The promise and *reality* of Next-Generation Sequencing (NGS)-based testing for diagnosis of Infectious Diseases

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Disclosures

None

We do not endorse any commercial products discussed in the presentation

Learning Objectives

- Compare NGS-based testing to traditional nucleic acid amplification methods
- List advantages and limitations of NGS-based testing for diagnosis of infectious diseases
- Identify clinical scenarios in which NGS-based testing should be considered
- Identify strategies to improve appropriate use of NGS-based testing for infectious diseases

Outline

- Overview of classical infectious disease (ID) testing
- Evolution of sequencing
  - Sequencing technologies
  - Practical applications for ID
- Currently available NGS tests for ID
  - Clinical performance of metagenomic NGS (mNGS)
  - Clinical impact and utility of mNGS
  - Diagnostic utilization criteria for mNGS
- Summary
- Future prospects

Diagnostic techniques in the microbiology laboratory

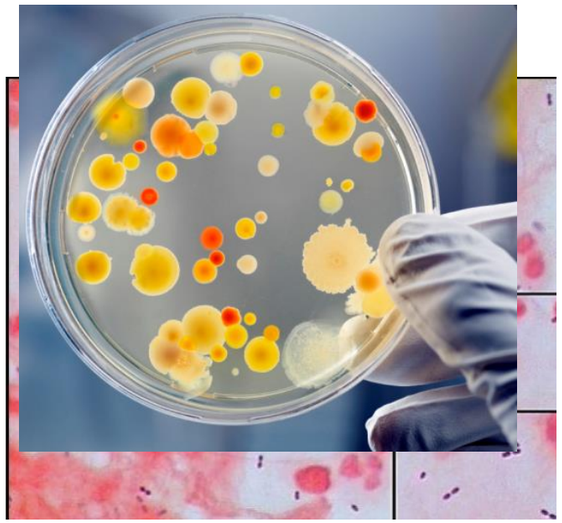
Classical microbiology

Microscopic examination
~1hr TAT

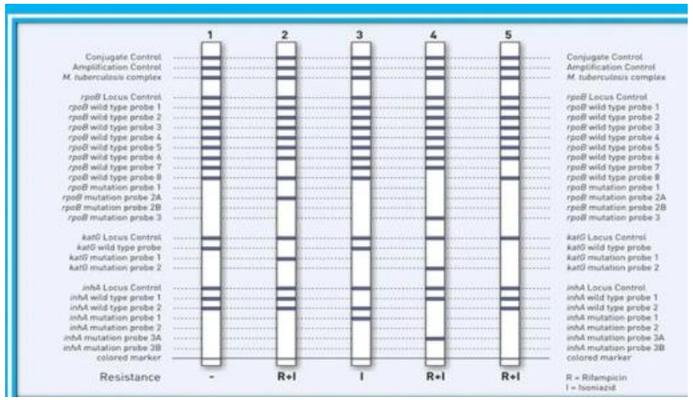
- Inflammation response
- Organisms
- Presumptive diagnosis

Cultivation and identification:
~2-14 days

- Enzymology, biochemistry or molecular method
- Antibiotic susceptibility testing
- Definitive diagnosis



CDC.org



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Molecular microbiology

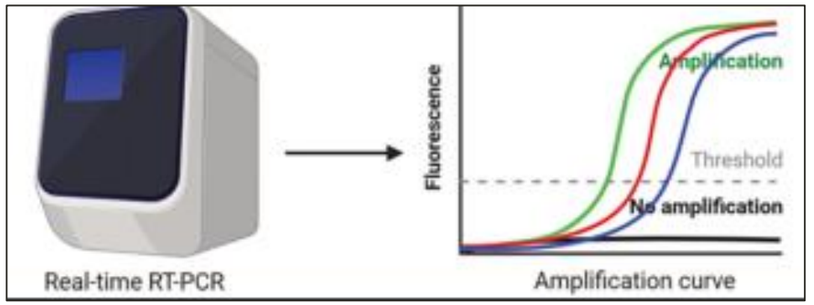
Direct detect viral genome/genes

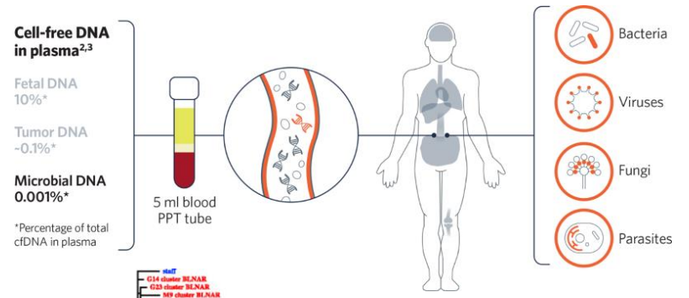
~1-3 day TAT

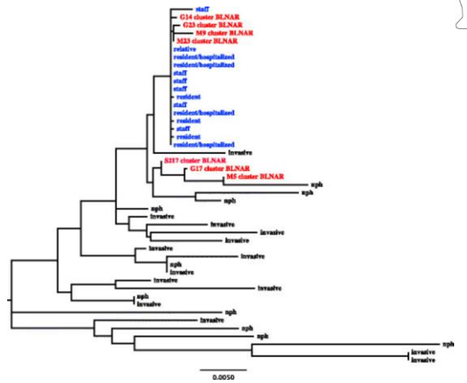
- DNA probes
- PCR
- DNA sequencing
- Definitive diagnosis

Epidemiology:

- Outbreak investigation
- Newly emerged pathogen







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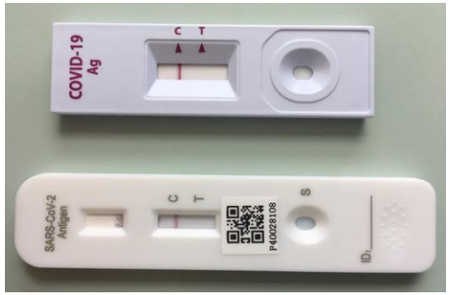
Immunoserology

Antigen tests
~ 1hr TAT

Antibody tests
~7 days



What is Sequencing?

The process of determining the number and order of nucleotides (adenine, guanine, cytosine, thymine) that make up a molecule of DNA

- Identify a microorganism
- Analyze genetic mutations within genomes: antimicrobial resistant marker, virulent factors
- Investigate an outbreak
- Understand host response

It starts with Sanger sequencing

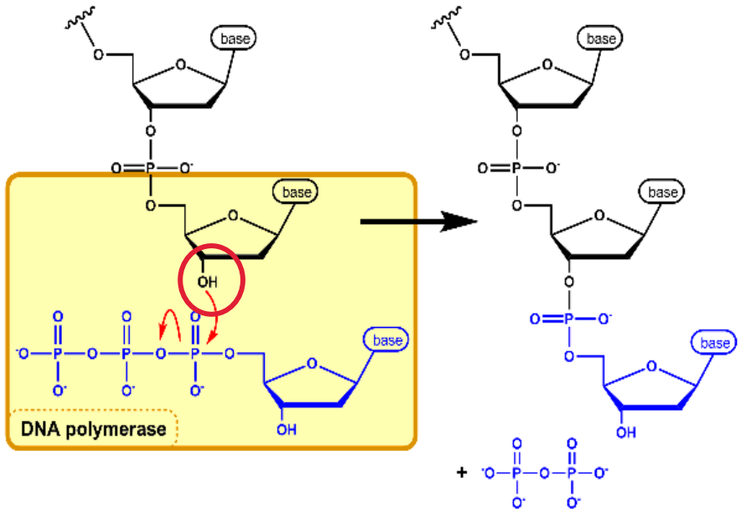
- 1953 Crick, Watson and Franklin discovered the structure of DNA

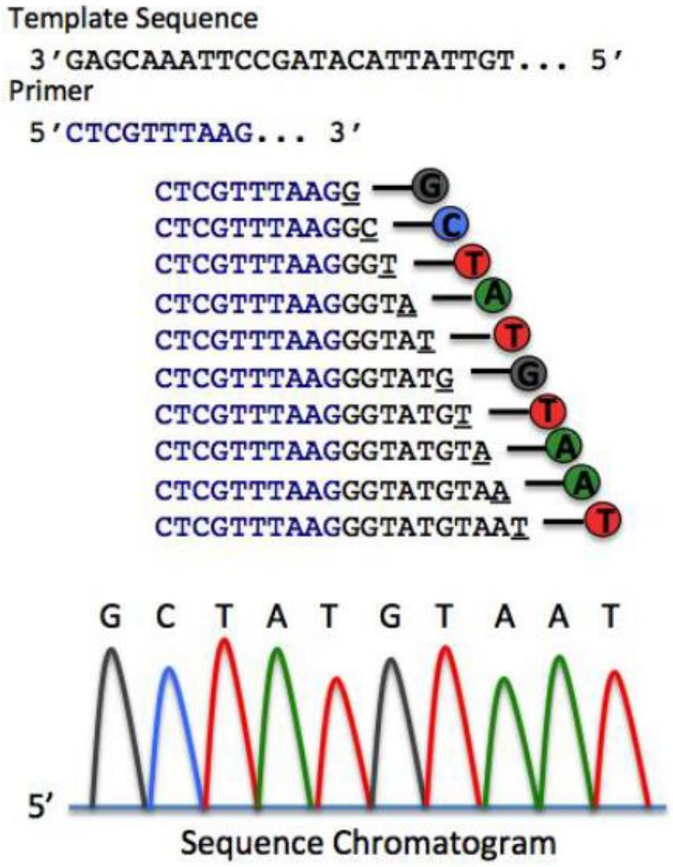
- 1977 Fredrick Sanger developed the first DNA sequencing method: chain termination method

Sanger sequencing dominates the field for three decades

Sanger sequencing: chain termination

DNA synthesis





- Modified DNA replication reaction
- Dideoxynucleoside triphosphates are fluorescently labelled and terminates DNA extension when incorporated
- Resulting DNA fragments are analyzed and sequences are determined by fluorescent signal
- Read-length: ~1000bp, highly accurate
- Poorly parallelizable

