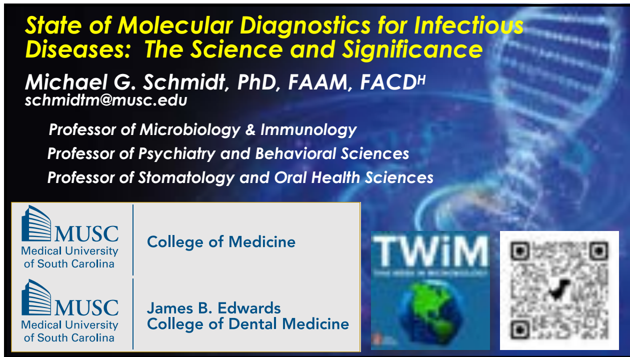


1

Learning Objectives

At the conclusion of this session the attendee will be able to -

1. Explain how meta-genomic analysis is an effective and affordable alternative to culture-based diagnostic approaches for infections.
2. Discuss the consequence of VBNC microbes in patient specimens offering how VBNC microbes impact the interpretation of culture-based diagnostics.
3. Describe the process required for the effective metagenomic analysis for the diagnosis of infections.
4. Recall data from the peer reviewed literature supporting the contention that meta-genomic analysis is an effective, an often superior, method over culture- based diagnosis for infection.

2

Financial Disclosures and Acknowledgements

Disclosures and Conflict of Interest Mitigation

- Member of the Scientific Advisory Board for MicroGenDX
- Unrelated to this presentation I am supported from the Office for Victims of Crime, Office of Justice Programs, US Department of Justice - 2016-RF-GX-0001 and OVC Co-Operative Agreement No. 2017-MU-GX-K114 and OVC Co-Operative Agreement No. Award No.2020-V7-GX-K002
  - Opinions expressed here are those of the author and not necessarily those of OVC, the U.S. Department of Justice, or my employer, MUSC.

3

What are the promises of molecular approaches for diagnostic microbiology?

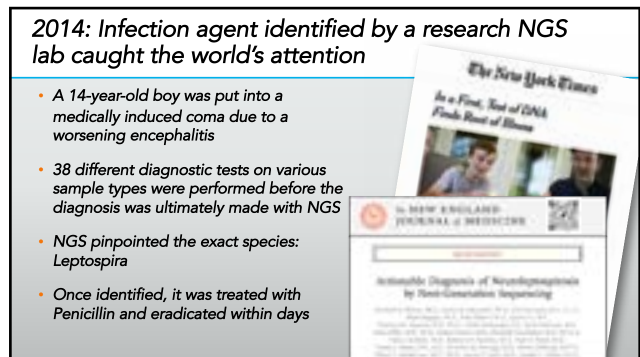
- Faster and more complete results
  - ✓ Beyond a Gram Stain
  - ✓ ± Genus and species like never before
- Additional information from the same specimen
  - ✓ Antibiotic resistance potential of the community within the specimen
- Potentially improved outcomes and + impact addressing ABX and Lab Stewardship

4

Ask yourself the following-

- What clinical evidence supports the utility of molecular approaches as effective diagnostic aides?
  - When should we consider employing this technology?
- Why are these molecular methods necessary for modern infection diagnosis?
  - How do different molecular testing approaches compare?
    - Are the limitations relevant?

5



6

Have we reached a tipping point warranting routine molecular diagnostics for infectious diseases?

- Molecular Methods offer definitive utility in the diagnosis of infectious diseases
  - ✓ PJI patients: Here NGS can provide valuable microbial information to inform treatment as confirmed by the multi-center study
  - ✓ Antimicrobial prophylaxis based on NGS results reduce infection incidence post kidney stone procedures
  - ✓ NGS offers higher microbial diagnostic sensitivity for infective endocarditis
- Evidence continues to propel us towards a new diagnostic paradigm ...

