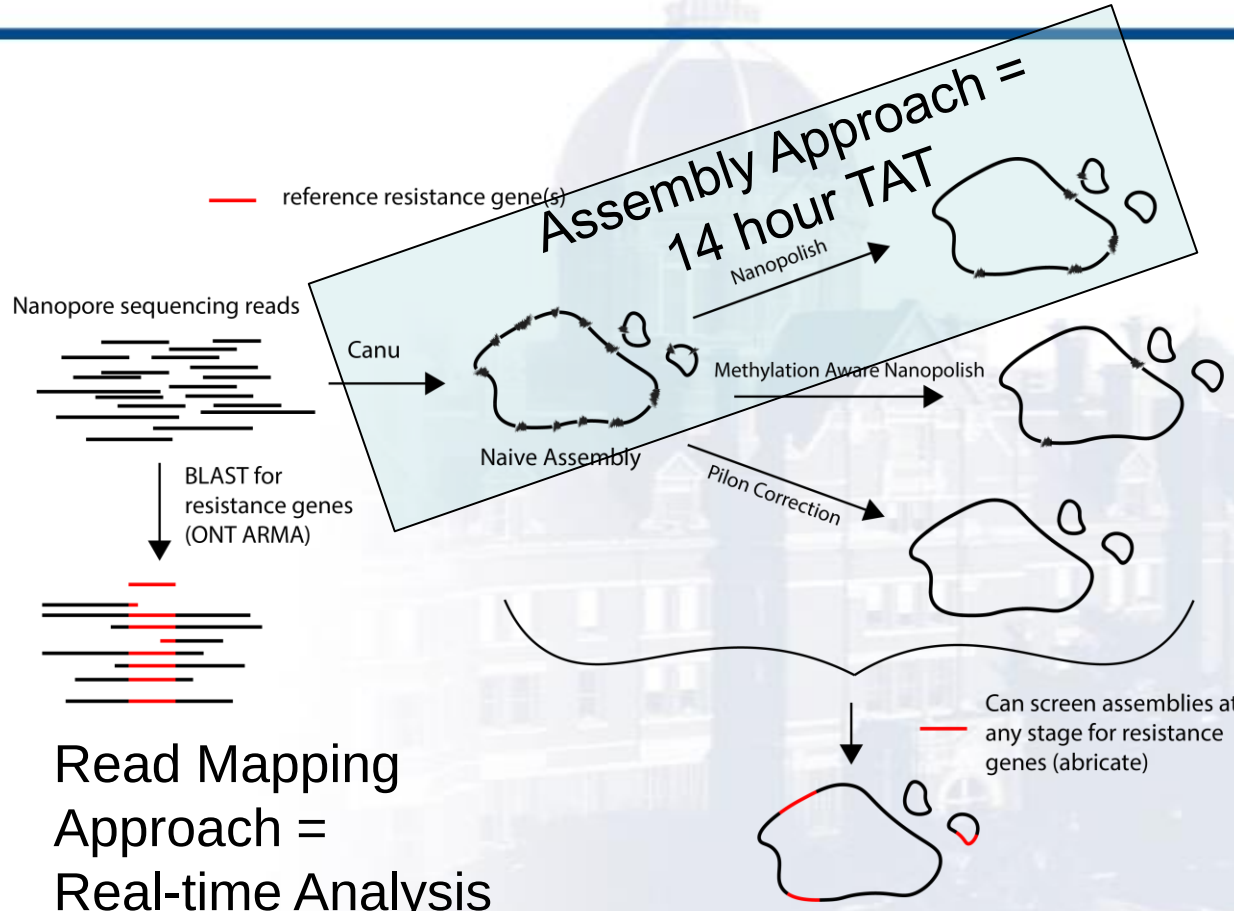


AST: antimicrobial susceptibility testing; ID: identification; whole genome sequencing-AST: WGS to predict AST

Can WGS Be Applied to Optimize Antimicrobial Therapy for MDR Gram-Negative Infections?

