Regulation of NGS-Based Diagnostics in the Academic Setting

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8th ICCMg – Geneva, Switzerland

Disclosures

- Research Contracts:
  - BD Diagnostics, OpGen Inc., Affinity Biosensors, Qiagen Sciences Inc, T2 Diagnostics, Accelerate 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, Day Zero Diagnostics, Next Gen Diagnostics

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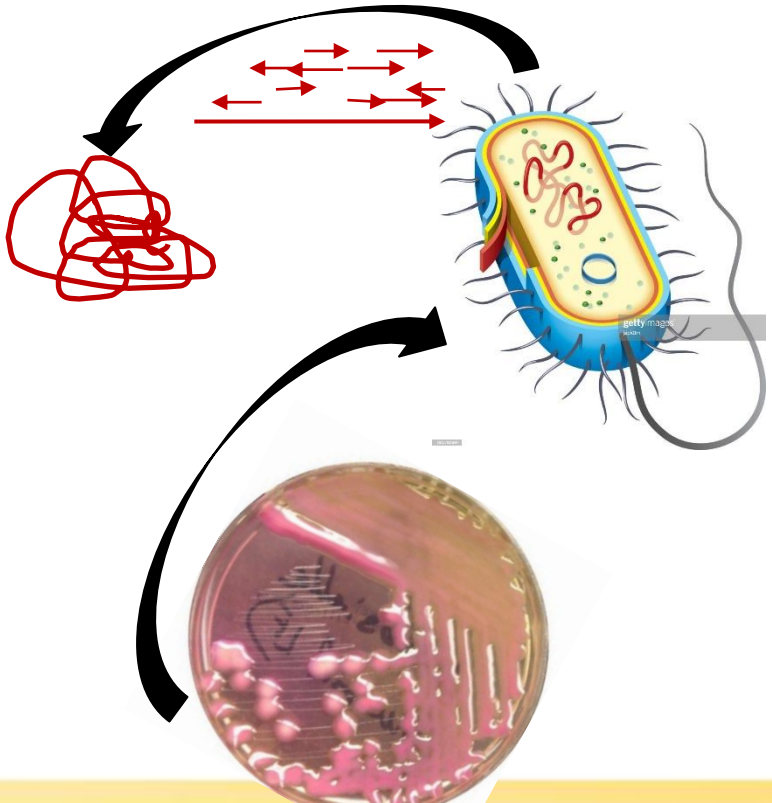
Objectives

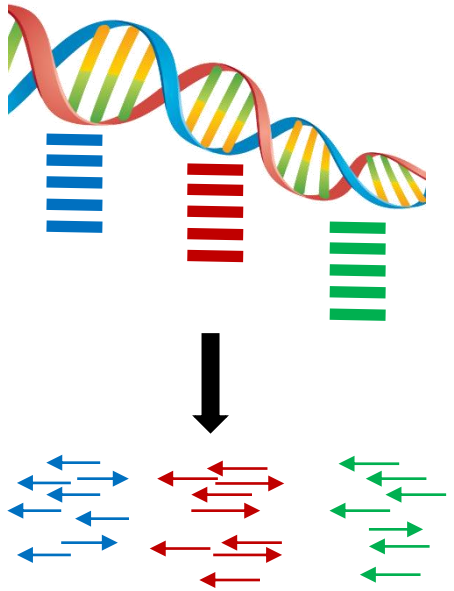
- Describe the current and future regulatory landscape of NGS-based diagnostics in academics
- Evaluate the pros & cons of regulation of NGS assays

NGS Applications for Infectious Diseases

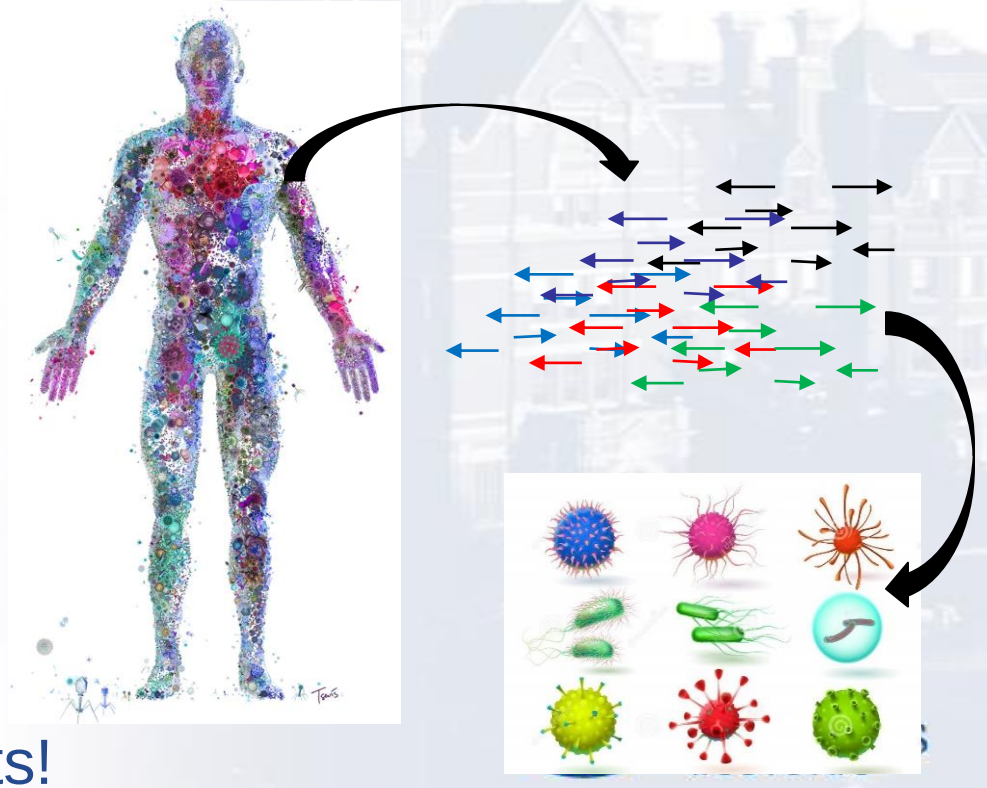
A. Whole Genome Sequencing



B. Targeted NGS (tNGS)



C. Metagenomic NGS (mNGS; aka clinical metagenomics)



Many methodologic variants!