McGovern RA 2015, DOI: 10.14288/1.0166738

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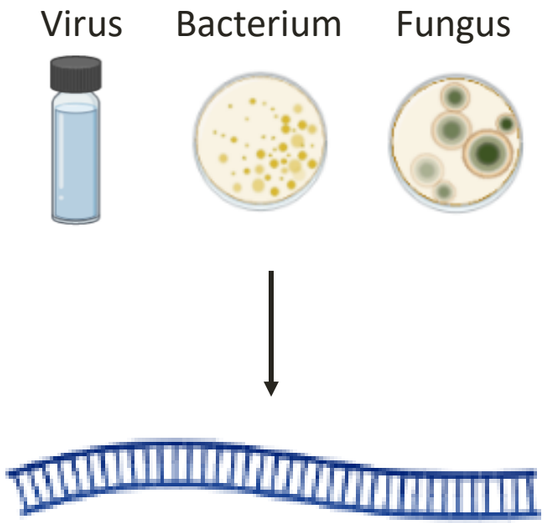
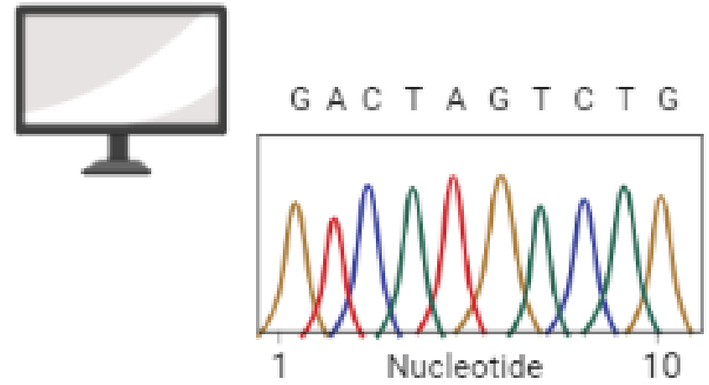
Application: Targeted sequencing

Strengths
- Lowest error rate
- Long read length (~1000bp)

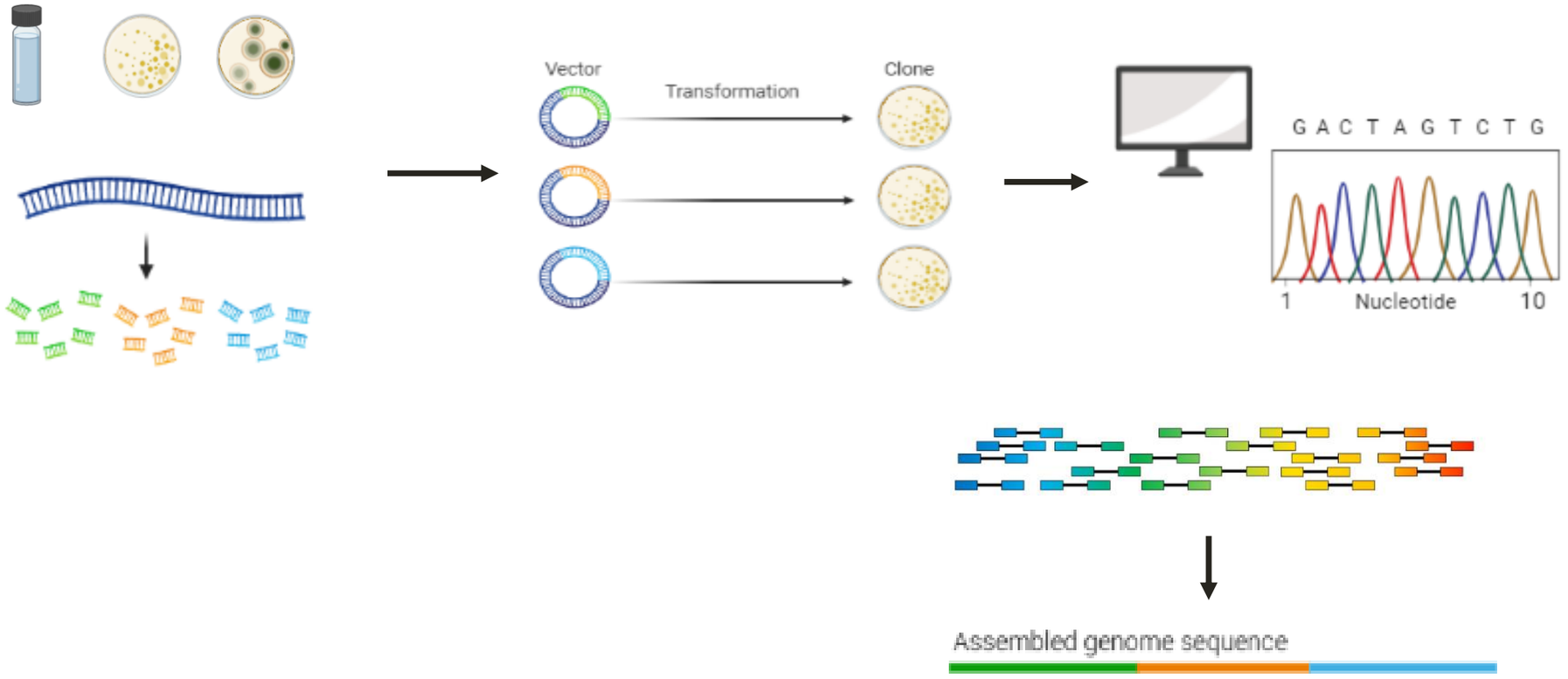
Limitations
- Long run time
- Can’t resolve mixed detections





- **16S ribosomal RNA**
- **HIV polymerase gene**

Application: Whole genome sequencing



Strengths

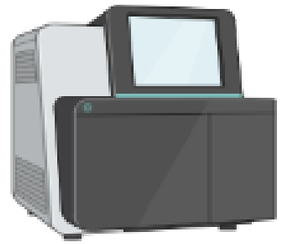
- Lowest error rate

Limitations

- Long run time
- Lower amount of data per run
- High case per base ($0.5 per kilobase)

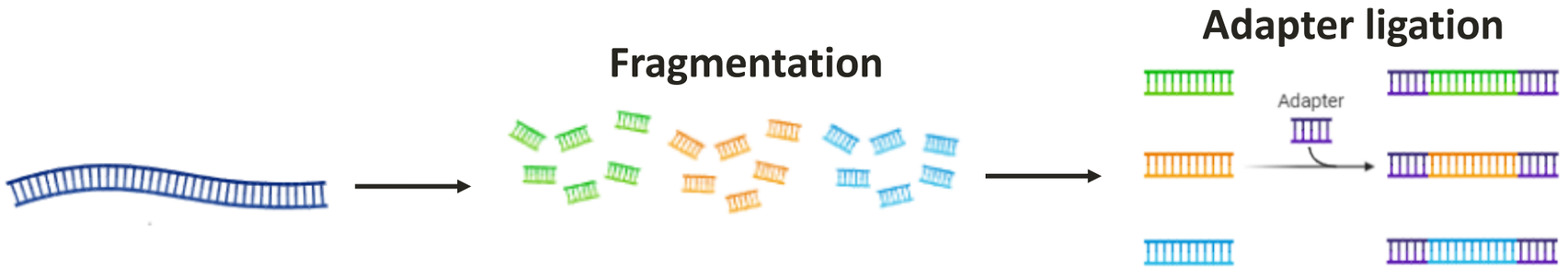
Next generation sequencing (NGS)

- 2005 The 454 system, first NGS platform to come to market
- 2007 Illumina acquired the company Solexa that developed sequencing by synthesis technology and graduate became the NGS platform market leader to this day
- 2007 SOLiD system introduces “sequencing by ligation” to the market
- 2011 Ion Torrent platform introduces “sequencing by synthesis” to the market

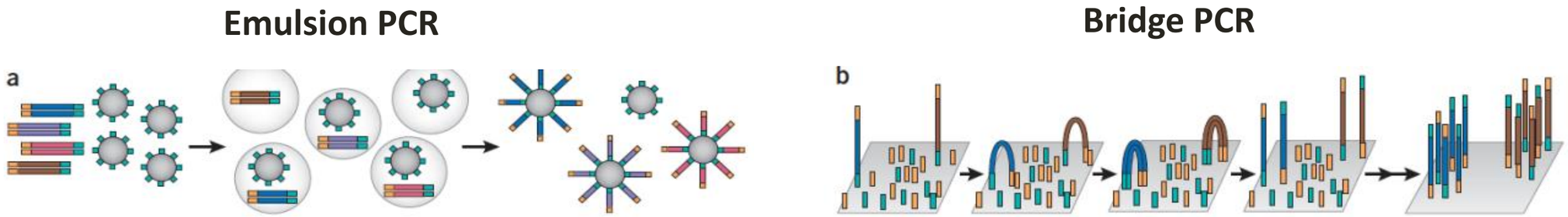


Next generation sequencing: massive parallel sequencing

1. Library preparation



2. Clonal amplification



Leong IUS et al. *Medical Science* 2014
Jay Shendure & Hanlee Ji *Nature biotechnology* 2008

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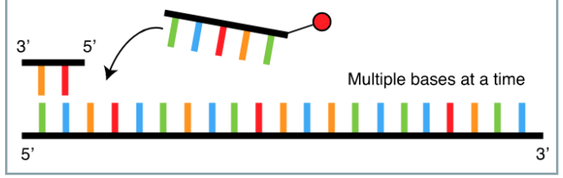
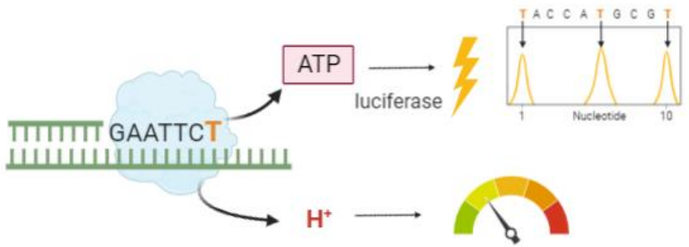
Next generation sequencing: massive parallel sequencing