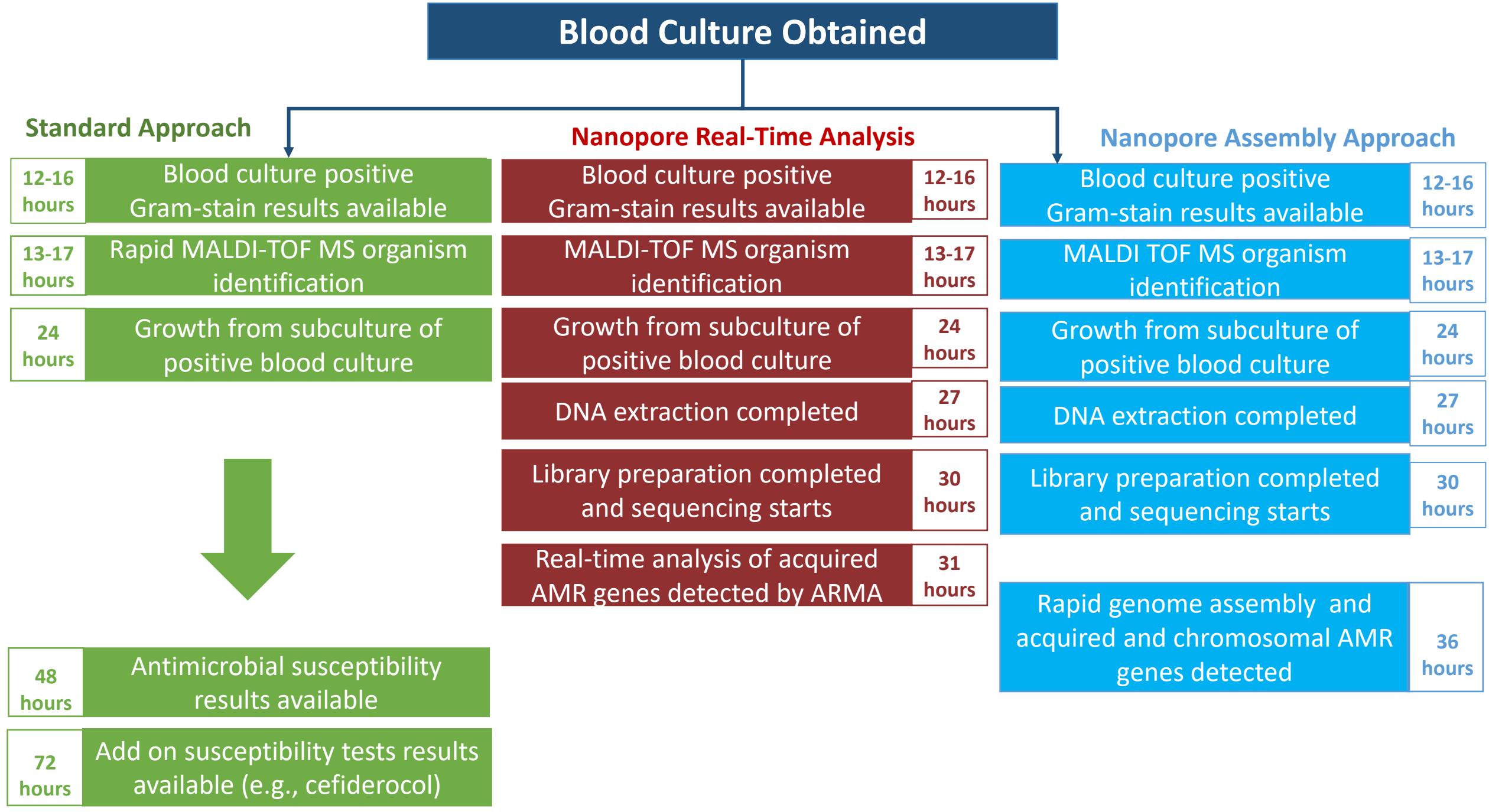
- 40 *Klebsiella pneumoniae*
  - 28 carbapenem-resistant
    - 19 carbapenemase producers
    - 9 non-carbapenemase producers
  - 12 carbapenem-susceptible





Read Mapping Approach =
Real-time Analysis
8 hour TAT

Tamma et al, AAC, 2018; PMID: 30373801. Yee et al, Eur J Clin Microbiol Infect Dis. 2021



How Did WGS Compare to BMD AST Results?