NGS Diagnostics for Infectious Diseases Timeline

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Where Do We Stand with Regulation of NGS Tests for Infectious Disease Diagnostics?

- At this point and time:
  - There are no United States Food and Drug Administration (FDA) cleared/approved, Conformité Européene (CE) marked and/or Health-Canada approved assays to date
  - All are considered laboratory-developed tests (LDT) & require validation prior to implementation for clinical use
  - No current comprehensive guidance on best approaches

Regulation of Diagnostic Devices in the US

- 2 main branches of government in the US that oversees diagnostic tests for human use in the US
  - Centers for Medicare and Medicaid Services (CMS)
    - Upholds the Clinical Laboratory Improvement Amendments (CLIA) law which defines quality standards for all laboratory testing
    - Laboratory-developed tests (LDTs) require validation but CMS does not dictate the number of samples required

    Academics

  - US Food and Drug Administration (FDA)
    - FDA has oversight of diagnostic devices sold and used in the US
    - Very strict requirements for tests are considered for FDA clearance or approval
    - At this time the FDA does not set any requirement for LDTs

    Industry

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What Do We Have in Terms of Guidance for Regulation of NGS-Based Diagnostics?

- Molecular standards & guidelines
  - ISO guidelines (ISO15189)
  - Clinical and Laboratory Standards Institute (CLSI; Various MM-03 to MM-24 documents)
- Accreditation bodies
  - College of American Pathologists (CAP) Microbiology & Molecular Pathology Checklists
  - New York State guidance
- Guidance from our peers
  - Peer-reviewed literature
  - Call a friend ☺

International Organization for Standardization
ISO
15189:2012
Medical laboratories

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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Validation of NGS Tests for Infectious Diseases

- Require validation
  - Establishment of the performance characteristics
    - Precision
    - Accuracy
    - Reportable Range
    - Reference Range
    - Analytical Sensitivity
    - Analytical Specificity
    - Other factors that affect the test: specimen stability, inferring substances, etc

PARR – AS AS

  - PARR AS AS definitions are fairly straight-forward, the ways to establish PARR AS AS are more open to interpretation
  - Ultimately it is up to the laboratory director to decide how test performance characteristics are established
    - Following traditional molecular testing guidance (ISO & CLSI)

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Methods-Based & Risk-Based Approach

- Traditional target based approach is not feasible for most metagenomic or large targeted NGS assay validations
  - Take a methods-based approach
  - Critical workflow steps are defined, and their risk of generating an incorrect result is assessed
  - Using this risk-based approach, representative pathogen types (eg, DNA viruses, RNA viruses, gram-positive bacteria, gram-negative bacteria, yeast, etc.) may be selected for testing of the entire workflow
    - Specimens (remnant, spiked with whole organism or simulated specimens)
    - *In silico* data

What Does CAP Have to Say About Validation?

Wet Bench Component

***REVISED** 10/24/2022*

MOL.36015 NGS Analytical Wet Bench Validation/Verification **Phase II**

The laboratory validates/verifies the analytical wet bench component.

NOTE: To determine acceptable beginning-to-end test performance, validation/verification of the NGS analytical wet bench component must be integrated with the bioinformatics component for the intended test (see MOL.36151), including all laboratories participating in distributive testing processes. Laboratories must also comply with applicable test method validation/verification requirements in the All Common Checklist and the Molecular Pathology Checklist, including MOL.31015 for each type of specimen expected for the assay.

NOTE: For infectious disease testing, the study must also comply with MIC.64770 for each type of specimen expected for the assay (eg, blood, fresh/frozen tissue, paraffin-embedded tissue, prenatal specimens, saliva, buccal swabs, culture isolates). Validation/verification studies can be complemented with, but not supplanted by, additional reference standards (eg, ATCC isolates).

Due to extensive microbial genetic variation and diversity, it is not possible to perform an NGS test validation/verification study that would assess the ability of the test to accurately and reliably detect every possible organism or variant that may be present in a specimen. Therefore, a methods-based approach can be used for validation/verification where the specimens included contain a representative spectrum of the types of organisms, resistance variants, pathogenic factors, and host-response markers that the test is designed to detect.

- *For tests that are designed for organism detection, the CAP recommends using common pathogens found in a particular specimen type, when feasible, in the validation to ensure their accurate detection.*
- *For tests that analyze genes with common pathogenic mutations (eg, HIV reverse transcriptase K103N or CMV UL97 M460V/I) or that identify common resistance genes (eg, S. aureus mecA gene), specimens with those common mutations should be included, when feasible, in the validation.*
- *For broad-range methods, organisms of all significant taxonomic classes (eg, viruses, bacteria, mycobacteria, and fungi) should be included, when feasible, in the validation/ verification.*

Evidence of Compliance:

- ✓ Records of validation/verification studies **AND**
- ✓ Records of review of referral laboratory validations/verifications, if applicable

Dry Bench Component

***REVISED** 10/24/2022*

MOL.36151 NGS Analytical Bioinformatics Validation/Verification **Phase II**

The laboratory validates/verifies the analytical bioinformatics component (pipeline).

