Metagenomics Next-Generation Sequencing (mNGS) in Transplant Infectious Disease Diagnostics

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Disclosures

1. Consultant fees from Karius Diagnostics
2. Research contracts (paid to institution)
    1. Symbio pharmaceuticals
    2. EAGLE pharmaceuticals

Objectives

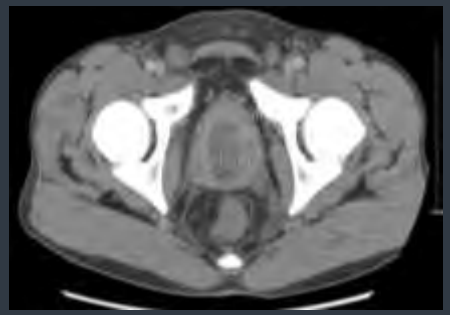
- Explain the Role of mNGS and its integration with conventional microbiology workflows in transplant patients.
- Address Diagnostic Challenges – Discuss limitations, test interpretation pitfalls (e.g., false positives), and barriers to widespread clinical implementation
- Assess Clinical Impact & Future Directions

Clinical Case 1

- Liver transplant recipient, admitted for persistent fevers
- Conventional testing and CT negative
- Hx of recent trip to Colorado (cabin)
- At 48 hrs, persist febrile, clinical deterioration
- Inflammatory markers trending up: Ferritin, CRP, thrombocytopenia
- Suspicion for HLH, No identifiable ID trigger.

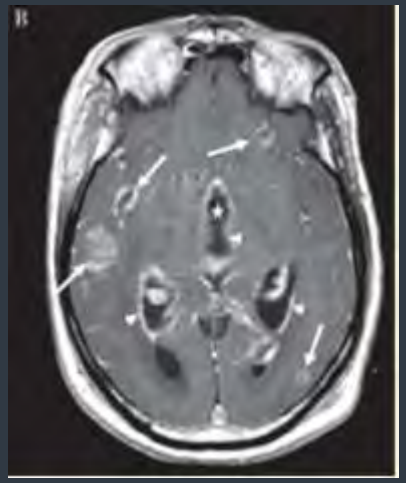
Plasma mNGS test ordered

Clinical Case 2



- Heart transplant males, > 20 yrs ago, stable IS, no recent urologic procedures, presenting with perirectal pain and prostatic abscess
- Blood and urine cx: negative 48 hrs
- Abscess fluid from TURP: Not collected for micro!
- Broad-spectrum antibiotics (TID wants to de-escalate and discharge)

Clinical Case 3



- Kidney transplant recipient (12 –months post transplant)
- Encephalopathy, fevers, pleuritic chest pain
- MRI: multiple supratentorial abscess-like lesions
- Impossibility for brain or lung biopsy
- Blood cx, fungal biomarkers, all negative
- CSF: not diagnostic (culture negative at 2 days, ME PCR panel negative
- Patient now on Broad-spectrum antibiotics and Ambisome

CSF mNGS testing requested

Let’s start with definitions first!

- **Metagenomics:** The study of genetic material recovered directly from environmental or clinical samples using NGS, providing a comprehensive analysis of microbial communities, their diversity, and functional potential in health and disease.

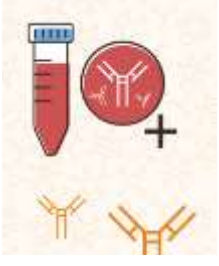
- **Next-Generation Sequencing (NGS):** A high-throughput sequencing technology that enables the rapid and parallel sequencing of millions of DNA or RNA fragments, allowing for comprehensive genomic analysis of pathogens and host responses.

- **Unbiased NGS:** A sequencing approach that does not require prior knowledge of the pathogen, enabling the simultaneous detection of all potential microbes (bacteria, viruses, fungi, and parasites) in a clinical sample, without specific primers or probes.