3. Sequencing and data acquisition

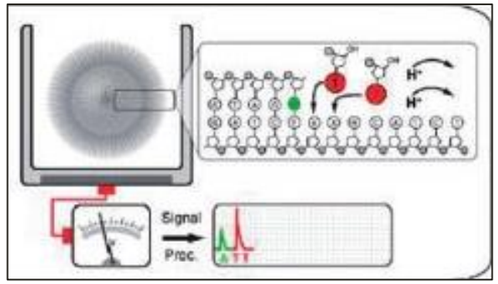
SOLiD platform:
Sequencing by ligation



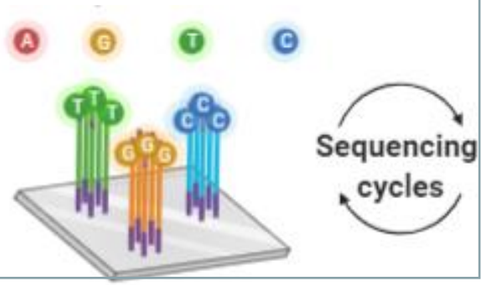
The 454 system:
Pyrosequencing



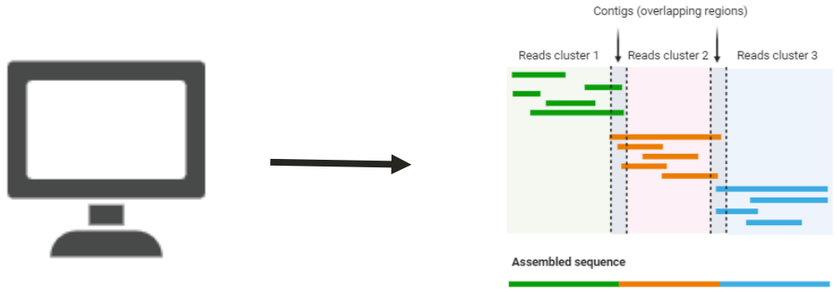
Ion Torrent:
Proton detection
sequencing



Illumina:
Reversible terminator
sequencing

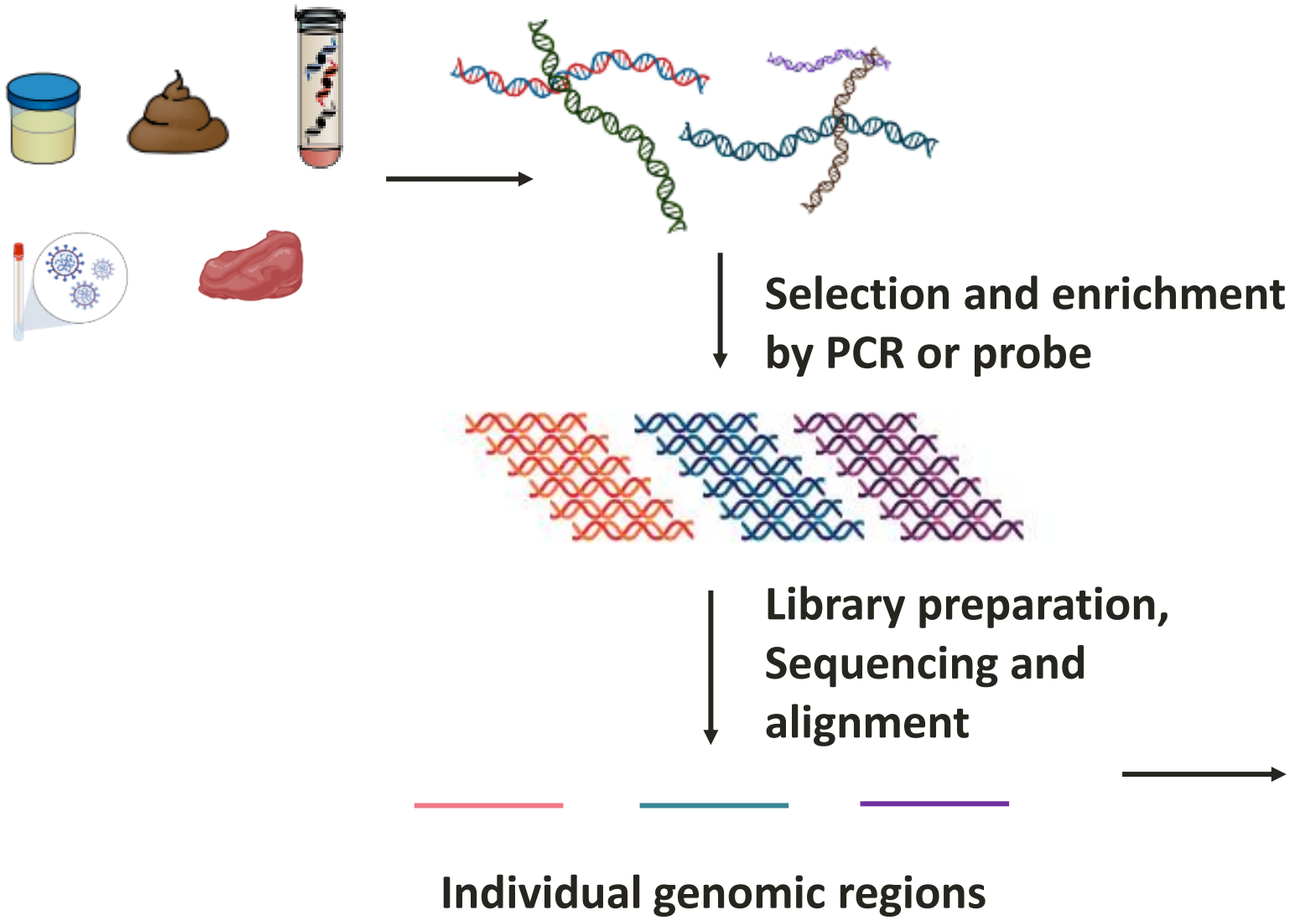


4. Data analysis and assembly



Golan D. and Medvedev P. Bioinformatics 2013

Application: Targeted NGS (tNGS)



Strengths
- Direct from clinical specimen without requirement of isolation
- Can resolve mixed detections
- High sensitivity

Limitations
- Long run time
- Relatively expensive

- Microorganism identification direct from clinical specimen
- Detect viral resistance in clinical sample

Chui CY and Miller SA. *Nature Reviews* 2019

Application: Whole genome NGS

Fragmentation

Sequencing and alignment

Individual genome

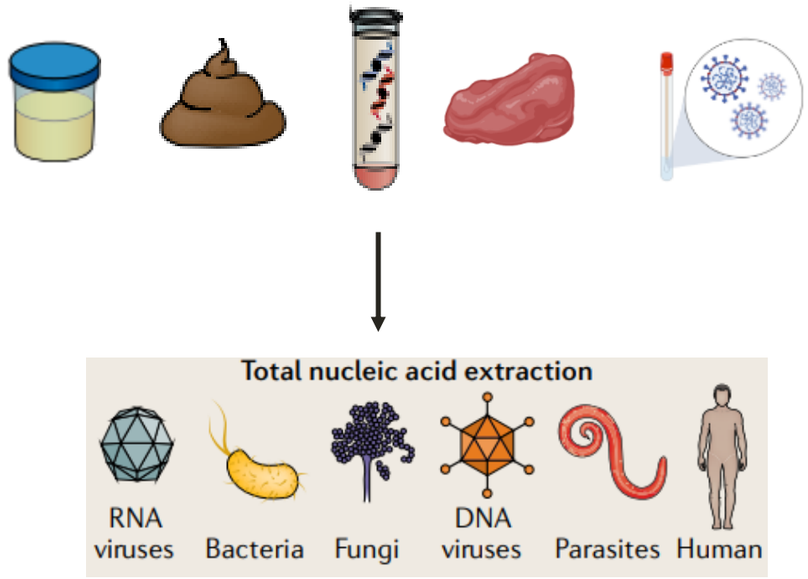
- Novel microorganism identification
- Bacterial typing and viral typing for outbreak investigation
- Antimicrobial resistance and virulence gene

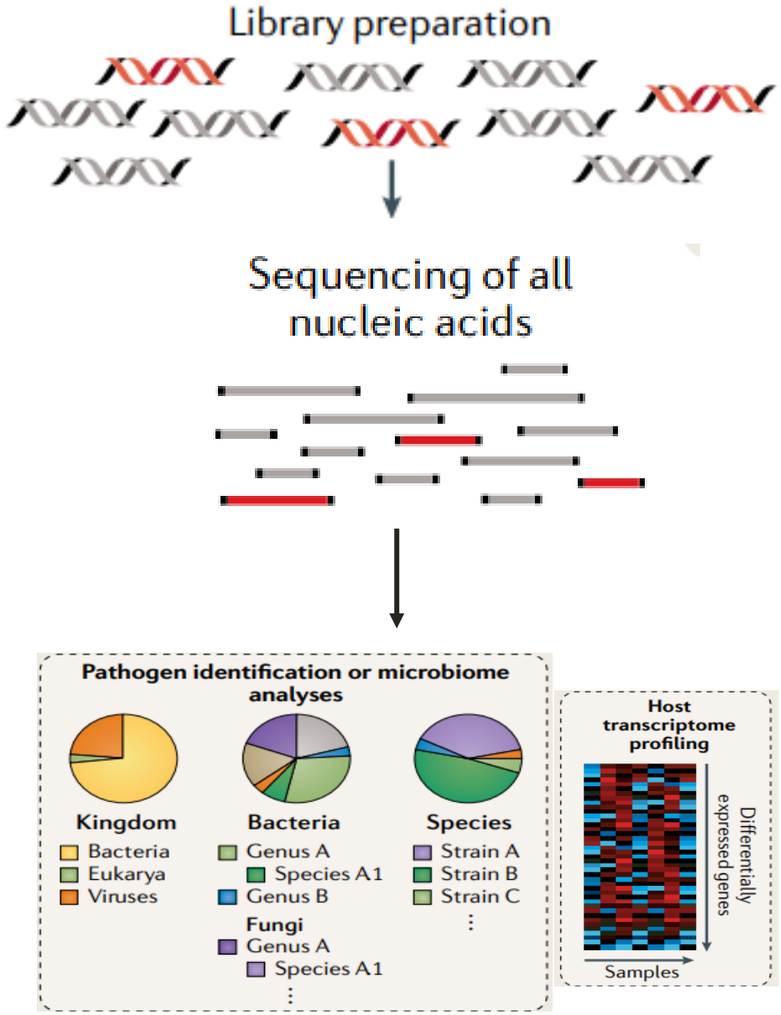
Strengths
- Relatively inexpensive ($2-10 per megabase)
- Relatively quick TAT