NOTE: The NGS analytical bioinformatics process data file output is used to determine if the sequence generated by the wet bench process is of sufficient quality and quantity for the intended test. To determine acceptable beginning-to-end test performance, bioinformatics component validation/verification must be integrated with the wet bench component (MOL.36015) for the intended test whether the testing is performed entirely in house or as part of a distributive testing processes.

Laboratories must comply with applicable test method validation/verification requirements in the All Common Checklist and the Molecular Pathology Checklist. As applicable, the laboratory also must:

Due to extensive microbial genetic variation and diversity, it is not possible to perform an NGS test validation that would assess the ability of the test to accurately and reliably detect every possible organism that may be present in a specimen. Therefore, a methods-based approach can be used for validation where the specimens included contain a representative spectrum of the types of organisms the test is designed to detect.

Evidence of Compliance:

- ✓ Records of validation/verification studies **AND**
- ✓ Records of review of referral laboratory validations, if applicable

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NGS- Specific Guidance

cofrac

Technical guide to accreditation of high throughput sequencing (NGS) technology

SH GTA 16 - Revision 00

Comité Français d'Accréditation

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Acting Executive Deputy Commissioner

March 2023

Validation of Next Generation Sequencing (NGS) Methods for Identification and/or Characterization of Infectious Agents

The following guidelines are applicable to whole genome sequencing (WGS) and other Next Generation Sequencing (NGS) methods for identification and/or characterization of infectious agents, including viruses, bacteria, fungi, and parasites. These guidelines should be used in conjunction with and not in lieu of the existing microbiology molecular guidelines available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.

The clinical validation of NGS assays should apply the same basic principles that have been established for validating most other complex molecular diagnostic procedures. These guidelines include considerations for both the NGS sequencing methods as well as the bioinformatic analysis. It is anticipated that these guidelines will evolve as the field matures and more experience is gained. Please utilize the most up-to-date version of these guidelines. (https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval)

https://www.wadsworth.org/sites/default/files/WebDoc/ID_WGS_NGS_Molecular_Guidance_update_032223.pdf

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Turning to Our Peers…

Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection

Robert Schlaberg, MD, MPH; Charles Y. Chiu, MD, PhD; Steve Miller, MD, PhD; Gary W. Procop, MD; George Weinstock, PhD; the Professional Practice Committee and Committee on Laboratory Practices of the American Society for Microbiology; the Microbiology Resource Committee of the College of American Pathologists

• *Context.*—Metagenomic sequencing can be used for detection of any pathogens using unbiased, shotgun next-generation sequencing (NGS), without the need for sequence-specific amplification. Proof-of-concept has been demonstrated in infectious disease outbreaks of unknown causes and in patients with suspected infections but negative results for conventional tests. Metagenomic NGS tests hold great promise to improve infectious disease diagnostics, especially in immunocompromised and critically ill patients.

Objective.—To discuss challenges and provide example solutions for validating metagenomic pathogen detection tests in clinical laboratories. A summary of current regulatory requirements, largely based on prior guidance for NGS testing in constitutional genetics and oncology, is provided.

Data Sources.—Examples from 2 separate validation studies are provided for steps from assay design, and validation of wet bench and bioinformatics protocols, to quality control and assurance.

Conclusions.—Although laboratory and data analysis workflows are still complex, metagenomic NGS tests for infectious diseases are increasingly being validated in clinical laboratories. Many parallels exist to NGS tests in other fields. Nevertheless, specimen preparation, rapidly evolving data analysis algorithms, and incomplete reference sequence databases are idiosyncratic to the field of microbiology and often overlooked.

(*Arch Pathol Lab Med.* 2017;141:776–786; doi: 10.5858/arpa.2016-0539-RA)

"2 separate validation studies are provided for steps from assay design, and validation of wet bench and bioinformatics protocols, to quality controls and assurance"

Schlaberg et al, Arch Pathol Lab Med, 2017 (PMID: 28169558).

Other Peer-Reviewed Examples of Validations

nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-018-0349-6

Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease

Timothy A. Blauwkamp[1,3*], Simone Thair[2,3], Michael J. Rosen[1], Lily Blair[1], Martin S. Lindner[1], Igor D. Vilfan[1], Trupti Kawli[1], Fred C. Christians[1], Shivkumar Venkatasubrahmanyam[1], Gregory D. Wall[1], Anita Cheung[1], Zoë N. Rogers[1], Galit Meshulam-Simon[1], Liza Huijse[1], Sanjeev Balakrishnan[1], James V. Quinn[2], Desiree Hollemon[1], David K. Hong[1], Marla Lay Vaughn[1], Mickey Kertesz[1], Sivan Bercovici[1], Judith C. Wilber[1,3] and Samuel Yang[2,3]

American Society for Microbiology | Journal of Clinical Microbiology®

VIROLOGY

Evaluation of Metagenomic and Targeted Next-Generation Sequencing Workflows for Detection of Respiratory Pathogens from Bronchoalveolar Lavage Fluid Specimens

David C. Gaston,[a]* Heather B. Miller,[a] John A. Fissel,[a]§ Emily Jacobs,[a] Ethan Gough,[b] Jiajun Wu,[c] Eili Y. Klein,[d] Karen C. Carroll,[a] Patricia J. Simner[a]

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[c]Department of Pediatrics, Biostatistics, Epidemiology and Data Management (BEAD) Core, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[d]Department of Emergency Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

American Society for Microbiology | Microbiology Spectrum

RESEARCH ARTICLE

Validation of a Metagenomic Next-Generation Sequencing Assay for Lower Respiratory Pathogen Detection

Zhenli Diao,[a,b,c] Huiying Lai,[d] Dongsheng Han,[e] Bin Yang,[f] Rui Zhang,[a,b,c] Jinming Li[a,b,c]