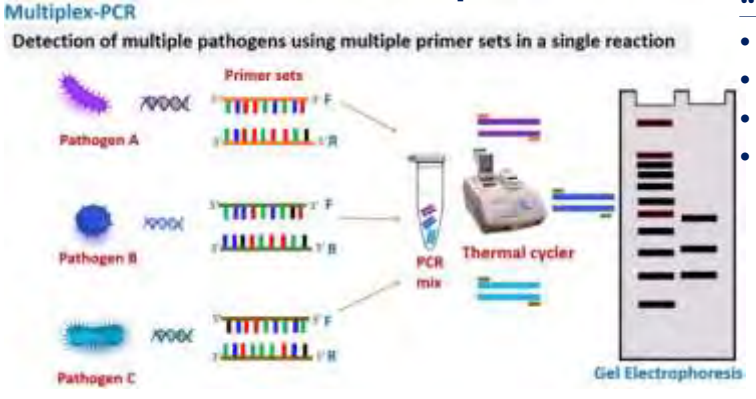
Current Landscape of Microbiology Testing

Cultures

Serology Antigen test



Targeted or Multiplex PCR



“Syndromic Panels”:

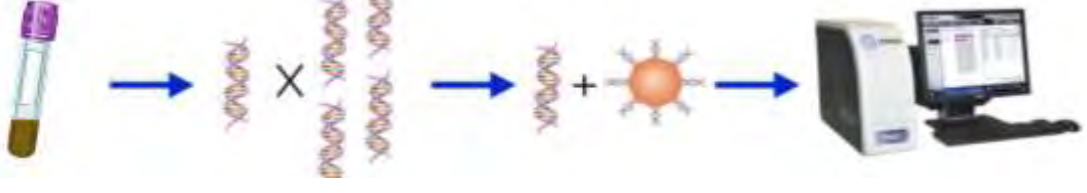
- Respiratory
- Pneumonia
- GI
- ME

Rapid Molecular tests (eg, flu or Crypto Ag)