1860: culture method created — 1860; Wright brothers' first flight — 1903; Chuck Yeager Supersonic Flight — 1947; The discovery of DNA — 1953; Sanger sequencing method — 1977; PCR Invented Cetus — 1984; NGS sequencing method; Human genome project completed; Human microbiome project; The beginning of space tourism — 2000, 2003, 2008, 2021

7

Value Proposition of Molecular Testing
Cost is no longer a significant issue – Consider Public Health and Wastewater Testing

- Cost is no longer an issue
  - Human Genome for less than $100
- Bioinformatics processing times have improved through distributed CPU burden sharing
  - Main issue – what to do with the data
  - Best Practices for EMR display and data mining
  - Consensus panels for common and expert interpretation

Cost per Human Genome

Sequencing of the human genome from between 500 million to 1 billion to ~$100 per genome

8

CDC and NIH have estimated that biofilm infections now constitute 65% to 80% (respectively) of bacterial infections treated by physicians in the developed world.

The Problems with Culture

1. Samples must follow strict guidelines of being at the lab within 2 hours and kept at room temperature.
2. Less than 1% of known microbes will grow in traditional culture methods.
3. Cultures have a high probability of returning with *"no growth"* results.
4. Anaerobes are extremely difficult to grow in culture.
5. Fungi can take over 20 days to get a result.
6. It takes several diagnostic tests to identify anaerobes, aerobes, and fungi, costing the patient for each test.
7. Prior Antibiotic Exposure of the Patient can limit recovery

9

Impact of Culture-Negatives

- Culture-negative rate:
  - PJI: 28.2% (MSIS, ICM2018 Criteria)[1]
  - IE-Blood: 53% (Duke's Criteria-Definite)[2]
  - IE-Valve: 80% (Duke's Criteria-Definite)[2]
  - Infectious Endophthalmitis[3]
  - Osteoarticular infections (OAI): 70%[4]
- Only ~1% of bacterial species cultivable in the laboratory
- Other Contributing Factors:
  - Empiric/Previous antibiotic therapy
  - Transport/growth requirements
  - Polymicrobial biofilms
  - VBNC

Culture Negative Rates

Category	Rate
Periprosthetic Joint Infection	30%
Infectious endocarditis-Blood	53%
Infectious Endophthalmitis	56%
Osteoarticular Infections	70%
Infectious endocarditis-Valve	80%

10

The problem with Persister Cells / Viable But Not Culturable (VBNC)

Variants for 'normal cells' that are tolerant to antibiotics and responsible for recalcitrance towards treatment with common antimicrobials

- ✓ ~0.3% of a microbial community are persister cells or in a VBNC state
  - In a community with a density of 1 x 10^9/ml ~ 3 x 10^6/ml are in a persistent state
  - Cells refractory to antimicrobials
  - Can revert and grow

Kim Lewis, University Distinguished Professor, Northeastern University College of Science

11

Clinical Significance of VBNC Populations Arise in biofilms established on foreign materials (catheter materials, PJ, Lines etc.)

- Now appreciate any environmental stressor could send a community into this unculturable but still virulent (able to cause disease) state
- Biofilm Development on Urinary Catheters Promotes the Appearance of Viable but Nonculturable Bacteria - Sandra A. Wilks, Verena V. Koerfer, Jacqui A. Prieto, Mandy Fader, C. William Keevil – mBio 12: e03584-20. https://doi.org/10.1128/mBio.03584-20.
- Antimicrobial urinary catheter materials have been developed
- Laboratory studies argue for their clinical utility
- None have significantly improved clinical outcomes
  - Why and How?
    - Poorly designed laboratory trials and failure to consider the impact of VBNC populations

C. William Keevil PhD FRSB FRSPH FAAM Professor of Environmental Healthcare, School of Biological Sciences, University of Southampton

$$\%VBNC = \frac{[TCC - (CFU + dead)]}{TCC} \times 100$$

12

Which of the following statements about VBNC bacteria is true?

A. VBNC bacteria are inactive bacteria that can be revived by adding nutrients.

B. VBNC bacteria are alive but cannot be cultured using routine clinical laboratory methods.

C. VBNC bacteria are dormant bacteria that can be reactivated by stress.

D. VBNC bacteria are a type of biofilm and cannot be cultured.

13

Which of the following statements about VBNC bacteria is true?