Limitations
- Uncultivable organism

Application: Metagenomic NGS (mNGS)

One technology for all





Strengths
- Unbiased detection
- Detects rare pathogens
- Relatively quick turnaround

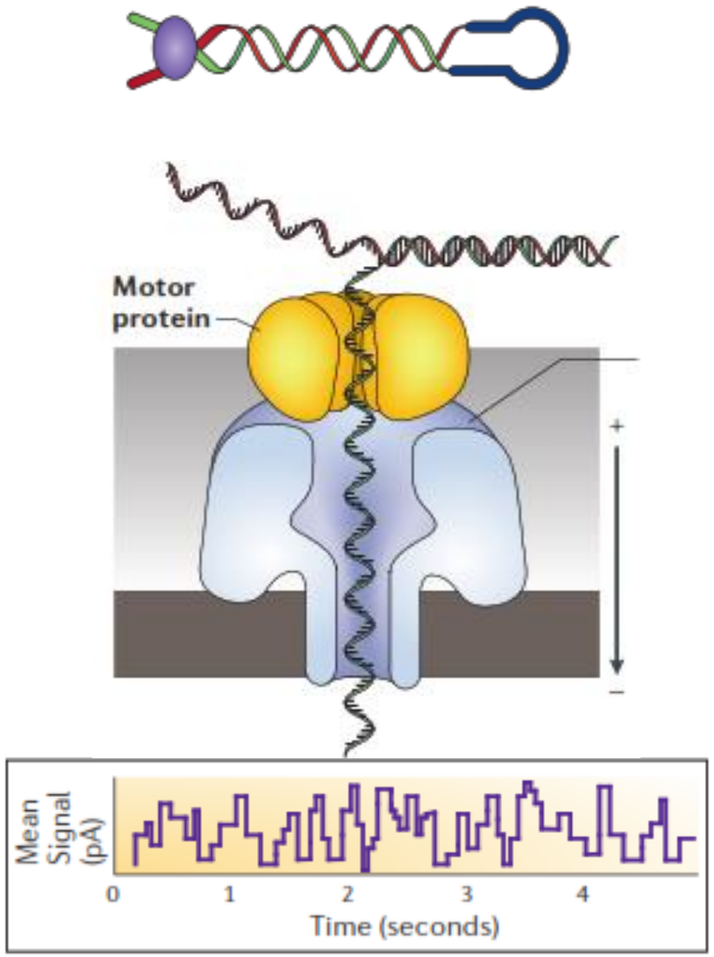
Limitations
- Low sensitivity
- Complicated result interpretation

Chui CY and Miller SA. *Nature Reviews* 2019

Third generation sequencing

- 2011 Pacific Biosciences introduces single molecular sequencing technology
- 2012 Oxford Nanopore technologies launches portable system for RNA and DNA sequencing

Single molecule sequencing



- Single molecules are sequenced. No requirement of DNA amplification
- Long reads: 10kb, allows for the resolution of large structural features
- Real-time base-calling and data assessment

McGovern RA 2015, DOI: 10.14288/1.0166738

Comparison of molecular infectious disease methods

	Real-time PCR	Sanger sequencing	tNGS	mNGS
Prior knowledge of the target	Yes	Yes* bacteria vs. fungus	Yes* bacteria vs. fungus	**No**

Comparison of molecular infectious disease methods

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Comparison of molecular infectious disease methods

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Availability **Turnaround time**	Most clinical labs <8h	Most clinical labs <8h	Large academic/Reference labs 1-7 days	Large academic/Reference labs 1-7 days

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Advantage	• **Quick TAT** • High sensitivity	• **Low error rate** • Long read	• **Highly sensitive** • Detect a group of pathogen simultaneously	• **Unbiased pathogen detection**
Example of clinical application	SA/MRSA PCR	16S rRNA sequencing of unknown isolate	Universal PCR from clinical sample	mNGS Pathogen detection from clinical sample

Available NGS tests for Infectious Disease

FDA-approved

- Sentosa SQ HIV Genotyping Assay
  - Targeted NGS technology to detect HIV drug resistance

Emergency Use Authorization (EUA)

- Clear DX SARS-CoV-2 Test
- Illumina COVIDSeq Test
- SARS-CoV-2 NGS Assay
- UCLA SwabSeq COVID-19 Diagnostic Platform
- Helix COVID-19 NGS Test

Available NGS tests for Infectious Disease

CLIA-certified lab offerings

	Test Name	Sample type	Targeted	Unbiased	Regulatory class
ARUP	Bacterial strain typing	Bacterial isolate		X	Diagnostic LDT
	HIV drug resistance	Blood- plasma	X		
Day Zero Diagnostics	epiXact strain typing	Bacterial isolate	X		Diagnostic LDT
Mayo Clinic Laboratory	Broad range bacterial sequencing	Normally sterile body fluid/tissue	X		Diagnostic LDT
	Bacterial strain typing	Bacterial isolate		X	
	CMV drug resistance	Blood-plasma	X		
	MTBC drug resistance	Bacterial isolate	X		
MicroGenDX	qPCR + NGS DNA^{DX}	Varies	X		Diagnostic LDT
UW Medicine Molecular Microbiology	Broad range PCR + NGS (bacteria, fungi, AFB)	Tissue, non-blood body fluids	X		Diagnostic LDT
	Bacterial strain typing by WGS	Bacterial isolate		X	

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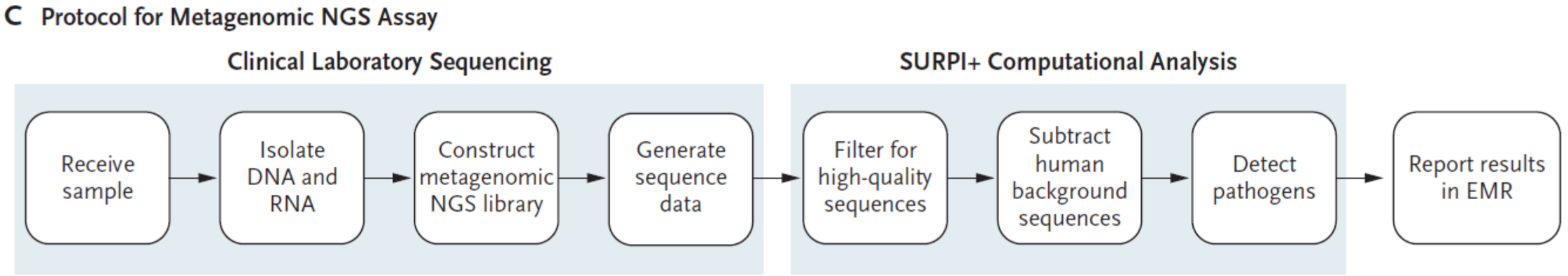
MicroGenDX	qPCR + NGS DNA^{DX}	Varies	X		Diagnostic LDT
UW Medicine Molecular Microbiology	Broad range PCR + NGS (bacteria, fungi, AFB)	Tissue, non-blood body fluids	X		Diagnostic LDT
	Bacterial strain typing by WGS	Bacterial isolate		X	
UCSF	mNGS Pathogen Dx	CSF		**X**	Diagnostic LDT
Karius	The Karius Test	Blood- plasma		**X**	Diagnostic LDT

Performance of UCSF mNGS Pathogen Dx for diagnosis of infectious meningitis and encephalitis

Study Design

- Prospective, multi-center study investigating usefulness of mNGS of CSF for diagnosis of meningitis and encephalitis
- **Inclusion criteria:** idiopathic meningitis, encephalitis, or myelitis without diagnosis at enrollment
- **Reference:** composite reference standard of conventional testing and orthogonal confirmatory testing of mNGS positive only samples



Wilson MR & Sample K et al., *N Engl J Med* 2019;380:2327-40. DOI: 10.1056/NEJMoa1803396