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[d]Department of Laboratory Medicine, Beijing Hospital, National Center of Gerontology Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, People's Republic of China
[e]Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Department of Clinical Laboratory, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, People's Republic of China
[f]Vision Medicals Center for Infectious Diseases, Guangzhou, People's Republic of China

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ANALYTICAL PROCEDURES

Validation and Application of Long-Read Whole-Genome Sequencing for Antimicrobial Resistance Gene Detection and Antimicrobial Susceptibility Testing

Thomas Weinmaier,[a] Rick Conzemius,[a] Yehudit Bergman,[b] Shawna Lewis,[b] Emily B. Jacobs,[b] Pranita D. Tamma,[c] Arne Materna,[a] Johannes Weinberger,[a] Stephan Beisken,[a] Patricia J. Simner[b]

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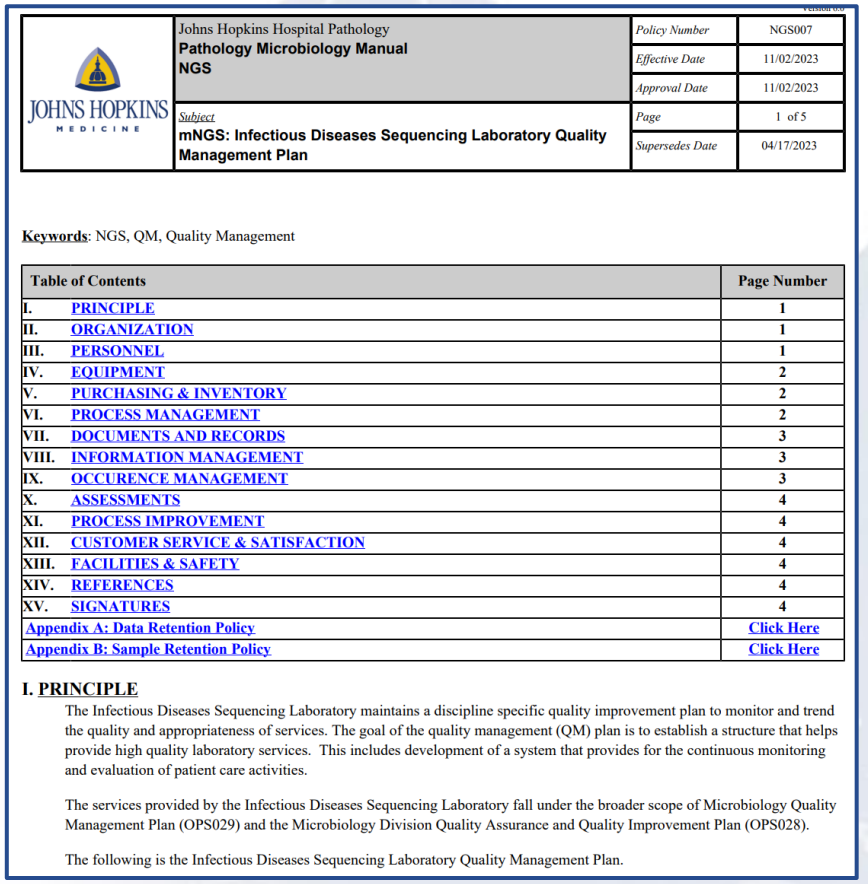
[b]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[c]Department of Pediatrics, Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Other Requirements to Consider

- Validation outline & summary
- Standard operating procedures
- Quality management plan
- QC requirements & established thresholds
- Competency assessment
- Proficiency testing
- Sample/Data retention policies



JOHNS HOPKINS MEDICINE	Johns Hopkins Hospital Pathology **Pathology Microbiology Manual** **NGS**	*Policy Number*	NGS007
		Effective Date	11/02/2023
		Approval Date	11/02/2023
	Subject **mNGS: Infectious Diseases Sequencing Laboratory Quality Management Plan**	*Page*	1 of 5
		Supersedes Date	04/17/2023

Keywords: NGS, QM, Quality Management

Table of Contents		Page Number
I.	PRINCIPLE	1
II.	ORGANIZATION	1
III.	PERSONNEL	1
IV.	EQUIPMENT	2
V.	PURCHASING & INVENTORY	2
VI.	PROCESS MANAGEMENT	2
VII.	DOCUMENTS AND RECORDS	3
VIII.	INFORMATION MANAGEMENT	3
IX.	OCCURENCE MANAGEMENT	3
X.	ASSESSMENTS	4
XI.	PROCESS IMPROVEMENT	4
XII.	CUSTOMER SERVICE & SATISFACTION	4
XIII.	FACILITIES & SAFETY	4
XIV.	REFERENCES	4
XV.	SIGNATURES	4
Appendix A: Data Retention Policy		Click Here
Appendix B: Sample Retention Policy		Click Here

I. PRINCIPLE

The Infectious Diseases Sequencing Laboratory maintains a discipline specific quality improvement plan to monitor and trend the quality and appropriateness of services. The goal of the quality management (QM) plan is to establish a structure that helps provide high quality laboratory services. This includes development of a system that provides for the continuous monitoring and evaluation of patient care activities.

The services provided by the Infectious Diseases Sequencing Laboratory fall under the broader scope of Microbiology Quality Management Plan (OPS029) and the Microbiology Division Quality Assurance and Quality Improvement Plan (OPS028).

The following is the Infectious Diseases Sequencing Laboratory Quality Management Plan.