A. VBNC bacteria are inactive bacteria that can be revived by adding nutrients.

B. VBNC bacteria are alive but cannot be cultured using routine clinical laboratory methods.

C. VBNC bacteria are dormant bacteria that can be reactivated by stress.

D. VBNC bacteria are a type of biofilm and cannot be cultured.

- The answer is B. VBNC bacteria are alive but cannot be cultured in the laboratory. They are in a state of very low metabolic activity and do not divide, but they are still capable of surviving for long periods of time. VBNC bacteria have been found in a variety of environments, including water, soil, and food. *They can also be found in the human body, where they can cause chronic infections.*
- VBNC bacteria are a challenge to traditional methods of diagnosis and treatment. They cannot be cultured in the laboratory, so it is difficult to identify them. Additionally, they are often resistant to antibiotics. However, new methods of detection and treatment are being developed.

14

Evolution of microbial identification —From tube and plate to a molecular signature

Balancing sensitivity, discovery power, timeliness and affordability

Sensitivity
Targeted NGS/TMS
Discovery Power
Metagenomic
Speed
Low Cost

All methods share sampling and nucleic acid extraction limitations

Comparison of DNA sequencing technologies

	Strengths	Limitations
qPCR ***Good, Bbiased but Fast***	• High sensitivity • Fastest processing speed	• Can only interrogate a limited set of mutations • No discovery power beyond the primer set
Shotgun metagenomics ***Better***	• Base-by-base view of the genome, capturing both large and small variants • Provides strain-level information, most relevant for viral typing and epidemiology tracing and research	• Lowest overall sensitivity (In illumine sequencing, for the same region, targeted sequencing achieves 5,000x reads as compared to 75x for shotgun sequencing) • Most expensive • Sequence library is relatively small
Targeted 16S/ITS NGS ***Best, Unbiased and highly sensitive***	• High sequencing depth enables high sensitivity • High discovery power • High mutation resolution • High relative processing speed	• Can't detect viruses or parasites • No strain-level specificity

15

qPCR + Targeted NGS:

Balancing sensitivity, discovery power, timeliness and affordability

- **qPCR is rapid, custom built for clinical needs, incredibly sensitive**
  - Quantitative identification of panel organisms
  - Antimicrobial resistance gene detection
  - 24 hour Turn around time
- **NGS/TMS process affords much greater discovery power than PCR**
  - 16S/ITS Targeted NGS
  - >50,000 organisms in reference database
  - 3.5-day Turn around time

16

Database Curation is Vital

- Look for accurate and extensive databases
- Often, they contains public and internally validated and curated sequences
- Look for continuous updates and curation

Remove incomplete and incorrect taxonomic information
Remove low quality reads over gene regions of interest
Remove suspect species matches

17

What information should you look?

Assess MEAN ACCURACY of the service

Example of proficiency data often presented on the websites of send-out labs

18

Assess acceptance of specimen types

Can they process what you will be sending them? accepts a wide selection of specimen types/samples-

- *Blood*
- *Bronchoalveolar Lavage (BAL)*
- *Cerebrospinal Fluid (CSF)*
- *Hardware*
- *Heart valve tissue*
- *Nails*
- *Sinus Specimens*
- *Sputum*
- *Synovial Fluid*
- *Tissue Drainage*
- *Urine*

- An appropriate patient specimen and collection technique are most critical for achieving a clinically valid molecular result
  - Avoid using DNA degradation agents, such as 4% lidocaine

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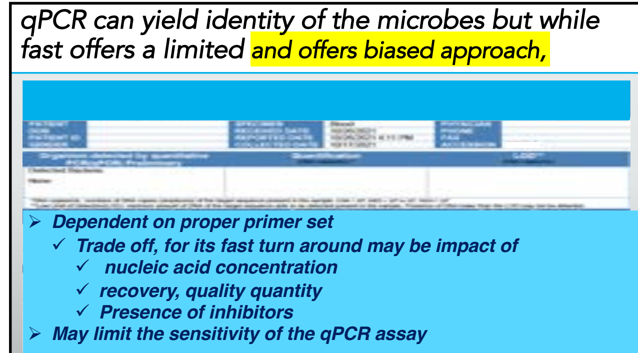