Antimicrobial	Real-time Approach % Agreement	Assembly Approach % Agreement
Ceftriaxone	93	95
Cefepime	95	98
Ertapenem	83	88
Meropenem	93	98
Piperacillin-tazobactam	80	83
Gentamicin or Tobramicin	45	95
Amikacin	78	88
Doxycycline	63	80
Ciprofloxacin or Levofloxacin	30	95
Trimethoprim-sulfamethoxazole	68	95
Colistin	93	98
Overall Agreement	**75% (range: 30-95)**	**91% (range: 80-98)**

Tamma et al, AAC, 2018; PMID: 30373801.

BMD: broth microdilution

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What Is the Potential Impact on Patient Care?

- Hypothetical trial design – to evaluate time to effective therapy
  - 28 patients with carbapenem-resistant *K. pneumoniae* infections
  - 22 (79%) received inadequate empiric therapy
    - Median time to effective therapy with conventional AST: **61 hours** (IQR 43-82)
  - Rapid WGS-AST had the potential to impact 20 patients (91%)
    - Median time to effective therapy with real-time approach: **41 hours** (IQR 33-44) ($p<0.05$ when compared to traditional AST)
    - Median time to effective therapy with assembly approach: **35 hours** (IQR 32-42) ($p<0.05$ when compared to traditional AST)

The time to effective therapy was reduced with the assembly approach because it provided more comprehensive data to infer AST activity than the real-time approach for which there were delays on awaiting additional AST results.

Tamma et al, AAC, 2018; PMID: 30373801.

Applying Automated Analytics Using Illumina vs Nanopore

- Study # 1: 144 *K. pneumoniae*
  - CP: carbapenemase-producers (n:66), Non-CP CRE (n:44);
    CS: carbapenem-susceptible (n:34)
- Study #2: 181 *Enterobacteriaceae*
  - CR: carbapenem-resistant (n:132)
  - CS: carbapenem-susceptible (n:49)
- Performed WGS using both Illumina and Oxford Nanopore technologies (ONT)
- Predictions using the ares-genetics.cloud
- Compared to broth microdilution
  - 1982 comparisons between predicted and observed resistance phenotypes for up to 22 antimicrobials

Simner et al, ASM NGS conference, 2020; Conzemius et al, Front Microbiol, 2022

JOHNS HOPKINS MEDICINE

How Does It Perform For the *Enterobacteriaceae*?

TABLE 2 Overall performance of the WGS-AST models across all antimicrobials, broken down by sequencing platform.

Platform	CA	VME	ME	TP	FP	FN	TN	*n*
Illumina	90%	10%	11%	1,646	161	178	1,315	3,300
ONT	88%	11%	13%	1,619	194	205	1,282	3,300

CA: Categorical agreement, VME: Very major error, ME: Major error, TP: True positive, FP: False positive, FN: False negative, TN: True negative, and *n*: number of evaluated species-antimicrobial pairs.

- Predictive AST demonstrated comparable performance between ONT & Illumina platforms
- Difference mostly driven by higher per base error rate for ONT
  - SNP based AMR detection (e.g., mutations in *gyrA/parC* leading to fluoroquinolone resistance or mutations in efflux pumps/porin genes)

Conzemius et al, Front Microbiol, 2022.

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Understanding the Mechanism of AMR Matters

- Treatment guidance based on whether AMR mechanism testing is performed or not
- Recommended treatment will differ based on the mechanisms mediating resistance

AMR	Recommended Treatment
ESBL	Meropenem
AmpC	Cefepime
Carbapenemase	≠ based on genotype

Table 2. Recommended Antibiotic Treatment Options for Presumed or Confirmed Extended-spectrum β-Lactamase–Producing Enterobacterales, Assuming In Vitro Susceptibility to Agents in Table