Point-of-care tests / lateral flow assay
Specific quantitative PCR

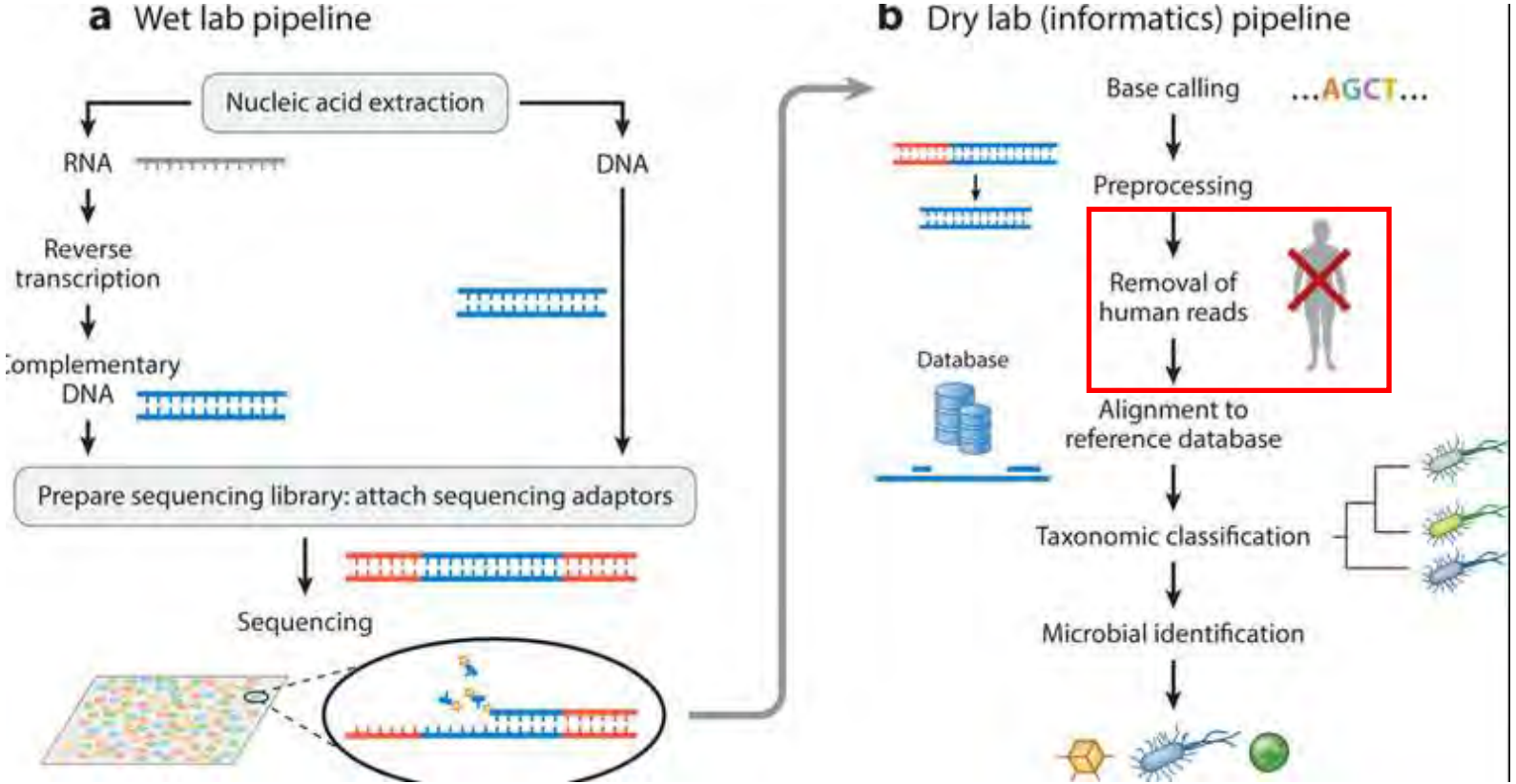
Unbiased mNGS

Sample



Pathogen ID

mNGS


a Wet lab pipeline
Nucleic acid extraction
RNA
DNA
Reverse transcription
Complementary DNA
Prepare sequencing library: attach sequencing adaptors
Sequencing
b Dry lab (informatics) pipeline
Base calling
...AGCT...
Preprocessing
Removal of human reads
Database
Alignment to reference database
Taxonomic classification
Microbial identification

Applications of mNGS in ID

1. Unbiased pathogen detection
   - Bacteria, viruses, fungi, parasites
   - No need for culture or targeted PCR (primers)
2. Diagnosis of culture-negative infections
   - Fastidious or difficult to culture pathogens
   - IE, FUO, meningitis, deep-seated infections
3. Pathogenesis discovery via Microbiome characterization
   - GI microbiome
4. Outbreak surveillance and epidemiology/Antimicrobial resistant prediction
   - whole-genome sequencing (WBS), can guide antimicrobial therapy and infection control