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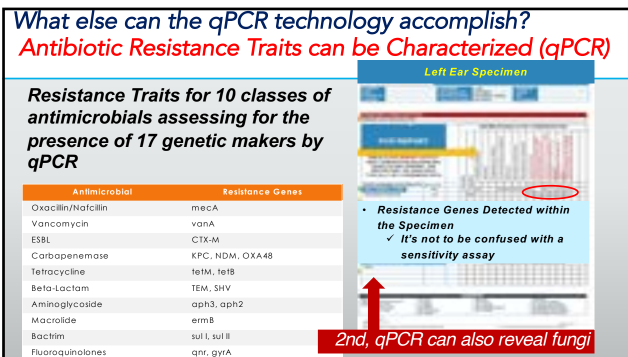

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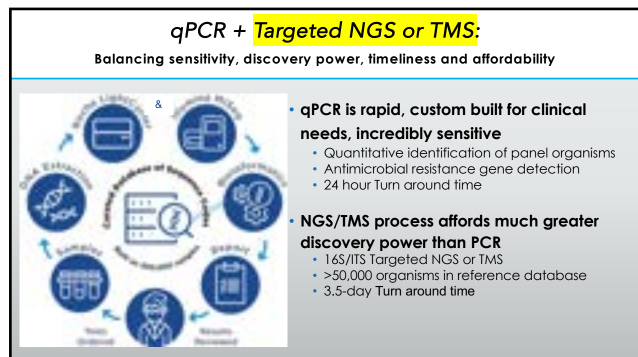

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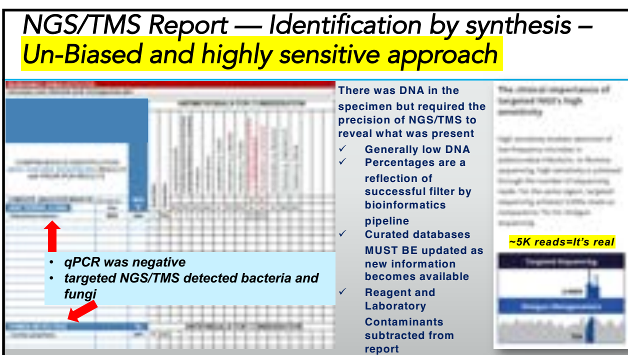

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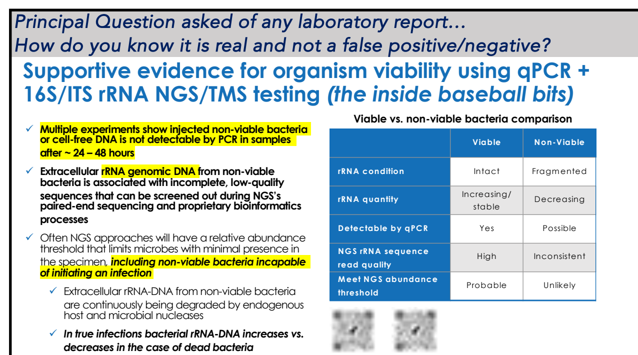

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Value Proposition- Laboratory Stewardship & Formulary Considerations

	Mayo Clinic	U of Washington	Karius	MicroGenDX
Species nucleic acid Identification from direct patient samples				
16S NGS Sequencing platform	Illumina MiSeq	Illumina MiSeq	SMg Platform	**Illumina MiSeq**
Bacteria	✓	✓	✓	✓
Mycobacteria	✓		✓	✓
Fungi			✓	✓
Antimicrobial resistance genes			✓	✓
Database (# of sequences)	NCBI (~100K)	NCBI (~100K)	Curated (~1.6K)	**Curated (~50K)**
Turnaround	14 – 21 days	10 – 12 days	24 - 48 hours	**24 hours (Level 1) 3-5 days (Level 2)**
Cost	**$400 - $800**	**$800-$1,000**	**$2,000**	**~$356**
Sample types				
CSF	✓			✓
Synovial Fluid	✓			✓
Tissue	✓	✓		✓
Respiratory		✓		✓
BAL		✓		✓
Urine		✓		✓
Swab				✓
Blood			✓	✓

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Value Proposition- Laboratory Stewardship & Formulary Considerations