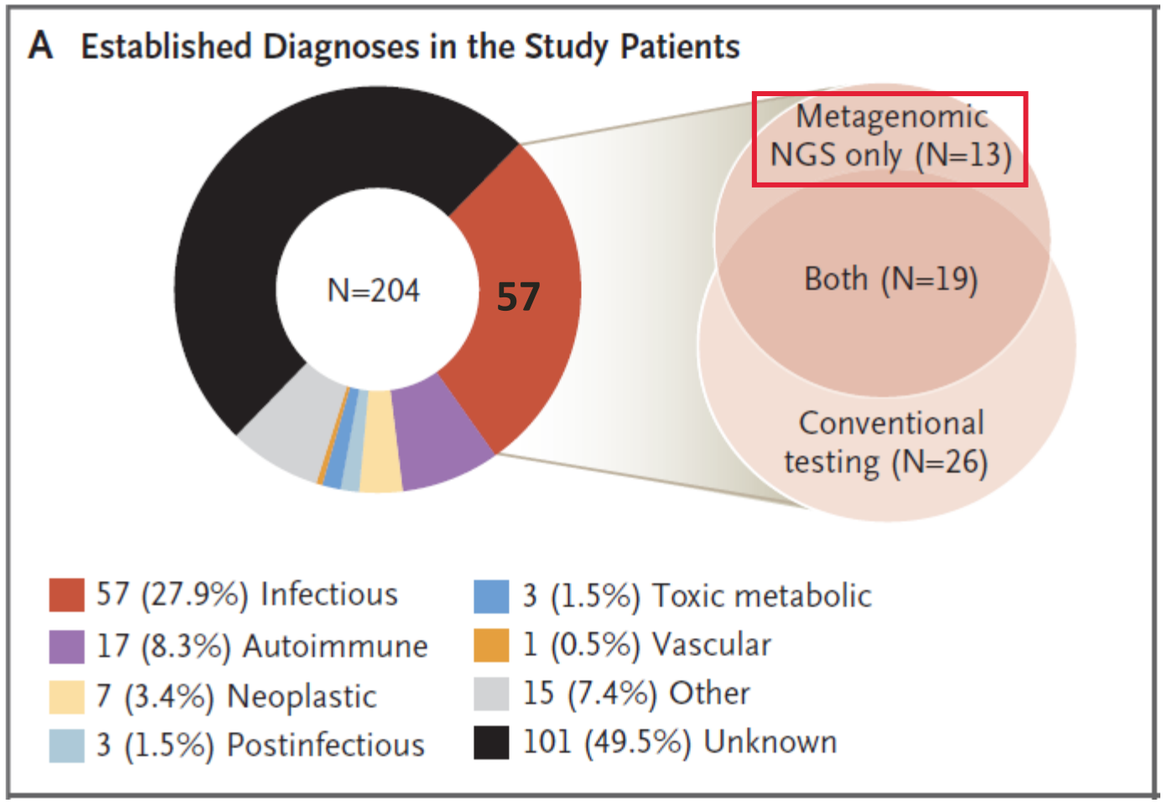
Characteristics of study patients

Table 1. Demographic and Clinical Characteristics of the 204 Patients.*

Characteristic	Value
Age	
Mean — yr	39.6
Distribution — no. (%)	
0–2 yr	5 (2.5)
3–12 yr	25 (12.3)
13–18 yr	16 (7.8)
19–25 yr	17 (8.3)
26–40 yr	40 (19.6)
41–60 yr	53 (26.0)
>60 yr	48 (23.5)
Male sex — no. (%)	114 (55.9)
Syndrome — no. (%)	
Meningitis alone	70 (34.3)
Encephalitis with or without meningitis	130 (63.7)
Myelitis with or without meningitis	4 (2.0)
Exacerbation of chronic condition — no. (%)†	28 (13.7)
Institution — no. (%)	
University of California, San Francisco	110 (53.9)
University of California, Los Angeles	36 (17.6)
University of California, Davis	31 (15.2)
Children's Hospital Los Angeles	8 (3.9)
Zuckerberg San Francisco General Hospital	8 (3.9)
Children's Hospital Colorado	6 (2.9)
St. Jude Children's Research Hospital	3 (1.5)
Children's National Medical Center	2 (1.0)
Immunocompromised — no. (%)	83 (40.7)
HIV-1	21 (10.3)
Solid-organ transplant	14 (6.9)
Bone marrow transplant	13 (6.4)
Chemotherapy	14 (6.9)
Immunosuppression for non-neoplastic condition	14 (6.9)
Congenital condition	3 (1.5)
Other	4 (2.0)
Existing CNS hardware — no. (%)‡	27 (13.2)
ICU admission — no. (%)	99 (48.5)
Death within 30 days — no. (%)	23 (11.3)
Mean Karnofsky performance-status score at time of discharge§	64.6
Mean length of stay (range) — days	
In hospital	27.9 (1–246)
In ICU¶	17.8 (1–71)
Percentage of hospitalization time spent in ICU¶	32.2
Median no. of days after hospital admission that CSF was collected for metagenomic NGS (range) — days‖	3.0 (0–219)

Wilson MR & Sample K et al., *N Engl J Med* 2019;380:2327-40. DOI: 10.1056/NEJMoa1803396

mNGS detections in confirmed CNS infections



mNGS only detections—22.8%, 13/57

- *Candida tropicalis*
- EBV
- Echovirus 6
- Echovirus 30
- *Enterovirus aerogenes*
- *Enterococcus faecalis*
- Hepatitis E Virus
- MW polyomavirus
- *Neisseira meningitidis*
- *Nocardia farcinica*
- Saint Louis Encephalitis Virus
- *Streptococcus agalactiae*
- *Streptococcus mitis*

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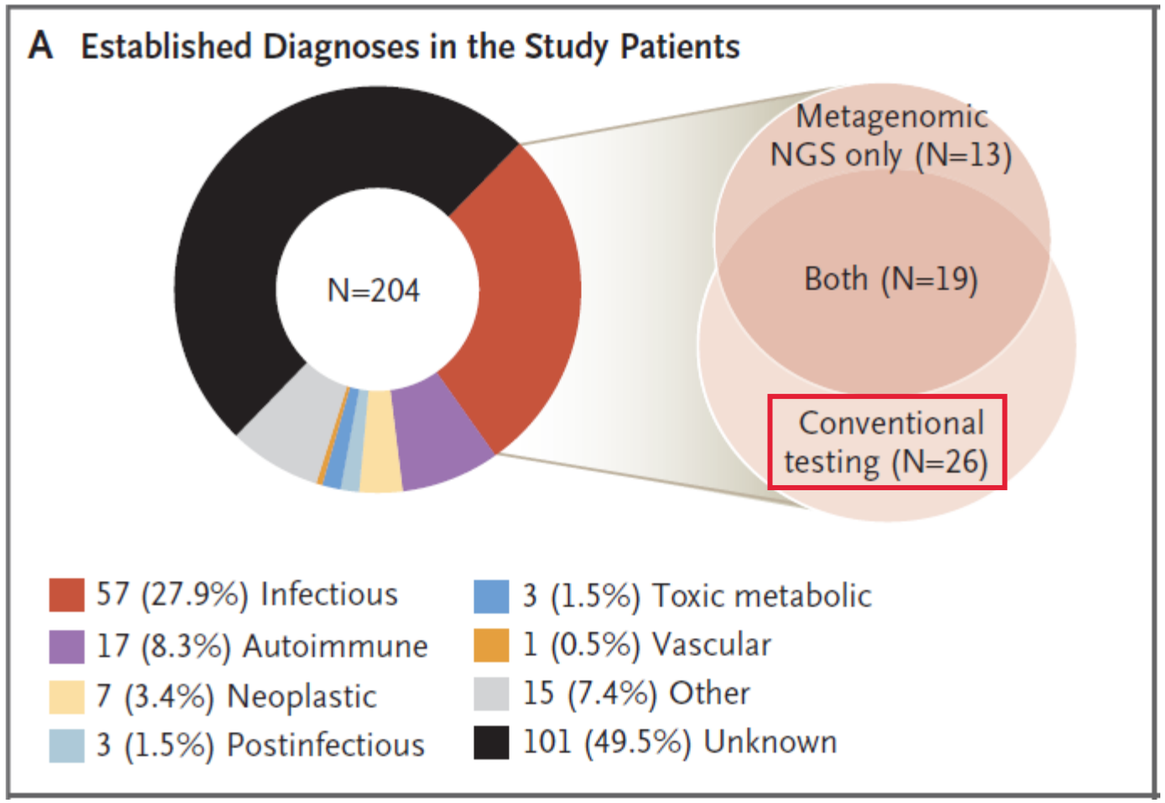
Wilson MR & Sample K et al., *N Engl J Med* 2019;380:2327-40. DOI: 10.1056/NEJMoa1803396

mNGS missed CNS infections



mNGS missed detections—45.6%, 26/57

Serology

- *Baylisascaris procyonis*
- Dengue virus
- *Treponema pallidum* (x2)
- WNV (x4)
- VZV (x3)

Non-CSF sample

- *Aspergillus* sp.
- *Bacillus cereus*
- *Fusobacterium* sp.
- *Mucor* sp.
- Polymicrobial empyema

Low-level pathogen

- CMV
- *Cryptococcus neoformans*
- *Fusobacterium* sp.
- HSV-2
- *Mycobacterium bovis*
- *Mycobacterium tuberculosis*
- *Cutibacterium acnes*
- *Staphylococcus aureus*

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Wilson MR & Sample K et al., *N Engl J Med* 2019;380:2327-40. DOI: 10.1056/NEJMoa1803396

Strengths and Limitations of mNGS of CSF for diagnosis of meningitis and encephalitis

Strengths

- High specificity of CSF mNGS detections
- Identifies organisms not previously considered

Limitations

- CSF mNGS does not replace conventional testing
  - Infections normally detected by serology often missed by mNGS (WNV, VZV, neurosyphillis)
- High levels of host DNA in CSF can interfere with mNGS pathogen detection
- Low-levels of pathogen can reduce sensitivity of CSF mNGS

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Wilson MR & Sample K et al., *N Engl J Med* 2019;380:2327-40. DOI: 10.1056/NEJMoa1803396

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

Cell-free DNA sequencing

- Fragments of genomic DNA from pathogens causing infections at various locations can be detected in purified plasma cell free DNA (cfDNA)
- Promise of non-invasive sampling for detection of deep-seated infections within rapid TAT, even with pre-treatment
- mNGS facilitates detection of >1,000 pathogens

The Karius test workflow

Blauwkamp TA & Thair S, et al., 2019. *Nature Microbiology* 4:663–674. DOI: 10.1038/s41564-018-0349-6

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Clinical validation of a microbial cell-free DNA sequencing (Karius) test for infectious disease

Study Design

- Prospective clinical trial to determine etiology of sepsis using infectious disease diagnostic sequencing assay
- **Inclusion criteria:** Adult patients, presenting to Stanford University Hospital Emergency Department with 2/4 sepsis criteria
- **Reference:** 1) initial blood culture 2) all microbiological testing 3) composite reference standard with clinical adjudication of Karius pathogen only
- **Primary outcome measure:** Accuracy of sequencing assay in diagnosing etiology of sepsis within 7 days

Blauwkamp TA & Thair S, et al., 2019. *Nature Microbiology* 4:663–674. DOI: 10.1038/s41564-018-0349-6