mNGS in Clinical Grounds

- Plasma
- CSF
- BAL

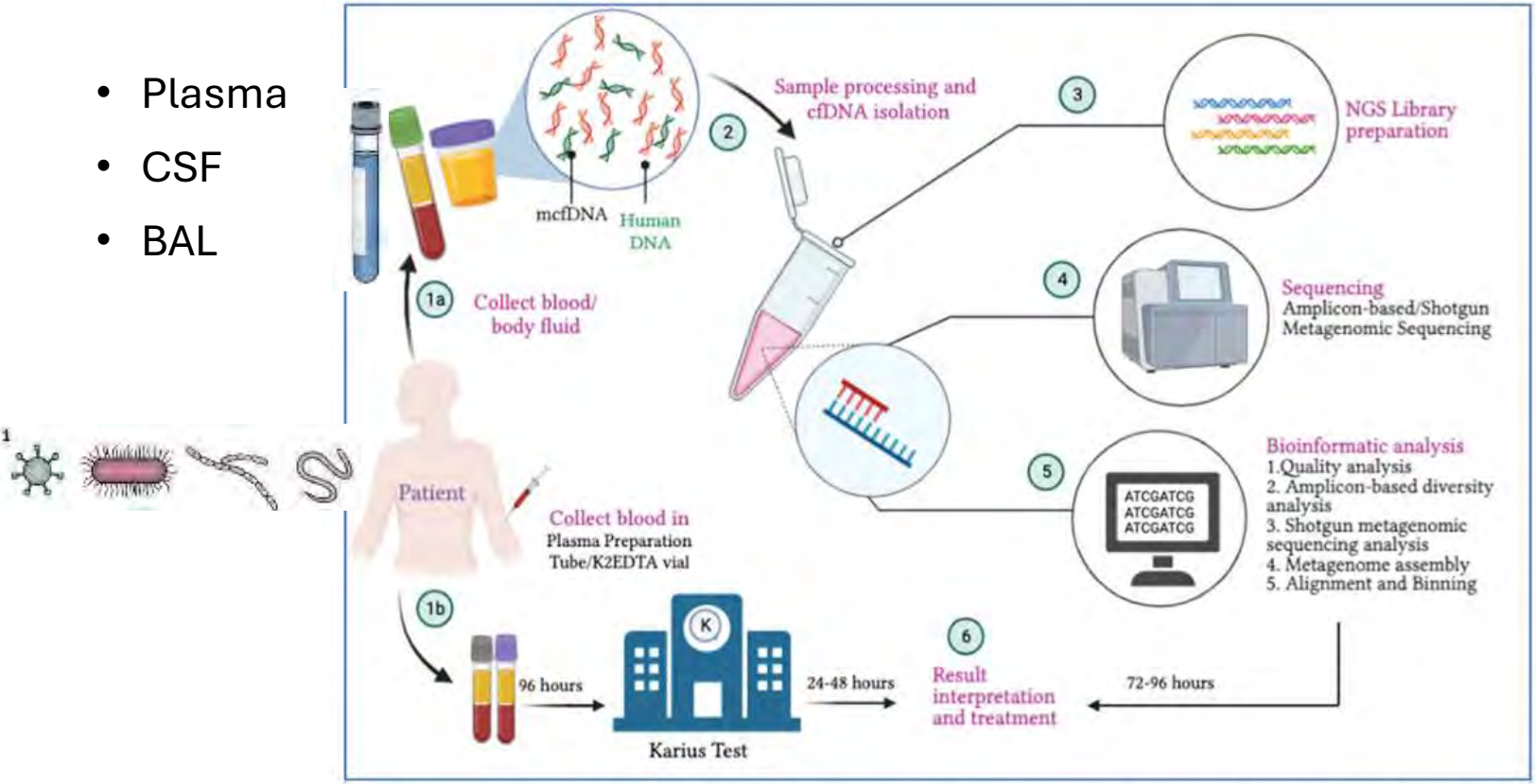
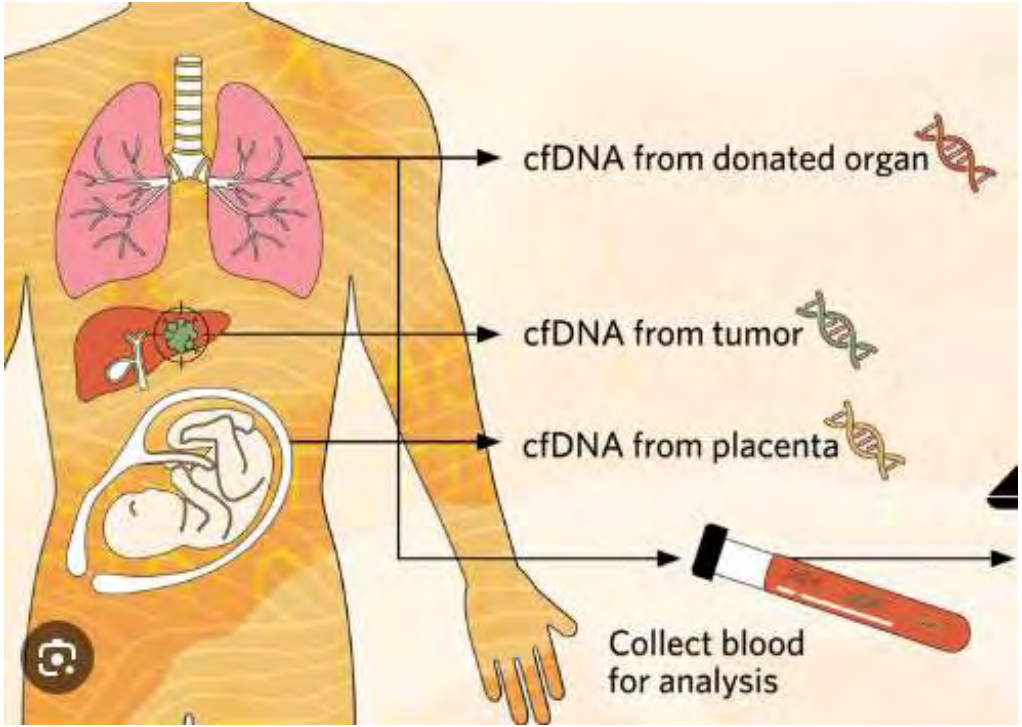


Fig. 2. Diagrammatic illustration of sample collection, mcfDNA isolation, and data analysis for the diagnosis of infections from clinical samples.