	Mayo Clinic	U of Washington	Karius	MicroGenDX	Traditional Culture
Species nucleic acid Identification from direct patient samples					
16S NGS Sequencing platform	Illumina MiSeq	Illumina MiSeq	SMg Platform	**Illumina MiSeq**	***Estimated Costs***
Bacteria	✓	✓	✓	✓	**Aerobic BC - $332**
Mycobacteria	✓		✓	✓	**Anaerobic BC - $434**
Fungi			✓	✓	**Fungal -$194**
Antimicrobial resistance genes			✓	✓	**AFB - $352**
Database (# of sequences)	NCBI (~100K)	NCBI (~100K)	Curated (~1.6K)	**Curated (~50K)**	**Tissue Processing $185**
Turnaround	14 – 21 days	10 – 12 days	24 - 48 hours	**24 hours (Level 1) 3-5 days (Level 2)**	**Sub-total Culture Costs ~$1493**
Cost	**$400 - $800**	**$800-$1,000**	**$2,000**	**~$356**	**~$2,603 Plus Sensitivities**
Sample types					
CSF	✓			✓	**MALDI–TOF Per Isolate $185**
Synovial Fluid	✓			✓	**Assume ~6-10 isolates ~$1,110- $1,850**
Tissue	✓	✓		✓	***Sensitivities***
Respiratory		✓		✓	**Per isolate**
BAL		✓		✓	
Urine		✓		✓	
Swab				✓	
Blood			✓	✓	

26

Which of the following is true about metagenomic analysis?

A. It is a more effective and affordable alternative to culture-based diagnostic approaches for infections.

B. It is a less effective and more expensive alternative to culture-based diagnostic approaches for infections.

C. It is a more effective and more expensive alternative to culture-based diagnostic approaches for infections.

D. It is a less effective and less expensive alternative to culture-based diagnostic approaches for infections.

27

Which of the following is true about metagenomic analysis?

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B. It is a less effective and more expensive alternative to culture-based diagnostic approaches for infections.

C. It is a more effective and more expensive alternative to culture-based diagnostic approaches for infections.

D. It is a less effective and less expensive alternative to culture-based diagnostic approaches for infections.

The answer is A. Metagenomic analysis is a more effective and affordable alternative to culture-based diagnostic approaches for infections. It can detect even small amounts of DNA or RNA from pathogens, while culture-based methods require a large number of organisms to grow in a laboratory. Additionally, metagenomic analysis can identify multiple pathogens in a single sample, while culture-based methods can only identify one pathogen at a time. Metagenomic analysis is also becoming more affordable as the cost of sequencing technology decreases.

28

What does the ID literature say?

- **Only about 30% of septic patients have a causative organism identified, even in cases with high clinical suspicion for infection, limiting targeted and maximally effective treatment**
  - In this study, 16S rRNA gene NGS identified a potentially pathogenic organism in 47% (n = 28) of cases compared to 32% (n = 19) with blood cultures.
  - A novel finding was the ability of NGS to possibly point to the source of sepsis (abdominal or genitourinary) in several cases based on the profile of identified organisms.

https://pubmed.ncbi.nlm.nih.gov/33893492/ ***2021 – Mayo- Robin Patel***

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30

Challenging the Paradigm of 142 yr. old culture technique

- ***A multicenter Study (14 sites)***
- ***301 Patients met ICM Criteria for PJI***
- ***56 of 85 (66%) culture-negative patients had positive NGS results***
- ***Named significant manuscript of 2022***

JB JS — The Journal of Bone & Joint Surgery

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Utility of NGS supplementing culture in PJI diagnosis

JB JS — The Journal of Bone & Joint Surgery

- ***301 patients from 14 institutions***, who met the ICM[1] criteria for PJI were included in the study[2]
  - ✓ 28% were culture-negative
  - ✓ A pathogen was detected in 66% of culture-negative PJI cases
  - ✓ 91% of NGS-positive cases were polymicrobial
- *E. coli, C. acnes, S. epidermidis* and *S. aureus* were the most commonly identified species

Total confirmed PJI patients	Culture-negative PJI patients	NGS - positive PJI patients
28% culture negative	66% NGS positive	91% poly-microbial
72% culture positive	34% NGS negative	9% single microbe

"The results of this collaborative research endeavor, involving multiple academic centers support the utility of NGS in the diagnosis of complex orthopaedic infections, in particular in the setting of culture-negative PJI".