Characteristics of study patients

Characteristic	Data (N=350)
Age, median (range), years	54 (18-97)
Sex, n (%)	
Male	179 (51.1)
Female	171 (48.9)
Race, n (%)	
White	197 (56.3)
Asian	74 (21.1)
Black or African American	15 (4.3)
Native Hawaiian or other Pacific Islander	7 (2)
American Indian or Alaskan Native	1 (0.3)
Not reported	55 (15.7)
Medical Comorbidities, n (%)	
≥ 1 concurrent chronic medical condition	227 (64.9)
Hypertension	97 (27.7)
Diabetes mellitus	61 (17.4)
Chronic heart disease	54 (15.4)
Hyperlipidemia	53 (15.1)
Lenght of Hospital Stay	
Mean length of stay in days, n (range)	4.7 (1-117)
Median length of stay in days, n (IQR)	3 (1-5)
Hospitalization Survival Status, n (%)	
Discharged	346 (98.9)
Died	4 (1.1)
Antimicrobial treatment[1] within 2 weeks of sepsis alert	97 (27.7)

Blauwkamp TA & Thair S, et al., 2019. *Nature Microbiology* 4:663–674. DOI: 10.1038/s41564-018-0349-6

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Clinical performance of the Karius test

Compared to blood culture—PPA: 93.7% (84.5-98.2), NPA: 40% (34.3- 45.9)

	Blood culture positive	Blood culture negative
Karius positive	59	**171**
Karius negative	4	114

Compared to all microbiology testing (SOC)—PPA: 84.8% (77.6-90.5), NPA: 48.2% (44.3-55.0)

	SOC positive	SOC negative
Karius positive	112	**112**
Karius negative	20	104

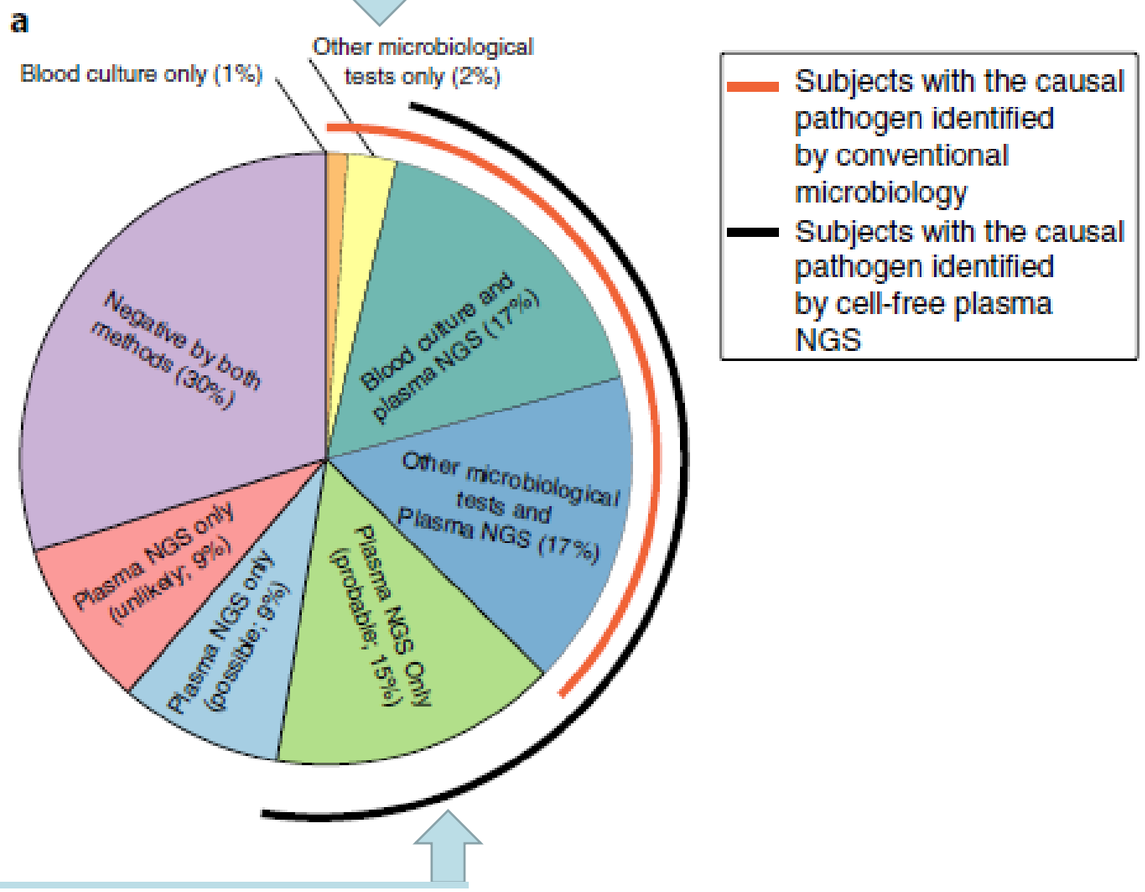
Compared to composite reference standard (CRS) —PPA: 92.9% (88.1-96.1), NPA: 62.7% (54.8-70.0)

	CRS positive	CRS negative
Karius positive	169	**62**
Karius negative	13	104

Blauwkamp TA & Thair S, et al., 2019. *Nature Microbiology* 4:663–674. DOI: 10.1038/s41564-018-0349-6

Clinical performance of the Karius test

False negative Karius



Adjudicated True positive

Blauwkamp TA & Thair S, et al., 2019. *Nature Microbiology* 4:663–674. DOI: 10.1038/s41564-018-0349-6

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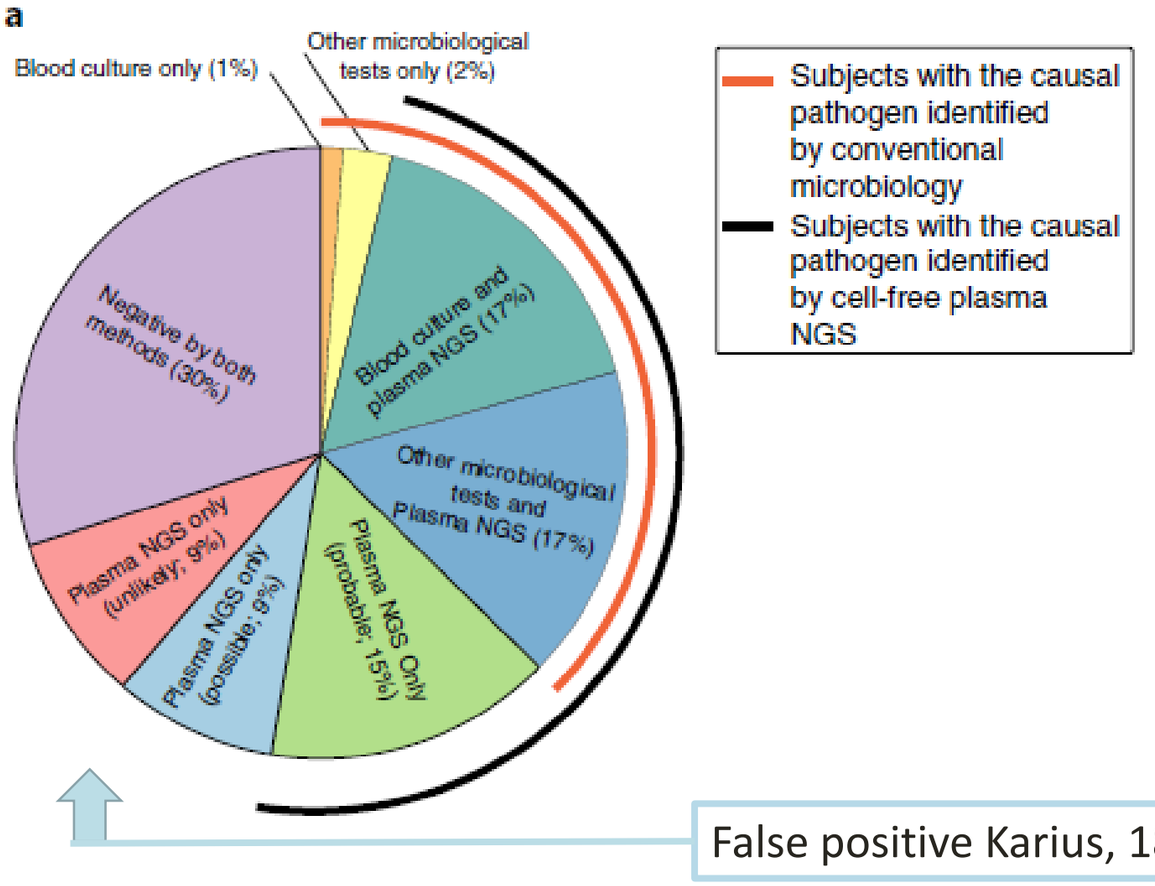
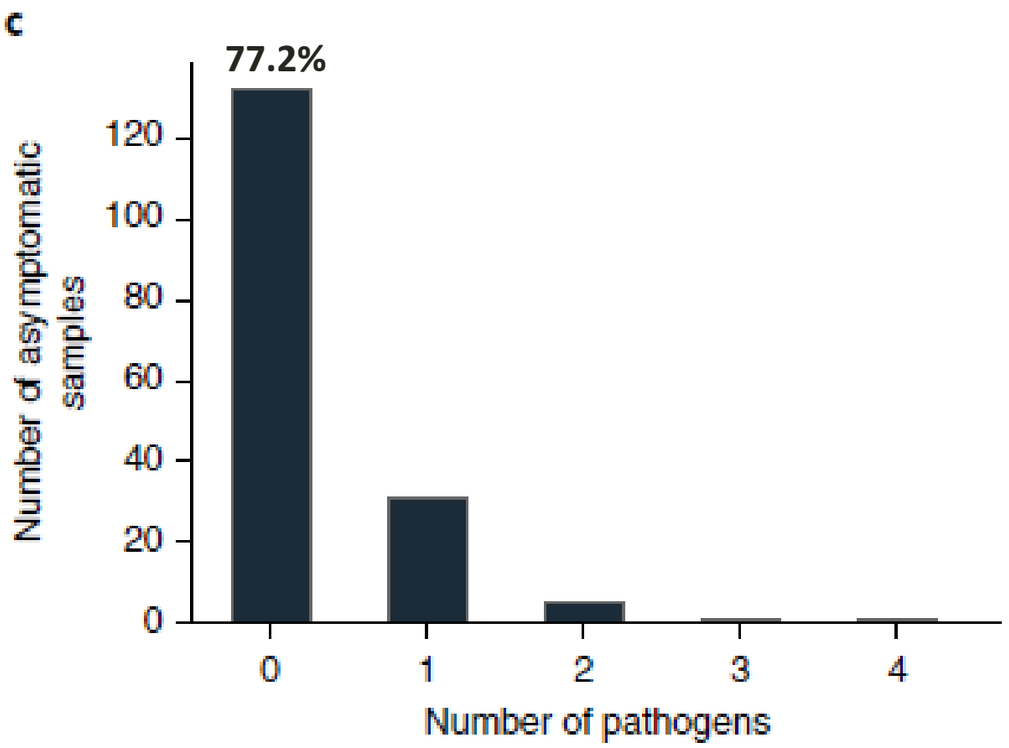
Clinical performance of the Karius test