Microbial cell-free DNA (mcfDNA)



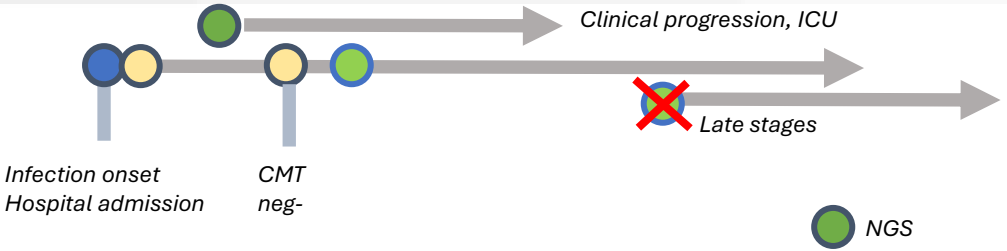
- Cell-free DNA in plasma
  - Fetal DNA : 10%
  - Tumor DNA: ~0.1%
  - Donor-derived: <1%
  - Microbial DNA: ~0.001%

Commercial Lab-Developed Assays for mNGS in the USA

Laboratory	Specimen	Cost	Location HD	Turnaround time
KARIUS®	Plasma	$2000	Redwood City, CA	48 hs from sample receipt
delvebio IN PARTNERSHIP WITH UCSF	CSF	$2000-$3100	Burlington, MA	48 hs from sample receipt

Clinical applications NGS

- Clinical syndromes with reported utility:
  - Culture-negative IE
  - Fever-unknown origin (FUO)
  - Persistent Febrile Neutropenia
  - Deep-seated infection
    - Vertebral osteomyelitis
    - Vascular graft infection
    - Organ abscess (eg, liver, spleen)
  - Rapid-progressive pneumonia
  - Sepsis/Infectious syndromes with negative CMT



- When to consider:
  - CMT not diagnostic and rapid clinical deterioration
  - Contraindications to invasive procedures
  - Imaging highly suggestive of infection
  - Critical illness

CMT: Conventional microbiological testing

mNGS and CNS infections

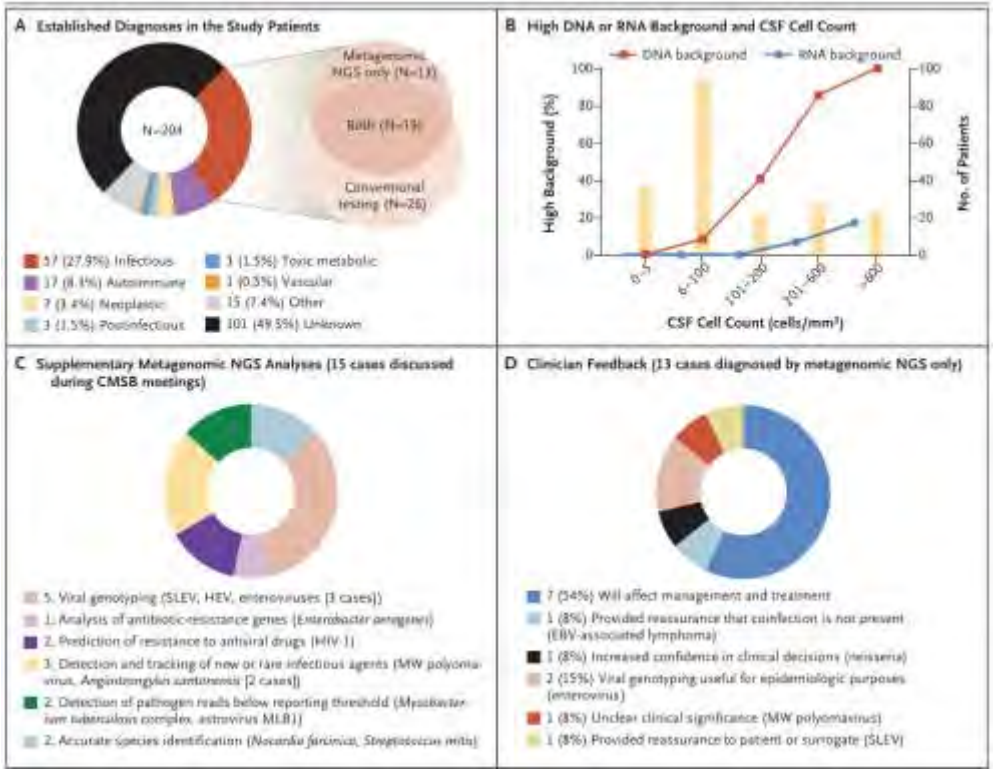
- ~50% of M/E cases: no identifiable cause