1. *International Consensus Meeting 2018-2018 International Consensus Meeting on Musculoskeletal Infection: Research Priorities from the General Assembly Questions*
2. *An Enhanced Understanding of Culture-Negative Periprosthetic Joint Infection with Next-Generation Sequencing from J Bone Joint Surg Am. 2022;00:1-7 d http://dx.doi.org/10.2106/JBJS.21.01061*

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An Enhanced Understanding of Culture-Negative Periprosthetic Joint Infection with Next-Generation Sequencing from J Bone Joint Surg Am. 2022;00:1-7 d https://pubmed.ncbi.nlm.nih.gov/35726882/

JB JS — The Journal of Bone & Joint Surgery

Species detected and dominance frequency

Frequency of species detected

Frequency of dominant species

Only species that were identified as common on the basis of having a minimum study-wide incidence of at least 5% were included for plotting

33

Why presumed culture-negative PJIs fail to elaborate microbes?

JB JS — The Journal of Bone & Joint Surgery

There are several, not necessarily mutually exclusive, explanations:

- ✓ Microbes are in ***low abundance***, such that they are below the detection limits afforded by routine culture;
- ✓ The organisms are in a ***VBNC*** (*viable but not culturable*) state and yet can still ***elicit symptoms*** in the patient;
- ✓ Microbe(s) may be fastidious and displaying a ***biofilm phenotype***, ***reducing likelihood of being detected*** by routine culture;
- ✓ The culture media used ***MAY NOT support the growth*** of the microbes present in the patient specimen; and
- ✓ The multiple microbes ***may compete*** against each other ***for resources***, making the isolation of all pathogens by routine culture difficult.

An Enhanced Understanding of Culture-Negative Periprosthetic Joint Infection with Next-Generation Sequencing from J Bone Joint Surg Am. 2022;00:1-7 d https://pubmed.ncbi.nlm.nih.gov/35726882/

176 Microbial species detected from culture negative specimens

12
84
80

Gram positive
Gram negative
Fungi

34

NGS aids infection reduction post kidney stone procedures

- **Patients undergoing kidney stone lithotripsy were prospectively assigned to NGS intervention and standard of care groups**
- 125 who had negative urine culture or commensal growth were included in the study
  - *50 in NGS intervention group*
  - *75 in standard of care group*
- ***Results***:
  - ***NGS arm had 0 infections***
  - ***Standard of care arm had 6 cases of infections, including 2 requiring intensive unit care9***

35

NGS for infective endocarditis: Superior sensitivity and shorter turnaround time in identifying causative pathogens

Culture vs NGS sensitivity comparison

100%
50%
0%
Valve gram stain 51%
Blood culture 46%
Valve culture 17%
NGS 98%

https://pubmed.ncbi.nlm.nih.gov/30926543/

- ***Valve culture and blood culture sensitivity maybe biased by prior antibiotic use, fastidious and/or viable unculturable bacteria (VBNC)***
- ***Supplemented culture media, special growth conditions and prolonged incubation times are often necessary to reveal the presence of common IE pathogens***

36

Additional outcome-based studies to augment your justification for selecting a NGS based approach for infection diagnosis