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AN EXAMPLE OF THE SUCCESSES

Optimization & Development of mNGS

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

- Started in January, 2016
  - Hired a full-time Clinical Laboratory Specialist
  - Complex multi-step process with little data available in the literature on methods
- Locked down our method for validation in April, 2019
- Went live October, 2020

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BACTERIOLOGY

Check for updates

Development and Optimization of Metagenomic Next-Generation Sequencing Methods for Cerebrospinal Fluid Diagnostics

Patricia J. Simner,[a] Heather B. Miller,[a] Florian P. Breitwieser,[b] Gabriel Pinilla Monsalve,[c] Carlos A. Pardo,[a,c] Steven L. Salzberg,[b,e] Cynthia L. Sears,[d] David L. Thomas,[d] Charles G. Eberhart,[a,f] Karen C. Carroll[a]

Heather Miller

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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%

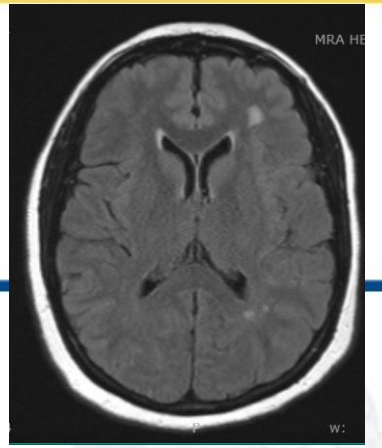
Limits of detection:

- 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

Apply using a diagnostic stewardship approach where mNGS serves as an adjunct test to standard-of-care methods – for rare, atypical or unsuspected cases

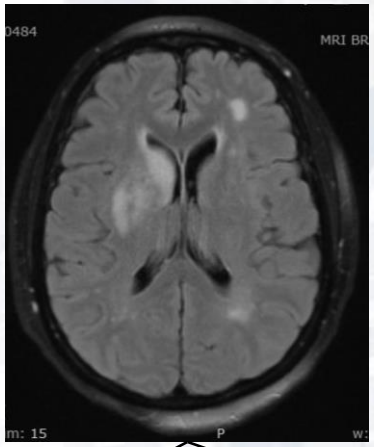
SOC: standard-of-care; PPA: positive percent agreement; NPA: negative percent agreement

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



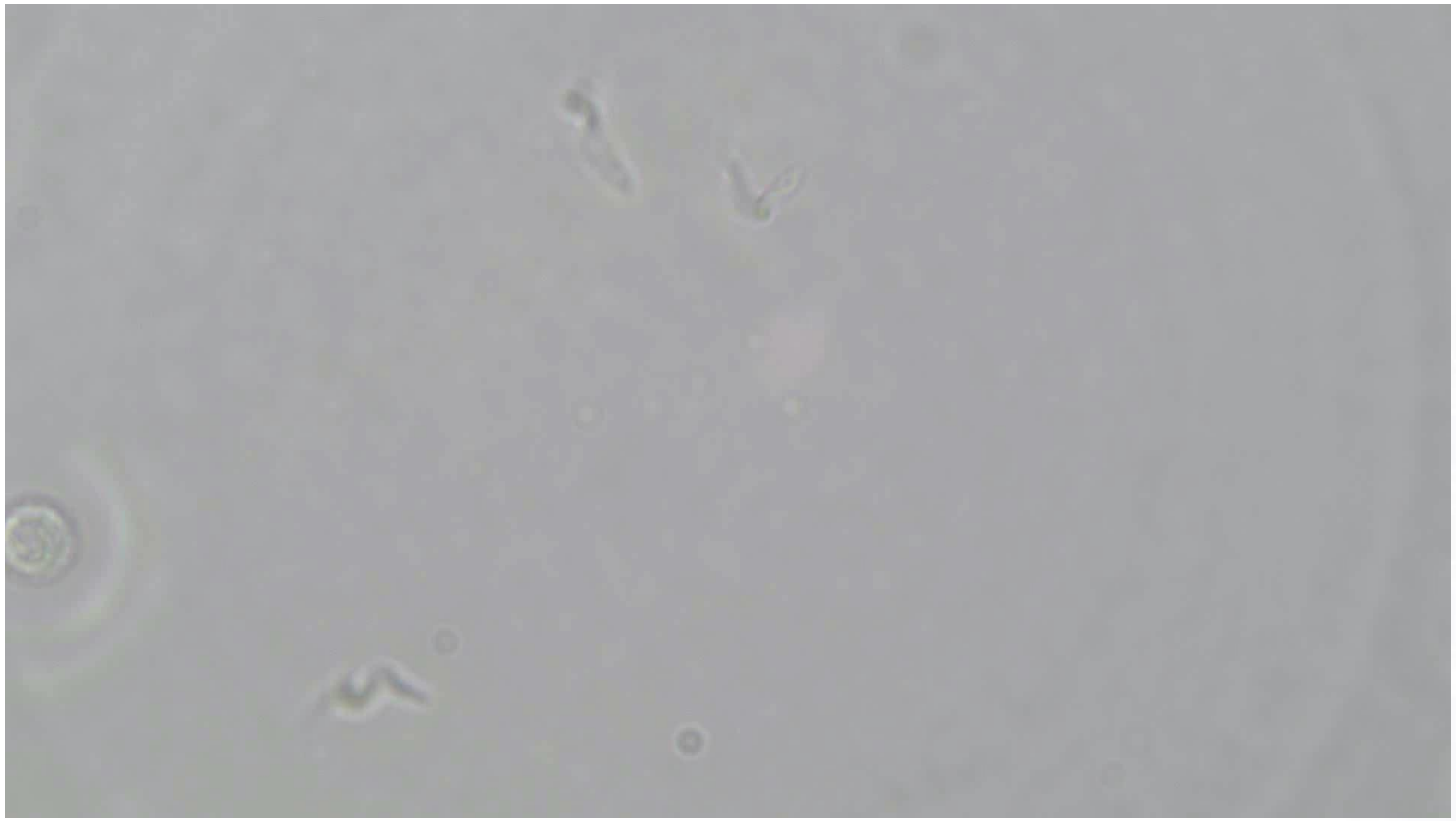
New T2 Flair hyperintensity of right anterior internal capsule & striatum