False positive Karius, 18%

22.8% Karius detection in asymptomatic donors



Blauwkamp TA & Thair S, et al., 2019. *Nature Microbiology* 4:663–674. DOI: 10.1038/s41564-018-0349-6

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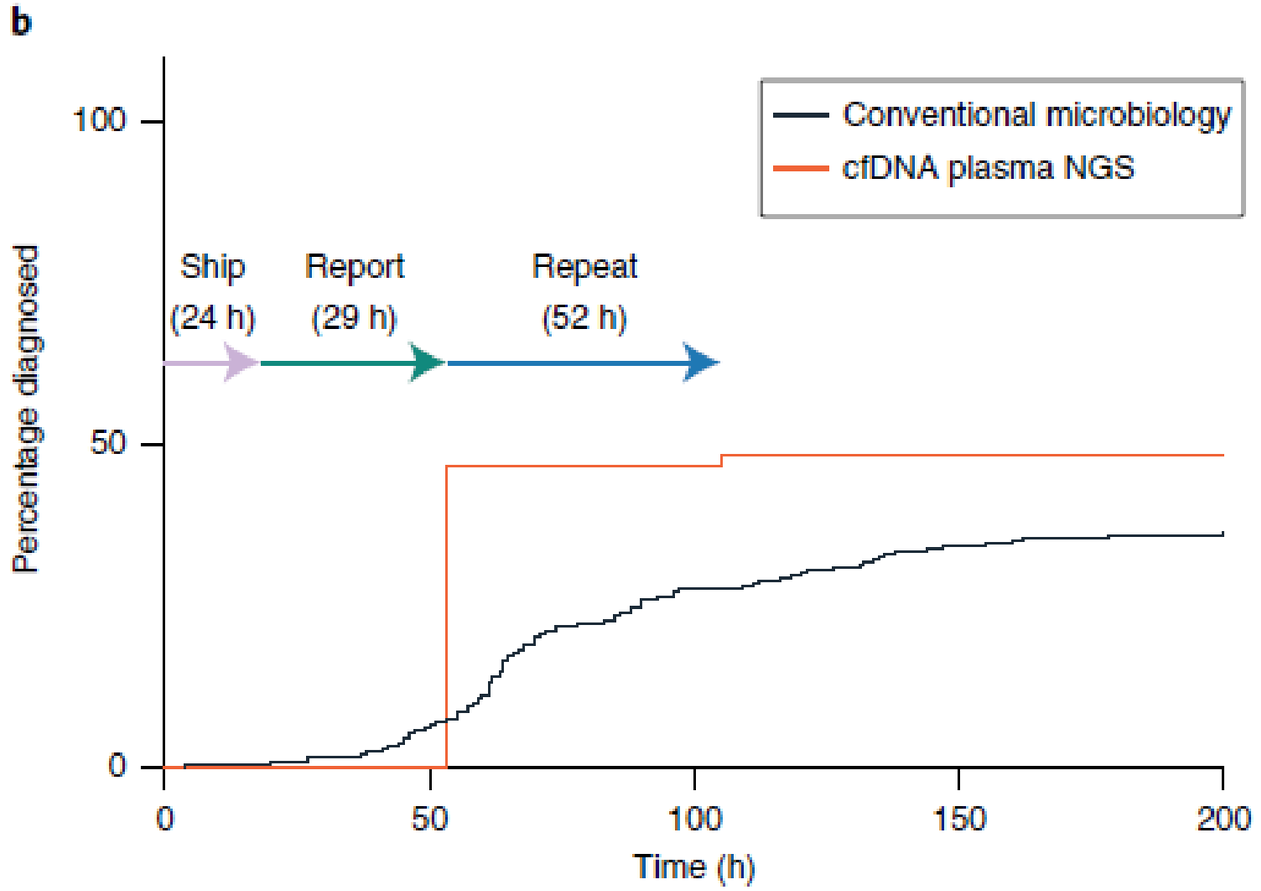
Karius test results are available within 3 days



Blauwkamp TA & Thair S, et al., 2019. *Nature Microbiology* 4:663–674. DOI: 10.1038/s41564-018-0349-6

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Strengths and Limitations of the Karius test

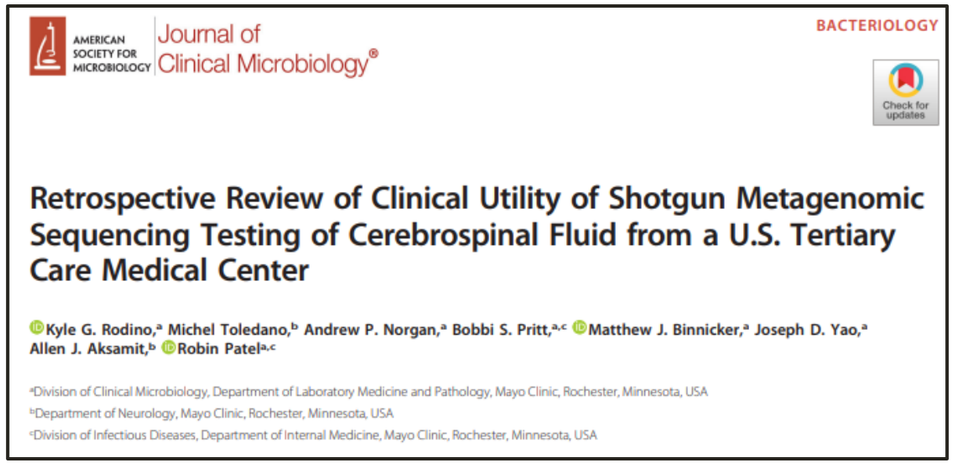
Strengths

- Rapid turnaround time
- High concordance with initial blood culture results

Limitations

- Low specificity (Karius only detections)
- Multiple detections can confound interpretation
- Susceptibility information not provided

Clinical impact and utility of mNGS in routine practice



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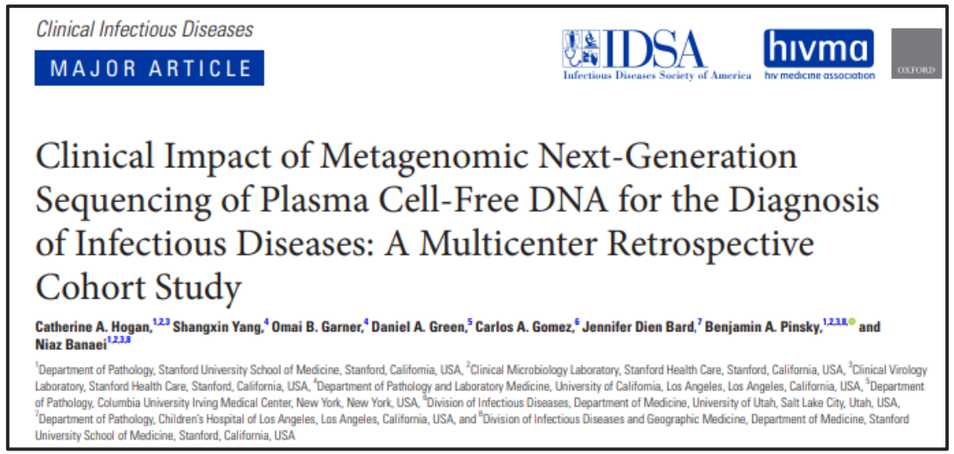
Retrospective Review of Clinical Utility of Shotgun Metagenomic Sequencing Testing of Cerebrospinal Fluid from a U.S. Tertiary Care Medical Center

Kyle G. Rodino,[a] Michel Toledano,[b] Andrew P. Norgan,[a] Bobbi S. Pritt,[a,c] Matthew J. Binnicker,[a] Joseph D. Yao,[a] Allen J. Aksamit,[b] Robin Patel[a,c]

[a]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA
[b]Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA
[c]Division of Infectious Diseases, Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota, USA

- Single center retrospective review of 80 cases submitted for CSF mNGS
  - 15% (12/80) positive result rate
  - 58% (7/12) interpreted as inconsistent with clinical presentation
  - 4% (2/53) altered patient management



Clinical Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America | hivma hiv medicine association | OXFORD

Clinical Impact of Metagenomic Next-Generation Sequencing of Plasma Cell-Free DNA for the Diagnosis of Infectious Diseases: A Multicenter Retrospective Cohort Study

Catherine A. Hogan,[1,2,3] Shangxin Yang,[4] Omai B. Garner,[4] Daniel A. Green,[5] Carlos A. Gomez,[6] Jennifer Dien Bard,[7] Benjamin A. Pinsky,[1,2,3,8] and Niaz Banaei[1,2,3,8]

[1]Department of Pathology, Stanford University School of Medicine, Stanford, California, USA, [2]Clinical Microbiology Laboratory, Stanford Health Care, Stanford, California, USA, [3]Clinical Virology Laboratory, Stanford Health Care, Stanford, California, USA, [4]Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, California, USA, [5]Department of Pathology, Columbia University Irving Medical Center, New York, New York, USA, [6]Division of Infectious Diseases, Department of Medicine, University of Utah, Salt Lake City, Utah, USA, [7]Department of Pathology, Children's Hospital of Los Angeles, Los Angeles, California, USA, and [8]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA

- Multicenter retrospective review of the clinical impact of 82 consecutive cases submitted for plasma cell-free mNGS (Karius)
  - 61% (50/82) positive result rate
  - 7.3% (6/82) positive clinical impact
  - 3.7% (3/82) negative clinical impact
  - 32.9% (27/82) diagnosis pre-established from conventional testing

Rodino KG, et al. 2020. Journal of Clinical Microbiology https://doi.org/10.1128/JCM.01729-20.
Hogan CA, et al. 2020. Clin Infect Dis https://doi.org/10.1093/cid/ciaa035.