1. Tarabichi M, Shohat N, Goswami K, Alvand A, Silibovsky R, Belden K, Parvizi J. ***Diagnosis of Periprosthetic Joint Infection: The Potential of Next-Generation Sequencing***. J Bone Joint Surg Am. 2018 Jan 17;100(2):147-154. doi: 10.2106/JBJS.17.00434. PMID: 29342065.
2. Haider AA, Marino MJ, Yao WC, Citardi MJ, Luong AU. ***The Potential of High-Throughput DNA Sequencing of the Paranasal Sinus Microbiome in Diagnosing Odontogenic Sinusitis***. Otolaryngol Head Neck Surg. 2019 Dec;161(6):1043-1047. doi: 10.1177/0194599819866692. Epub 2019 Aug 6. PMID: 31382814.
3. McDonald M, Kameh D, Johnson ME, Johansen TEB, Albala D, Mouraviev V. ***A Head-to-Head Comparative Phase II Study of Standard Urine Culture and Sensitivity Versus DNA Next-generation Sequencing Testing for Urinary Tract Infections***. Rev Urol. 2017;19(4):213-220. doi: 10.3909/riu0780. PMID: 29472825; PMCID: PMC5811878.
4. Dowd SE, Wolcott RD, Kennedy J, Jones C, Cox SB. ***Molecular diagnostics and personalised medicine in wound care: assessment of outcomes***. J Wound Care. 2011 May;20(5):232, 234-9. doi: 10.12968/jowc.2011.20.5.232. PMID: 21647068.
5. van Asten SA, La Fontaine J, Peters EJ, Bhavan K, Kim PJ, Lavery LA. ***The microbiome of diabetic foot osteomyelitis***. Eur J Clin Microbiol Infect Dis. 2016 Feb;35(2):293-8. doi: 10.1007/s10096-015-2544-1. Epub 2015 Dec 15. PMID: 26670675; PMCID: PMC4724363.
6. Werneburg GT, Nguyen A, Henderson NS, Rackley RR, Shoskes DA, Le Sueur AL, Corcoran AT, Katz AE, Kim J, Rohan AJ, Thanassi DG. ***The Natural History and Composition of Urinary Catheter Biofilms: Early Uropathogen Colonization with Intraluminal and Distal Predominance***. J Urol. 2020 Feb;203(2):357-364. doi: 10.1097/JU.0000000000000492. Epub 2019 Aug 20. PMID: 31430245; PMCID: PMC6952550.
7. Liang M, Fan Y, Zhang D, Yang L, Wang X, Wang S, Xu J, Zhang J. ***Metagenomic next-generation sequencing for accurate diagnosis and management of lower respiratory tract infections***. Int J Infect Dis. 2022 Sep;122:921-929. doi: 10.1016/j.ijid.2022.07.060. Ep

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Clinical evidence is building with each study

NGS affords higher sensitivity than culture in identifying microorganisms involved in PJI

- Among 28 MSIS* positive patients, **NGS detected bacteria in 25 (90%), while culture was positive in 17 (60%) – a significant improvement in sensitivity**
- NGS was positive in > 80% of culture-negative, MSIS confirmed PJIs

MSIS-positive patients
100%
80%
60%
40%
20%
0%
MicroGenDX NGS
Culture
Positive
Negative

* MSIS: Musculoskeletal Infection Society

https://pubmed.ncbi.nlm.nih.gov/29342065/

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Outcomes -Patients benefit

Etiology of Chronic Rhinosinusitis (CRS)

- **Odontogenic sinusitis often goes unrecognized and can be a source of failed endoscopic sinus surgery**
- **NGS has 85% sensitivity and 81% specificity to detect odontogenic sinusitis** – statistics comparable to FIT, a recommended screening test for colorectal cancer
- NGS has a negative predictive value of 99%

https://pubmed.ncbi.nlm.nih.gov/31382814/

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Outcomes drive evolution of standards

Randomized prospective cystitis outcome study: Better outcome with NGS

Antibiotics based on **NGS results** showed statistically **greater improvements** in symptom scores

Urine samples were collected from 44 patients with **acute cystitis symptoms**

Sensitivity comparison Culture vs NGS

Culture 30%
NGS 100%

Randomized to antibiotics based on **NGS or culture results**

https://pubmed.ncbi.nlm.nih.gov/29472825/

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Diagnosing and treating

Wound care study: DNA diagnostics + customized treatment show significant improvements in outcome

- **1378 patients were recruited into the study**
- In the standard of care group 48.5% of patients healed completely during the 7-month study period
- This increased to 62.4% in treatment group 1 and 90.4% in treatment group 2