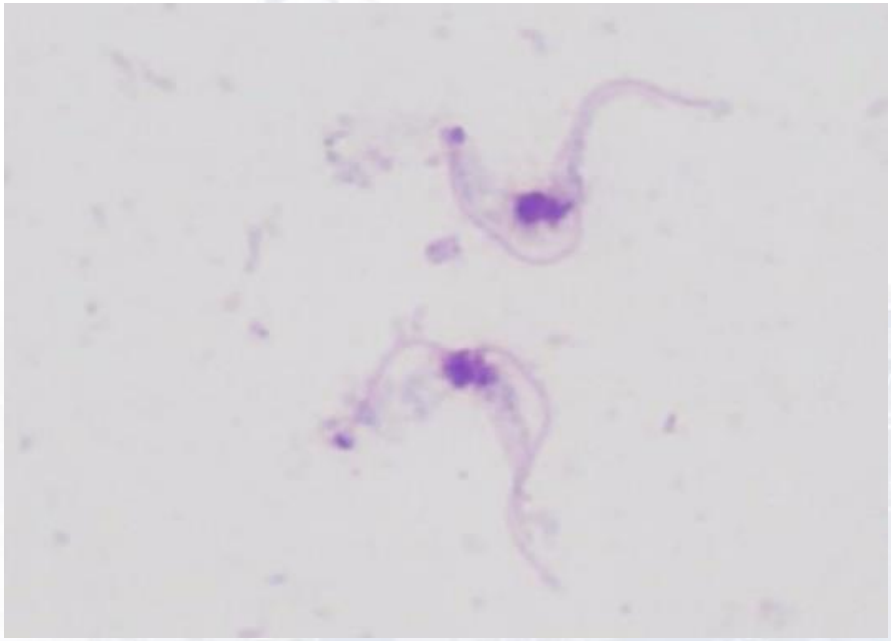
CSF #1
WBC: 48 cells/uL
Protein 58 mg/dL
Concerns for PML
JCV PCR negative

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

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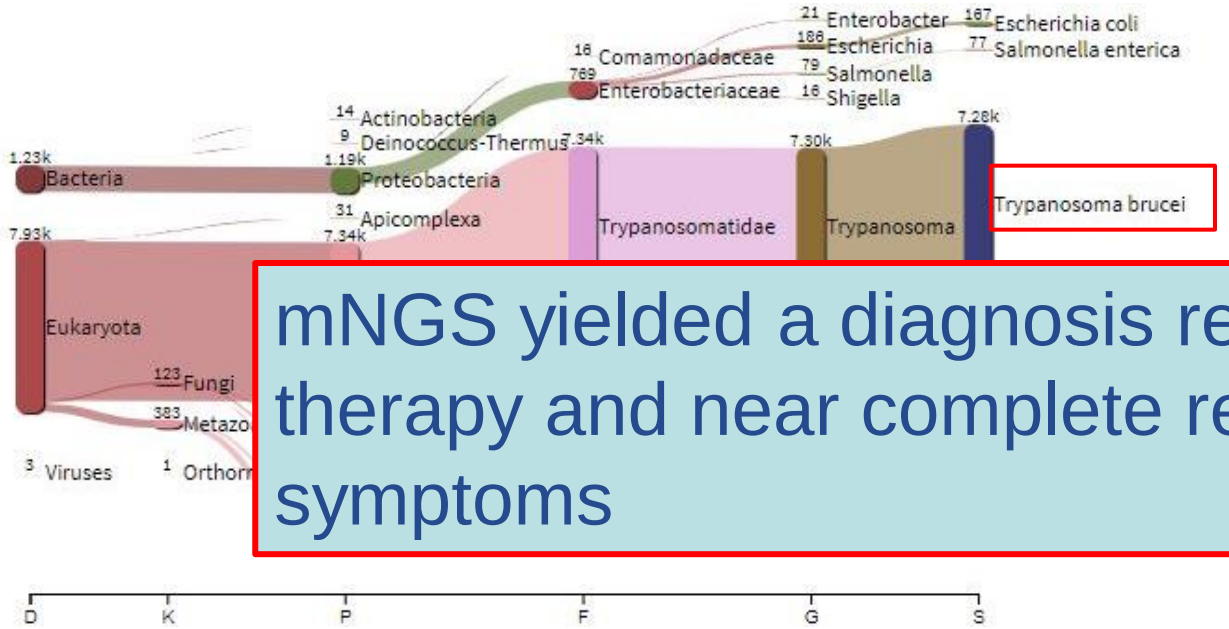
mNGS Yielded a Diagnosis of Human African Trypanosomiasis (HAT)



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

Confirmed by targeted PCR

Trypanosome brucei genome coverage

Parasitic protozoan hemoflagellate

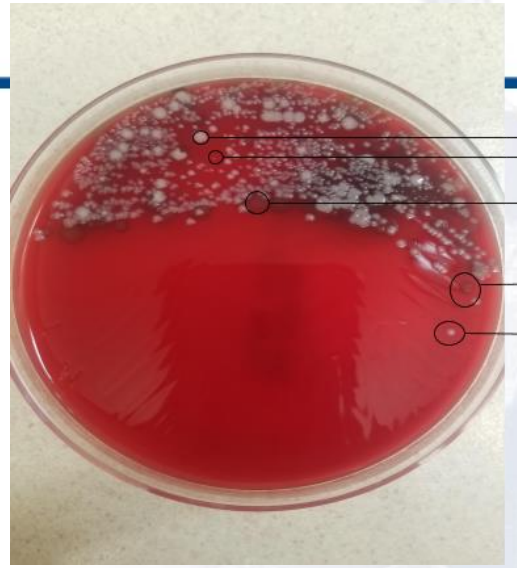
- Usually fatal if left untreated
- Treatment involves toxic agents & requires IND from the FDA (nifurtimox & efluornithine)