Source of Infection	Preferred Treatment	Alternative Treatment if First-line Options not Available or Tolerated
Cystitis	Nitrofurantoin, trimethoprim-sulfamethoxazole	Amoxicillin-clavulanate, single-dose aminoglycosides, fosfomycin (*Escherichia coli* only) Ciprofloxacin, levofloxacin, ertapenem, meropenem, imipenem-cilastatin
Pyelonephritis or complicated urinary tract infection[a]	Ertapenem, meropenem, imipenem-cilastatin, ciprofloxacin, levofloxacin, or trimethoprim-sulfamethoxazole	
Infections outside of the urinary tract	Meropenem, imipenem-cilastatin, ertapenem Oral step-down therapy to ciprofloxacin, levofloxacin, or trimethoprim-sulfamethoxazole should be considered[b]	

[a]A complicated urinary tract infection (UTI) is defined as a UTI that occurs in association with a structural or functional abnormality of the genitourinary tract, or any UTI in a male patient.

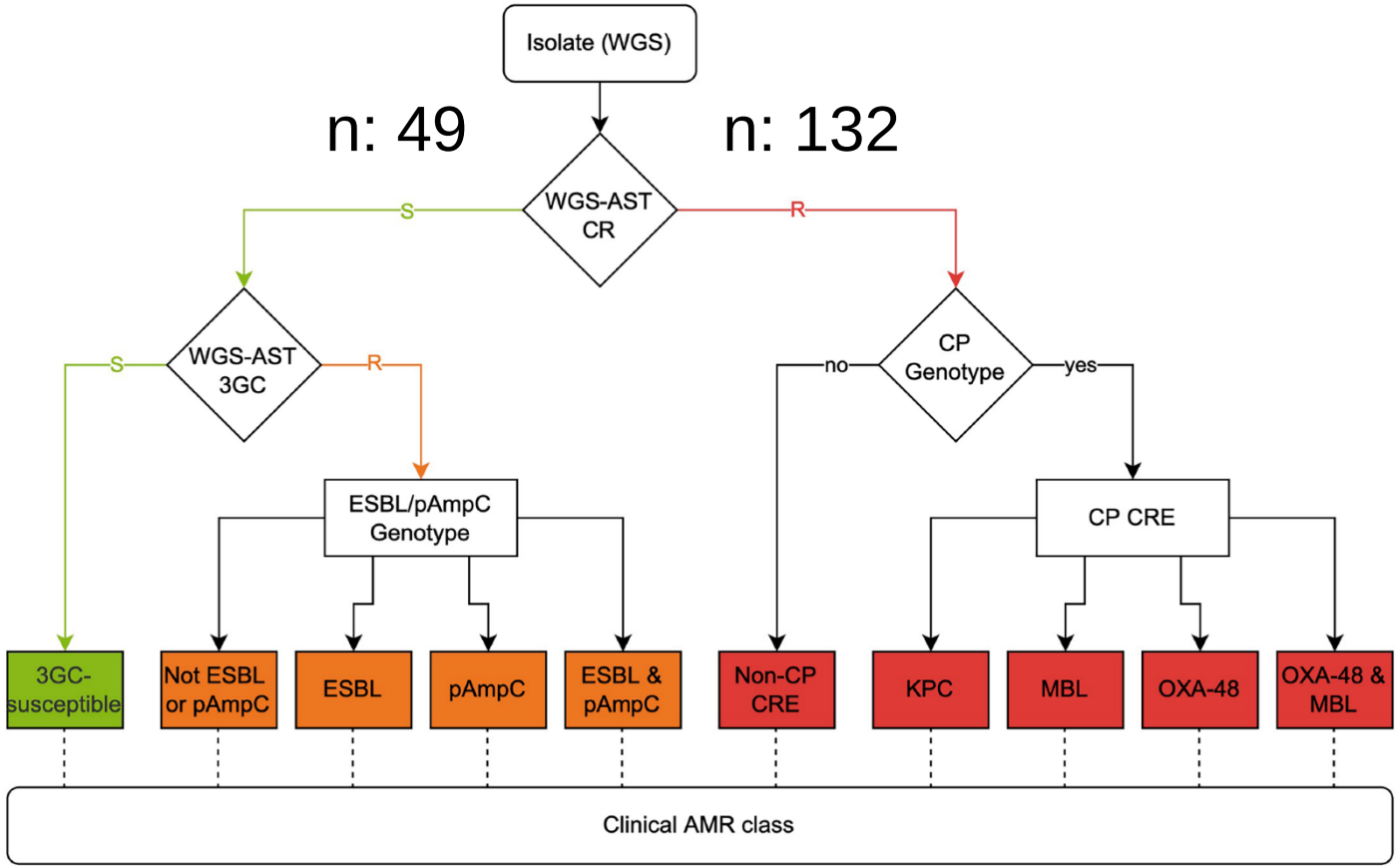
[b]Oral step-down therapy can be considered after susceptibility to the oral agent is demonstrated, patients are afebrile and hemodynamically stable, appropriate source control is achieved, and there are no issues with intestinal absorption.