Standard of Care Group	Group I	Group 2
Traditional Culture with Systemic Antibiotics	Molecular Diagnostics with Systemic Antibiotics	**Molecular Diagnostics with Customized Topical Antibiotics**
% of Patients Healed	**% of Patients Healed**	**% of Patients Healed**
48.5% 244/503	62.4% 298/479	**90.4%** 358/396

https://pubmed.ncbi.nlm.nih.gov/21647068/

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Diagnosing and treating

Wound care study: Median number of days to heal by type

Wound Type	~~Standard of Care~~ Traditional Culture with Oral Antibiotics	~~Group 1~~ DNA Diagnostics with Oral Antibiotics	~~Group 2~~ DNA Diagnostics with Customized Topical Antibiotics
Pressure Ulcer	N/A	107	**28**
Diabetic Foot Ulcer	168	84	**32**
Non-Healing Surgical Wound	176	75	**44**
Traumatic Abscess	39	33	**14**
Venous Leg Ulcer	177	98	**37**
TOTAL	177	77 ($p<0.001$)	**28** ($p<0.001$)

https://pubmed.ncbi.nlm.nih.gov/21647068/

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Osteomyelitis diagnosis

NGS assists in osteomyelitis diagnosis and management

NGS: 85% sensitivity for 34 admitted patients

Compared to culture, NGS detected:

- Significantly more anaerobes (86.9% vs. 23.1%) and
- Gram-positive bacilli (78.3% vs. 3.8%)
- The suggestion of a more significant role of anaerobic and fastidious organisms in osteomyelitis

https://pubmed.ncbi.nlm.nih.gov/26670675/

The microbiome of diabetic foot osteomyelitis

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Are we moving towards a new standard?

Next generation sequencing improves characterization of multispecies biofilms

"The presence of uropathogens in biofilm samples in indwelling urinary catheters **shows the utility of NGS platforms such as to identify bacteria which might be missed by conventional urine culture**"

"Next generation sequencing will have an expanded role due to superior ability to identify organisms which are nonculturable, anaerobic or present in the form of biofilms"

– Daniel A Shoskes MD, FRCSC
Professor of Urology, The Cleveland Clinic
President, Society for Infection and Inflammation in Urology

Cleveland Clinic

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Diagnostic performance of mNGS assay for pathogen detection

(a) Composition of pathogens in patients with positive mNGS results;
(b) Distribution of pathogens identified by mNGS;
(c) The number of patients with suspected LRTIs for various pathogens;
(d) Consistency analysis between mNGS and final diagnosis. mNGS, metagenomic next-generation sequencing; LRTI, lower respiratory tract infections.

Liang M, Fan Y, Zhang D, Yang L, Wang X, Wang S, Xu J, Zhang J. Metagenomic next-generation sequencing for accurate diagnosis and management of lower respiratory tract infections. Int J Infect Dis. 2022 Sep;122:921-929. doi: 10.1016/j.ijid.2022.07.060. Ep

45

Of the choices provided which offers sufficient justification to shift from one diagnostic standard to another?

A. The strength of the evidence: The evidence should be from high-quality studies with a large sample size.
B. The consistency of the evidence: The evidence should be consistent across different studies.
C. The clinical relevance of the evidence: The evidence should be relevant to the clinical setting.
D. The balance of benefits and harms: The benefits of shifting the diagnostic standard should outweigh the harms.
E. Evidence that the previous standard was not effective
F. Items A-E should each be considered taking into regard the desired outcome required

46

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F. Items A-E should each be considered taking into regard the desired outcome required

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How to employ this type of diagnostic tool to microbial ID?

Clinical Algorithm for NGS Utilization

High suspicion of infection, based on symptoms and biomarkers
Low-risk infection or low-risk patient
C&S & other necessary tests
Culture Definitive
Treatment
Treatment Failure
Culture Not Definitive
qPCR & NGS & Resistance markers
High-risk* infection or high-risk patient
C&S & other necessary tests
qPCR & NGS & Resistance markers
Report Interpretation
Treatment Change

* High risk: CNS, blood stream infections, PJI, vascular access device infections, complex wounds, complex pneumonia, complex UTI

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Conclusion- The Dx Paradigm has Shifted!

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What is Next for these Molecular Methods?

Future is in your hands

Thank you!!

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State of Molecular Diagnostics for Infectious Diseases The Science and Significance

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Thank you !

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TWiM

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