AND SOME CHALLENGES

Lack of Standardization

- Differences in validation processes
- Lack of standardization in definitions, methods & reporting
  - Clinical care teams may not understand the methodologies & how to appropriately interpret results
  - Results listing all organisms detected without interpretation nor with understanding of the clinical context
  - Antimicrobial resistance gene reports
    - No association to the organism
    - Interpreting associations vs true predictions

Examples of normal respiratory microbiota



mNGS

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

tNGS

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**

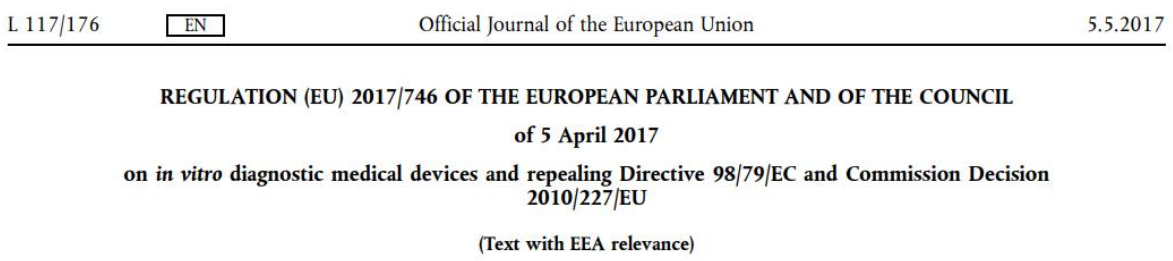
Analysis Performed: Explify® Respiratory Pathogen ID/AMR Panel (RPIP) - Data Analysis Solution
For Research Use Only. Not for use in diagnostic procedures.

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

Further Regulation of LDTs ☹

EU- IVDR



L 117/176 EN Official Journal of the European Union 5.5.2017

REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 5 April 2017

on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU

(Text with EEA relevance)

Lab-Developed Tests

Guidance on use of Lab-Developed Tests as described in REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 April 2017 on in vitro diagnostic medical devices

Task force IVDR

New FDA Proposed Rule

- Phase out enforcement discretion of LDTs

NATIONAL ARCHIVES

FEDERAL REGISTER

The Daily Journal of the United States Government

Proposed Rule

Medical Devices; Laboratory Developed Tests

A Proposed Rule by the Food and Drug Administration on 10/03/2023

This document has a comment period that ends in 21 days. (12/04/2023)

SUBMIT A FORMAL COMMENT

1686 comments received. View posted comments

https://www.federalregister.gov/documents/2023/10/03/2023-21662/medical-devices-laboratory-developed-tests

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Pros of Regulating NGS-Based Diagnostics

Standardized review of all NGS-based diagnostics in a particular setting/country

Potential to drive market pressures to prioritize regulatory approval by commercial entities

Assure appropriate reporting practices based on the intended use of the assay

Cons of Regulating NGS-Based Diagnostics

Impact on Clinical & Public Health Labs

- Regulation of NGS as LDTs exist in some capacity in clinical labs
- Potential for significant burden to laboratories (infrastructure, costs, resources)
- Increase dependence on reference labs

Impact on Advancing Patient Care

- Delay the understanding of the value of NGS-based (e.g. appropriate timing of testing, patient populations & clinical syndromes)
- Decrease innovation & leave diagnostics gaps

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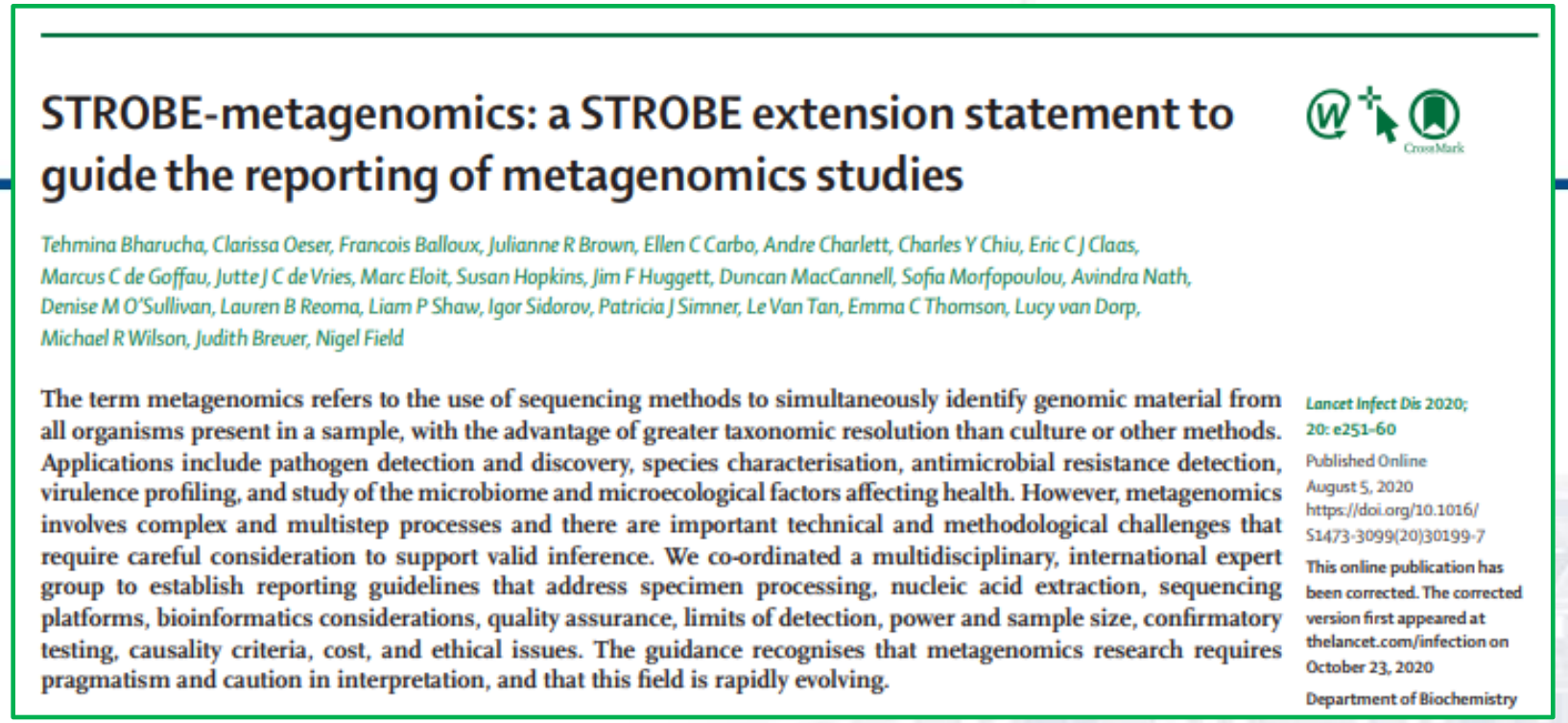