Infectious Diseases Society of America Antimicrobial Resistant Treatment Guidance: Gram-Negative Bacterial Infections. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Clin Infect Dis. 2020 Oct 27:ciaa1478. doi: 10.1093/cid/ciaa1478. Online ahead of print. Clin Infect Dis. 2020. PMID: 33106864

IDSA: Infectious Diseases Society of America

Combining AMR Detection & WGS-AST Predictions

n: 181 *Enterobacteriaceae*



	Illumina CA	ONT CA
Ceftriaxone	96%	96%
Ertapenem	94%	91%

- Combining AMR genotypes & WGS-AST approaches have the potential to help further guide therapeutic management

Conzemius et al, Front Microbiol, 2022.

Validation of AMR and WGS-AST

- Phase 1: What is the best WGS method?
  - Multicenter comparison sequencing methods – with a focus on extraction
- Phase 2: How does it work if we apply it to clinical samples?
  - Applied best method to 42 consecutive blood cultures positive with ESKAPE pathogens Weinmaier et al, AAC, *In press*.

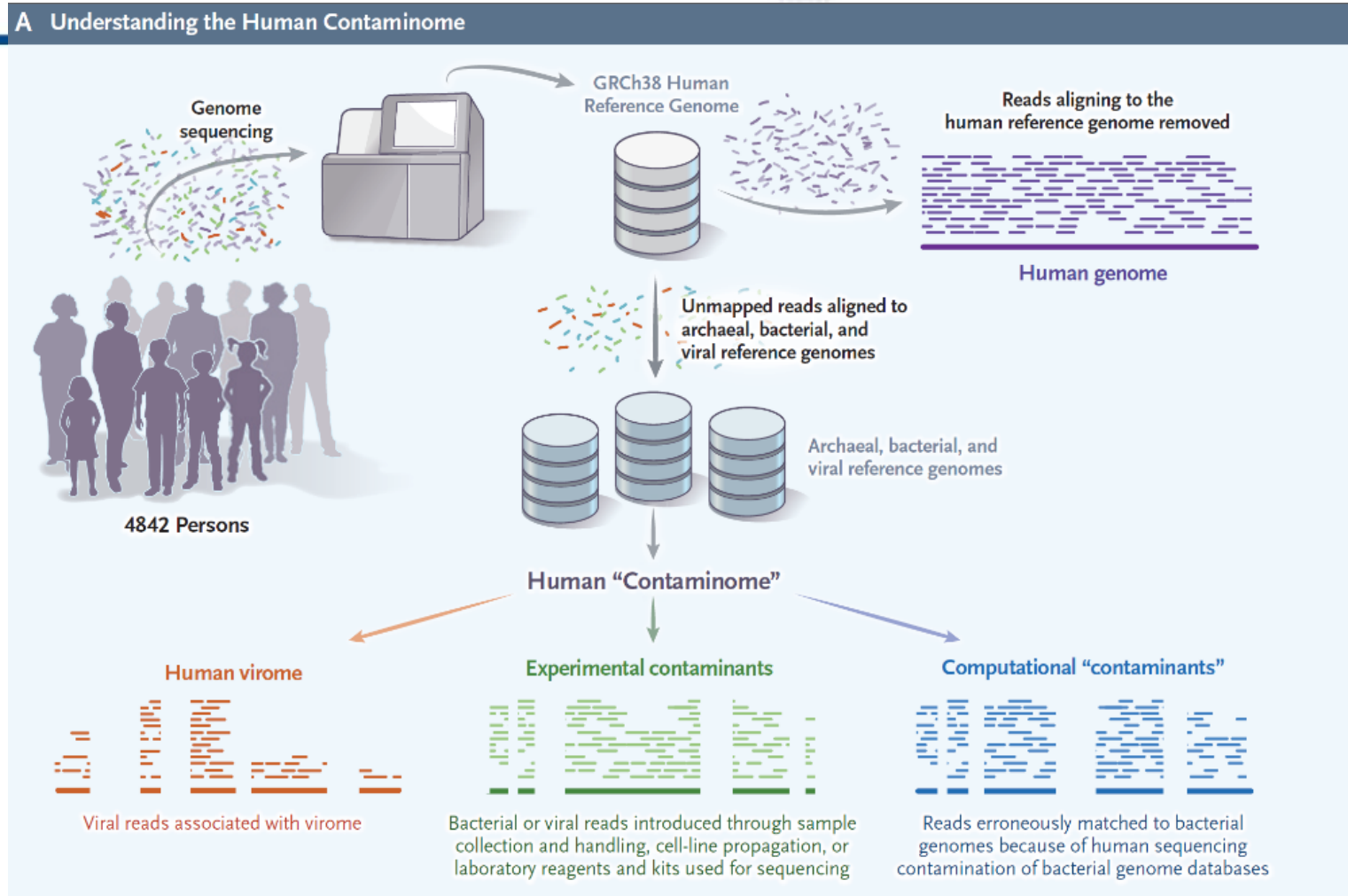