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

N ENGL J MED 380;24 NEJM.ORG JUNE 13, 2019



mNGS and CNS infections

nature medicine

Article

Seven-year performance of a clinical metagenomic next-generation sequencing test for diagnosis of central nervous system infections

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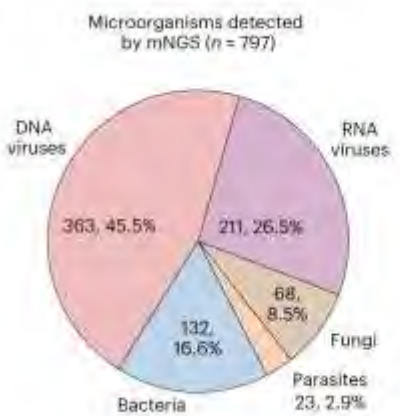
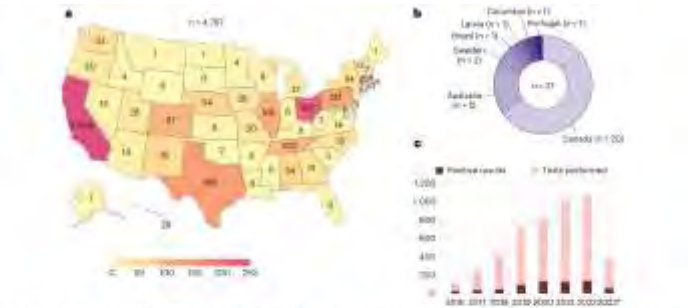
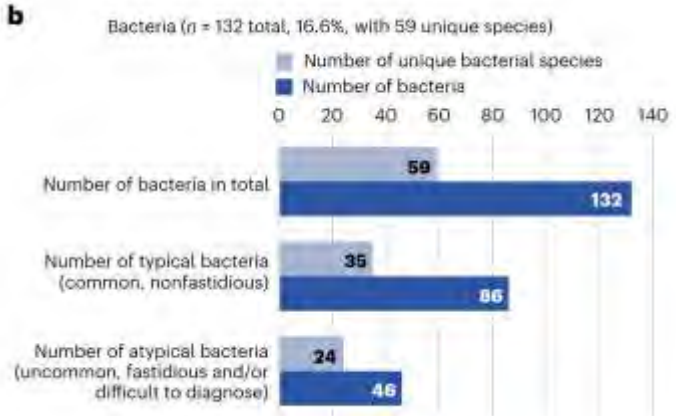
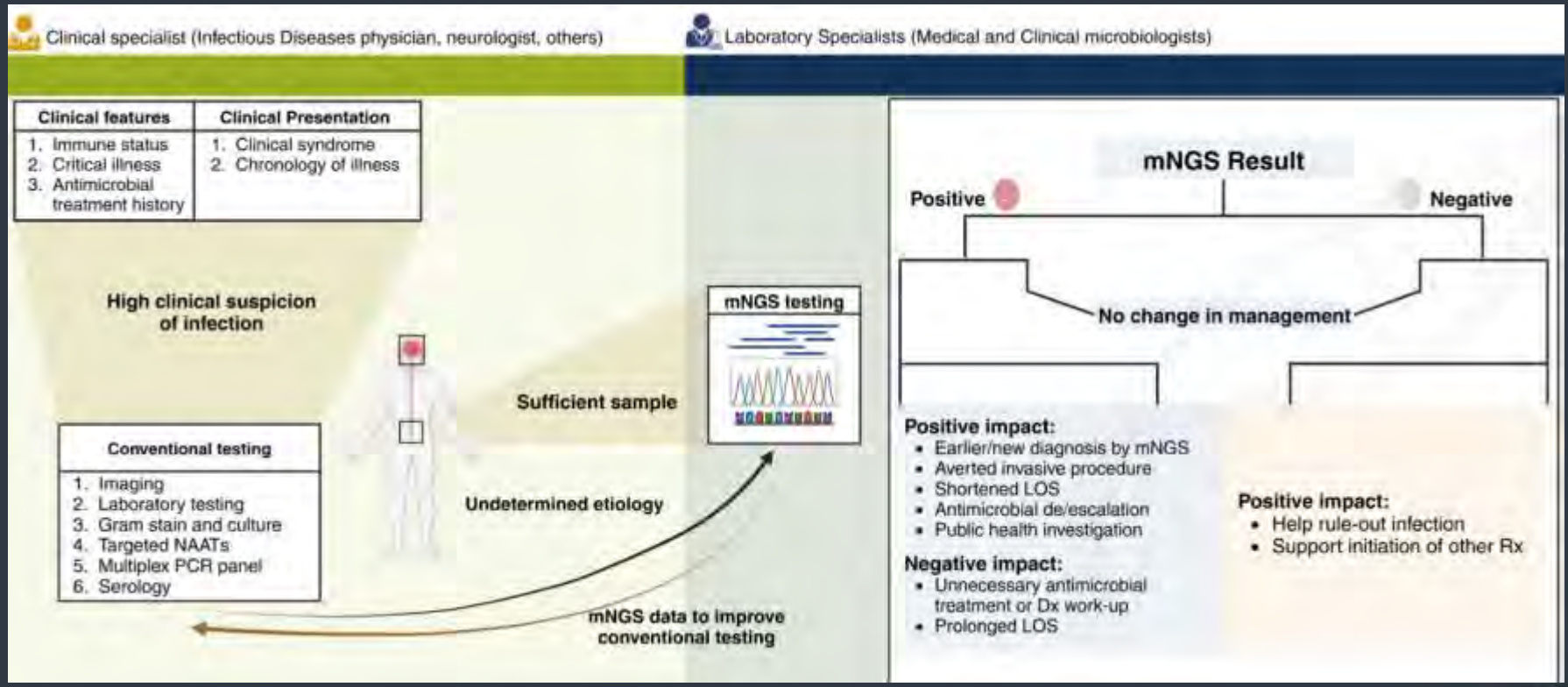
A list of authors and their affiliations appears at the end of the paper