STROBE-metagenomics: a STROBE extension statement to guide the reporting of metagenomics studies

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The term metagenomics refers to the use of sequencing methods to simultaneously identify genomic material from all organisms present in a sample, with the advantage of greater taxonomic resolution than culture or other methods. Applications include pathogen detection and discovery, species characterisation, antimicrobial resistance detection, virulence profiling, and study of the microbiome and microecological factors affecting health. However, metagenomics involves complex and multistep processes and there are important technical and methodological challenges that require careful consideration to support valid inference. We co-ordinated a multidisciplinary, international expert group to establish reporting guidelines that address specimen processing, nucleic acid extraction, sequencing platforms, bioinformatics considerations, quality assurance, limits of detection, power and sample size, confirmatory testing, causality criteria, cost, and ethical issues. The guidance recognises that metagenomics research requires pragmatism and caution in interpretation, and that this field is rapidly evolving.

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This online publication has been corrected. The corrected version first appeared at thelancet.com/infection on October 23, 2020

Department of Biochemistry

IDEALLY, WE WOULD HAVE BEST PRACTICE OR CONSENSUS STANDARDS TO GUIDE US

JOHNS HOPKINS MEDICINE

Summary

- There are no United States Food and Drug Administration (FDA) cleared/approved, Conformité Européene (CE) marked and/or Health Canada approved assays to date
- All NGS-based assays are viewed as laboratory-developed tests with little to no defined guidance on validation requirements
- Pros and cons to further regulation → stifle innovation
- Encourage the development of consensus guidelines created by an internationally group to help fill the gap

Thank you!

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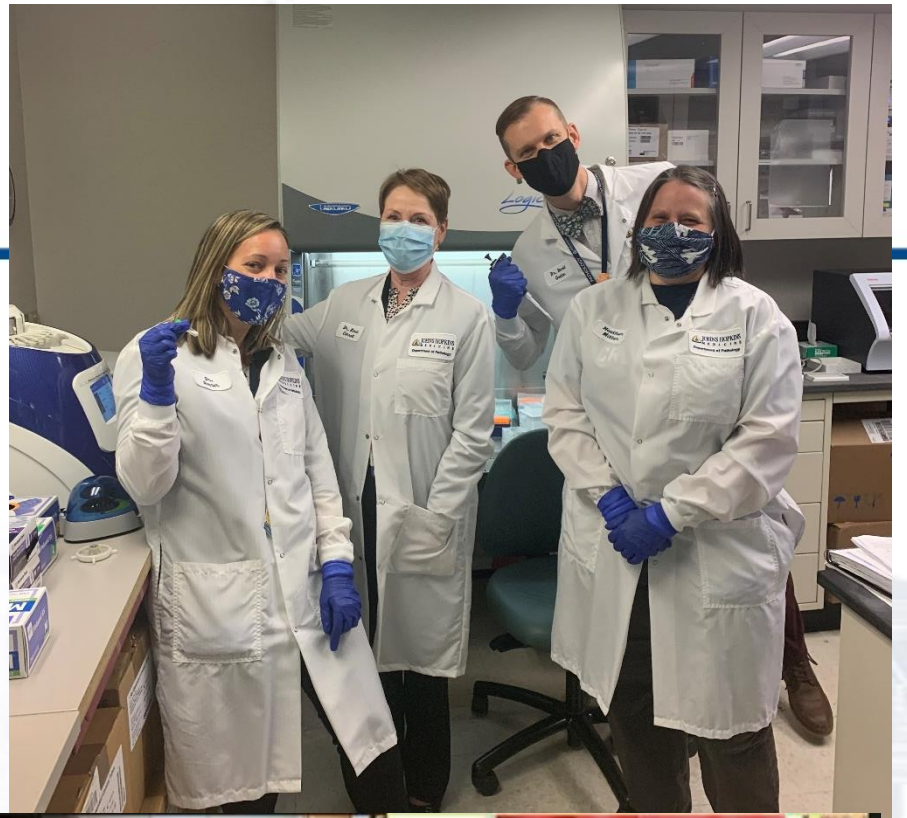

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