Organism	CA	VME	ME	mE	TN	FP	FN
All (n:42)	87.6%	4.8%	10.6%	3.8%	269	32	6
Acinetobacter baumannii (n:2)	87.0%	18.8%	0.0%	0.0%	7	0	3
Enterococcus faecium (n:5)	81.8%	15.4%	5.9%	9.1%	16	1	2
Escherichia coli (n:10)	87.2%	0.0%	12.6%	3.8%	97	14	0
Klebsiella pneumoniae (n:10)	81.7%	3.7%	17.2%	4.8%	77	16	1
Pseudomonas aeruginosa (n:10)	94.6%	0.0%	2.4%	3.6%	41	1	0
Staphylococcus aureus (n:5)	100.0%	0.0%	0.0%	0.0%	31	0	0

Part 2: Summary

- NGS methods are a powerful tool to study AMR & the resistome
- WGS:
  - Allows us to define mechanisms of resistance and to detect all AMR genes harbored by an isolate
  - WGS-AST has been demonstrated as an accurate tool to define and predict resistance among a variety of organisms
- tNGS & mNGS – AMR detection
  - Proof-of-concept & case report studies
  - Limitations: Abundance of organism, composition of specimen, unable to link AMR marker to specific organism
  - Further development is required to accurately detect antimicrobial resistance