Optimizing mNGS use



Adapted from Hogan, 2024.

Pros and Cons of mNGS

Box 4 | **Pros and cons of metagenomic next-generation sequencing**

Pros

- Single test that can diagnose infections from fungi, DNA and RNA viruses, bacteria and parasites
- Can identify emerging pathogens that are either novel to the region or highly divergent from known pathogens
- Can identify common infections presenting in an atypical manner or overlooked by the treating team
- Clinically validated assays are increasingly available

Cons

- Expensive: current costs of the clinical assay are ~US$2,000
- Dependent on the presence of microbial nucleic acid; therefore, it is insensitive for compartmentalized or transient infections
- Can be insensitive for low titre (<100 copies) infections or with high human DNA or RNA background (for example, pleocytosis (~500–1,000 cells/μl))
- Environmental contamination might lead to false positives; the clinical context and appropriateness of the result should always be considered

Clin Lab Med 44 (2024) 63–73

Diagnostic Stewardship at UNMC

Nebraska Medicine

Clinical Guidance on the Use of Next Generation Sequencing (NGS) Tests for Infectious Diseases

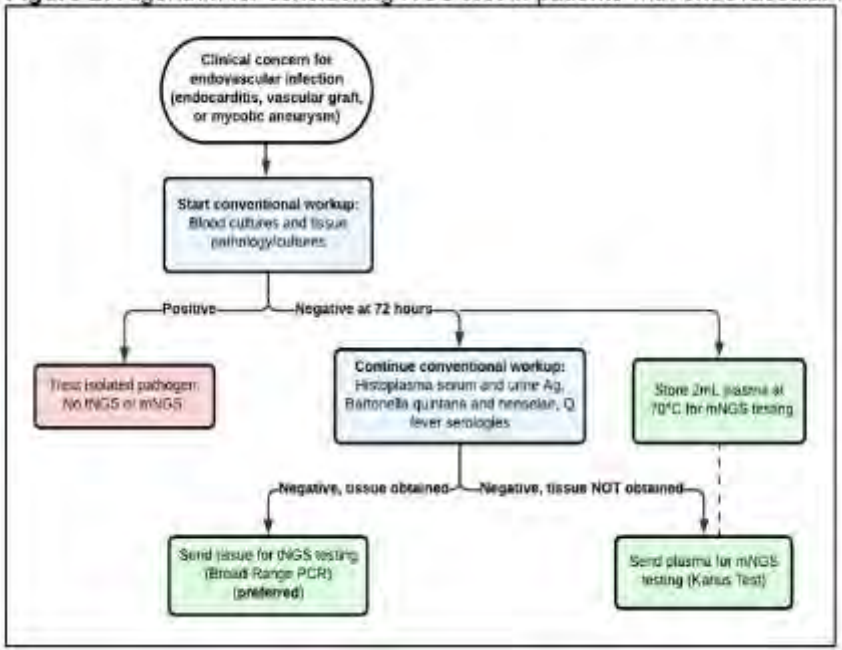
EXECUTIVE SUMMARY:

Next-Generation Sequencing tests should ONLY be considered when the following criteria are met:

1. **Clearly identifiable focus on infection** (do not use in undifferentiated clinical conditions)
2. **Anticipated prolonged course of antimicrobial therapy** (i.e. weeks to months; avoid NGS if anticipating short [*≤14 day*] course of empiric therapy or no plans to treat)
3. **Negative conventional workup at >48 hours** (or longer, depending on the clinical scenario, suspected pathogen, and type of conventional testing sent)

An Infectious Diseases consult is **required** to order these tests.

Figure 2: Algorithm for considering NGS test in patients with endovascular infections



Website: https://www.unmc.edu/intmed/_documents/id/asp/ngs.pdf

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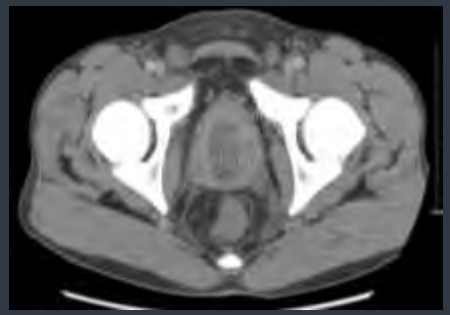
Bartonella spp

Clinical Case 2



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Ureaplasma spp

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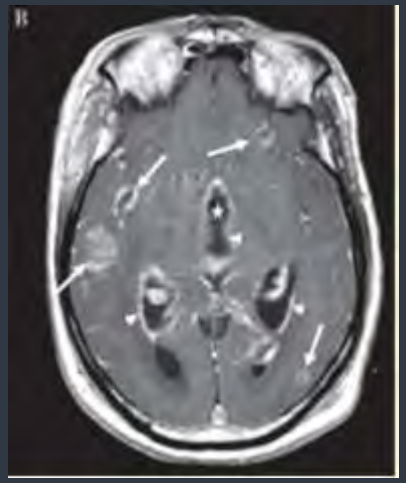

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Nocardia spp

Take-Home Messages: mNGS in Transplant ID

- **Unbiased Infection Detection** :

  Identifies a wide range of pathogens, including rare and unexpected infections

- **Non-Invasive & Early Diagnosis**

  –Detects infections from plasma or CSF before traditional tests, aiding faster clinical decision-making

- **Shortcomings: Potential for False Positives**

  –May detect pathogens of unclear clinical significance, leading to challenges in interpretation and possible overdiagnosis. $$$

- **Need for Future Studies**

  – More research is needed to determine diagnostic accuracy, cost-effectiveness, and clinical impact in routine transplant care.

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