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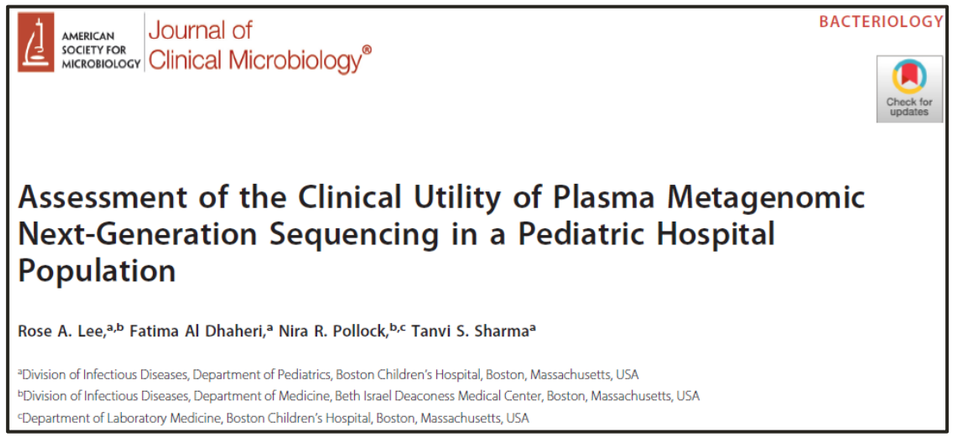
Clinical impact and utility of Karius in pediatric patients



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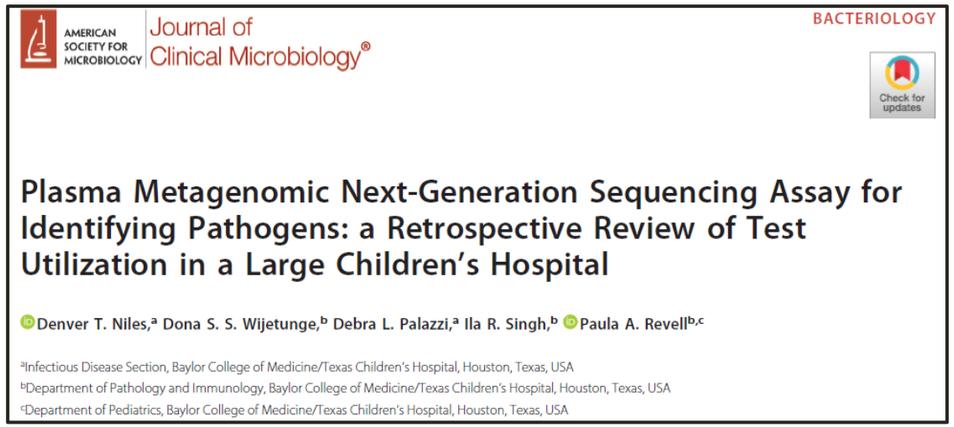
Check for updates

Assessment of the Clinical Utility of Plasma Metagenomic Next-Generation Sequencing in a Pediatric Hospital Population

Rose A. Lee,[a,b] Fatima Al Dhaheri,[a] Nira R. Pollock,[b,c] Tanvi S. Sharma[a]

[a]Division of Infectious Diseases, Department of Pediatrics, Boston Children's Hospital, Boston, Massachusetts, USA
[b]Division of Infectious Diseases, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
[c]Department of Laboratory Medicine, Boston Children's Hospital, Boston, Massachusetts, USA

- Single-center retrospective review of 59 cases submitted for Karius
  - 49% (29/59) positive result rate
  - 55% (28/51) clinically-relevant organisms
  - 14% impacted clinical management
  - 50% true negative agreement



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Check for updates

Plasma Metagenomic Next-Generation Sequencing Assay for Identifying Pathogens: a Retrospective Review of Test Utilization in a Large Children's Hospital

Denver T. Niles,[a] Dona S. S. Wijetunge,[b] Debra L. Palazzi,[a] Ila R. Singh,[b] Paula A. Revell[b,c]

[a]Infectious Disease Section, Baylor College of Medicine/Texas Children's Hospital, Houston, Texas, USA
[b]Department of Pathology and Immunology, Baylor College of Medicine/Texas Children's Hospital, Houston, Texas, USA
[c]Department of Pediatrics, Baylor College of Medicine/Texas Children's Hospital, Houston, Texas, USA

- Single-center retrospective review of 60 cases submitted for Karius
  - 63% (38/60) positive result rate
  - 26% (6/23) change in antimicrobial therapy
  - 73% of cases with positive agreement reported conventional testing earlier than Karius

Lee RA, et al. 2020. Journal of Clinical Microbiology https://doi.org/10.1128/JCM.00419-20.
Niles DT et al. 2020. Journal of Clinical Microbiology https://doi.org/10.1128/JCM.00794-20.

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Should everyone get mNGS sequencing?

NO!

Which patients benefit most from testing?

- Confirmed infectious process
- Previously negative SOC testing
  - Pre-treatment with antimicrobials
  - Deep-seated, difficult to sample infections
- Immunocompromised with high risk of infection

If mNGS is indicated:

- Also consider targeted NGS at affected sites
- Interpret results with caution!

Recommendations for test utilization

- Appropriate use criteria are actively being evaluated
- Restricted access to test ordering
  - Require Infectious Diseases consult/approval
  - Microbiology lab director approval
- Interpretation with experts
  - NGS review boards
  - Multidisciplinary team
    - Infectious disease consultants
    - Microbiology lab directors
    - Testing lab

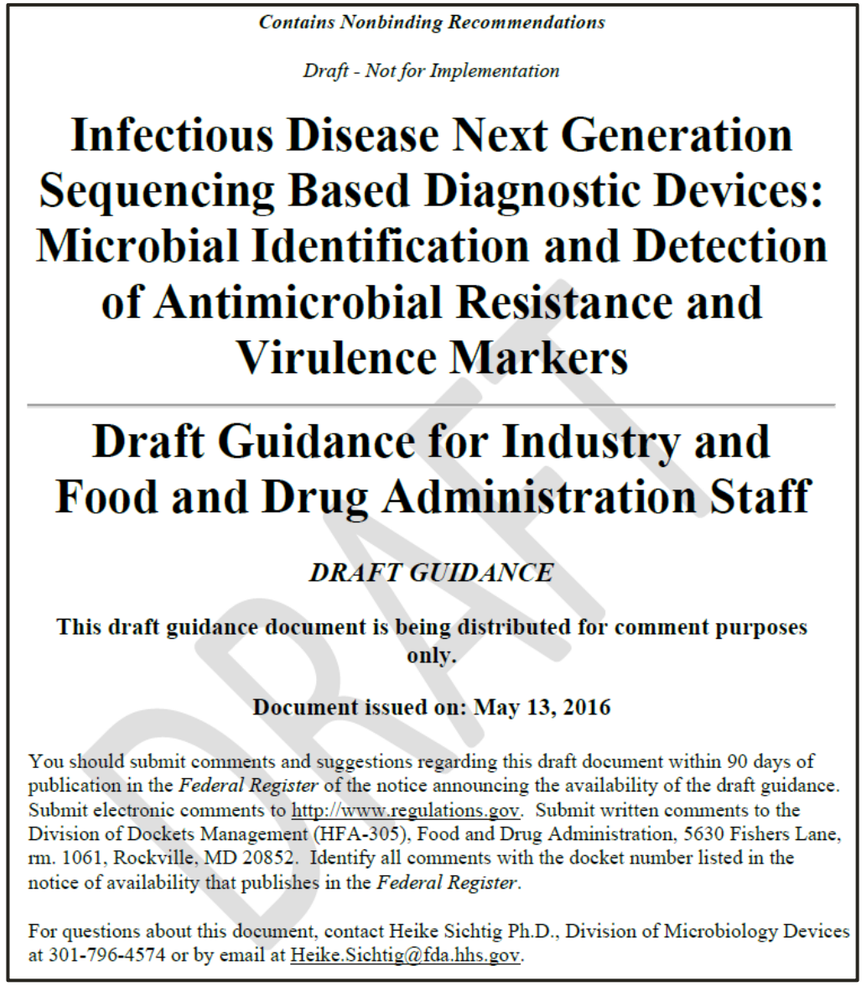
Babady NE. 2021. Clin Infect Dis 72:246–248.

In summary

- NGS technology:
  - Sequencing continues to rapidly evolve
  - More accurate, affordable and timely
- Advantages of Infectious Diseases NGS Dx:
  - Does not require prior suspicion
  - Identify pathogens not detected by routine testing
  - Generate large scale data in shorter turn around time
- Limitations of Infectious Diseases NGS Dx:
  - Not a standalone test
  - False positive detections of unclear significance
  - Still a reference lab test- requires specialized equipment and expertise, relatively expensive

The future of NGS for infectious disease

- As technology improves, cost and time for NGS analysis will continue to decline
- More NGS based testing in molecular microbiology
  - Only available large academic medical centers
  - Combined computer science and microbiology expertise
- Pathway to FDA-clearance/approval
- Studies establishing best practices for interpretation and utilization



Contains Nonbinding Recommendations

Draft - Not for Implementation

Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

Document issued on: May 13, 2016

You should submit comments and suggestions regarding this draft document within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions about this document, contact Heike Sichtig Ph.D., Division of Microbiology Devices at 301-796-4574 or by email at Heike.Sichtig@fda.hhs.gov.

DRAFT

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/infectious-disease-next-generation-sequencing-based-diagnostic-devices-microbial-identification-and

Thank you