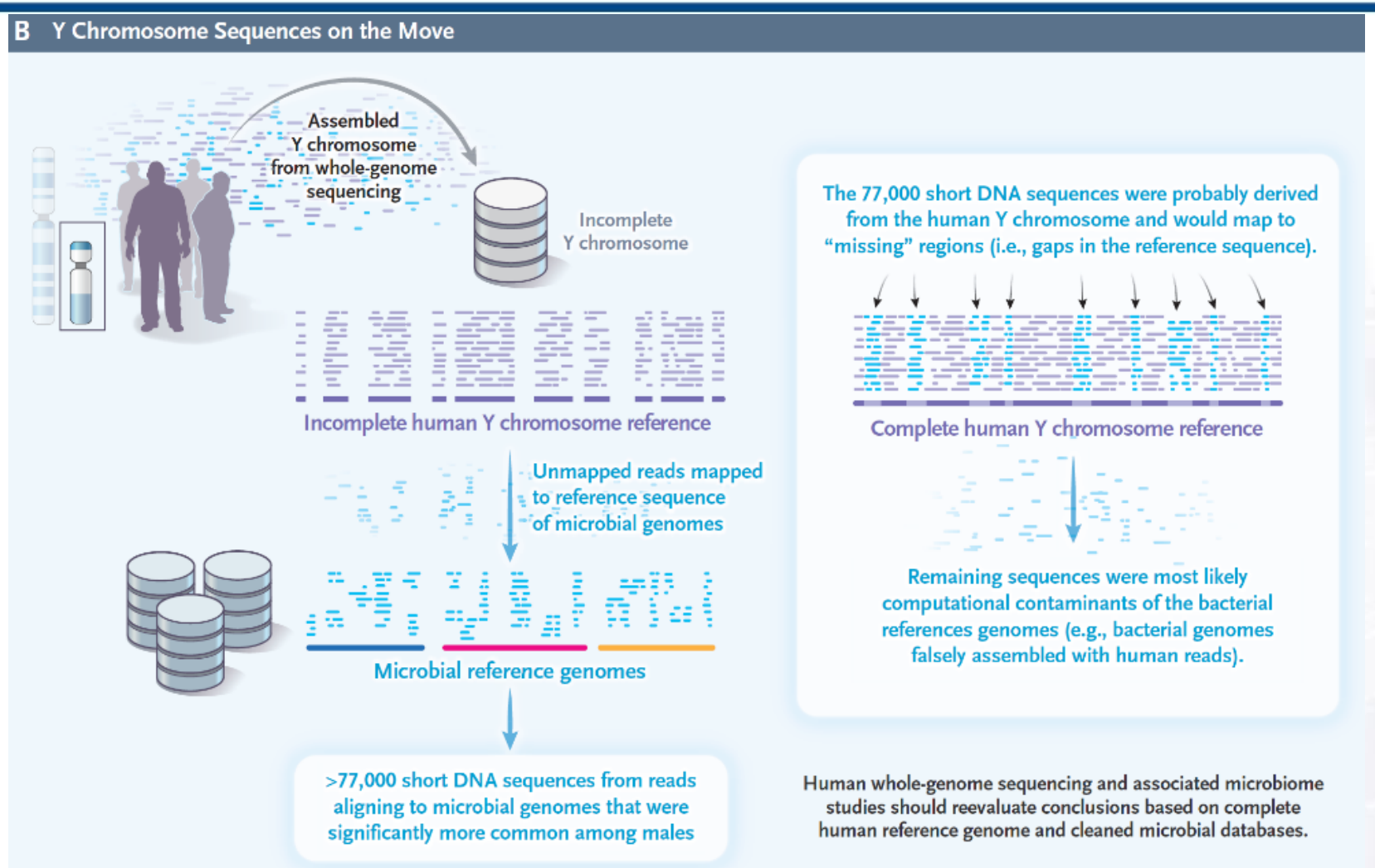
Beware of the Contaminome

- NGS applications frequently rely on curated genome databases for analysis and interpretation of sequencing result
- During the process of genome sequencing, small amounts of DNA not derived from the organism of interest are nearly impossible to avoid



Simner and Salzberg, NEJM, 2022; Chrisman et al, Sci Rep, 2022.

Cautious Interpretation of Sequencing Results



- Underscores the need for standard protocols to identify the contaminome to ensure the fidelity of sequencing-based studies and diagnostics

Simner and Salzberg, NEJM, 2022; Chrisman et al, Sci Rep, 2022.

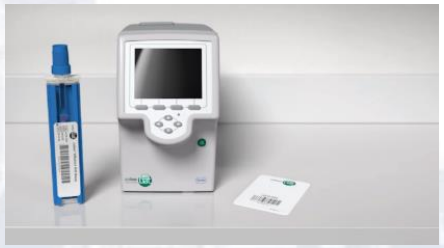
NGS is the Next Paradigm Shift

Conventional PCR

Real-time PCR

Moderately Complex – Closed Systems Sample- to-Answer

CLIA waived PCR POC devices



POC: Point-of-Care

Research → Academic/Reference Labs → Broad Scale Uptake → POC

Increased automation
Ease of use
Automated analytics
Outcome studies

JOHNS HOPKINS MEDICINE

Thank you!

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