MICROBIOLOGY DEVICES PANEL
MEDICAL DEVICES ADVISORY COMMITTEE

# Why Pathogen-Agnostic Sequencing is Difficult to Validate




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The barrier isn't the technology.

Our validation paradigm was built for targeted assays.

Pathogen-agnostic testing breaks foundational assumptions.

# Five Validation Problems, One Root Cause

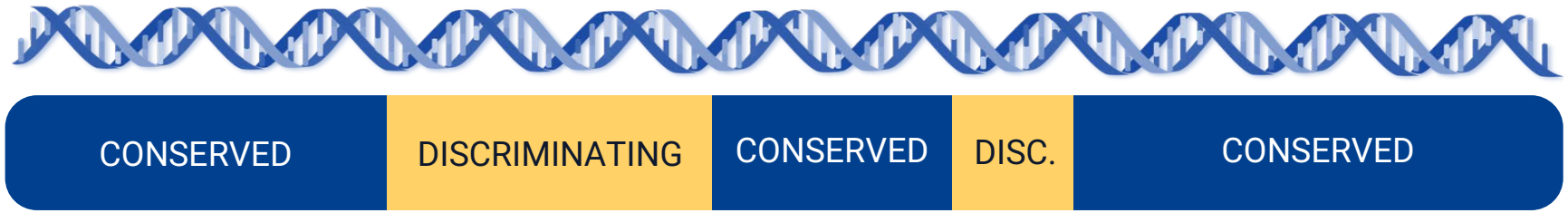
- What is a "positive"?
- Validating the untested
- Interpreting messy results
- Keeping the test current
- Proving it clinically

## Targeted Validation Framework

CHALLENGE 1: POSITIVITY

# What Is a "Positive"?

- A targeted assay has one target -a clean yes/no
- Pathogen agnostic approach returns reads, not a single signal
- Not every read carries the same information




- Shared across genus/family - low discriminating power
- Unique to species/strain - confirms identity

**Limit of Detection (LOD)** isn't how many copies.
It's how many copies to reliably hit the yellow regions.

We can reframe this as **Limit of Identification**, meaning a confidence measure, not just the presence/absence of reads.

CHALLENGE 2: EMPIRIC TESTING

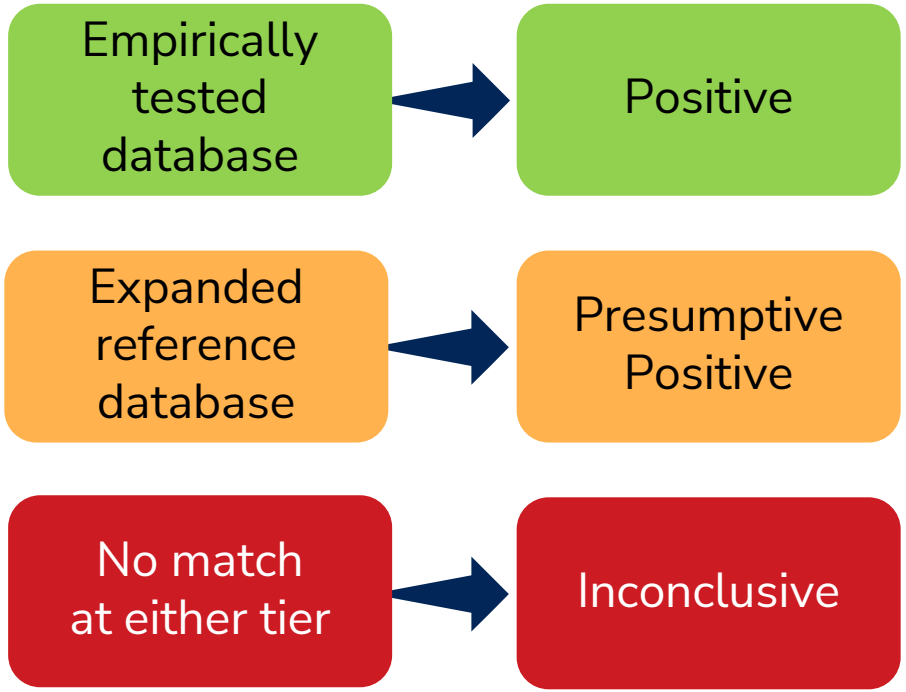
# Validating the Untested

- Targeted assays are validated organism-by-organism
- Pathogen agnostic testing's entire value is finding what **wasn't** validated
- No current system bridges that gap

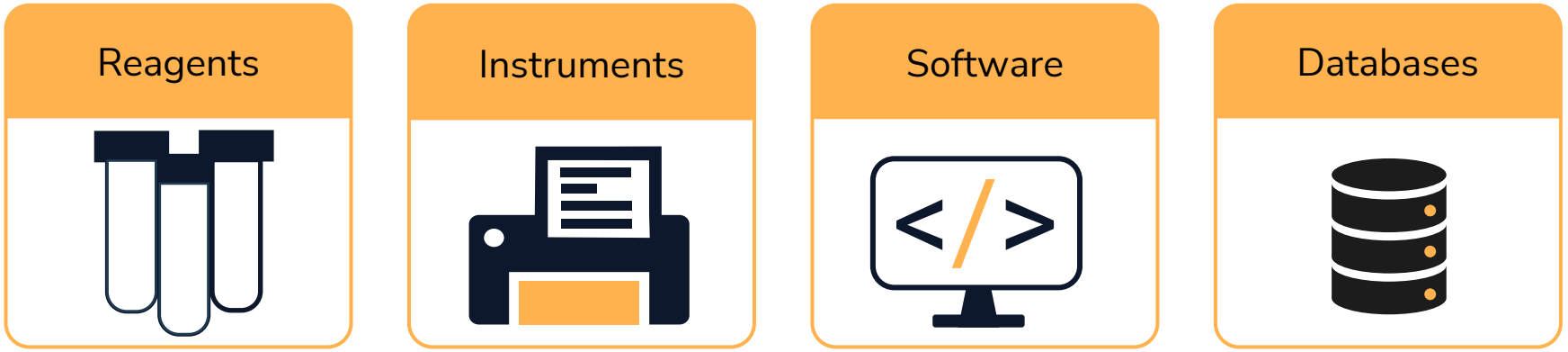
*To build a successful validation, we can include a focus on method-level performance, instead of only on "per organism" confirmation.*

## Potential solution:

**Tiered confidence instead of a single call**



# Consider the Components, Not Just the Whole Test




- This evaluates each component separately.
- It enables system-level oversight.
- It puts less emphasis on organism-by-organism validation.



When tests don't fit the rules: regulatory challenges for agnostic metagenomic next-generation sequencing in infectious diseases diagnostics.
Gauthier NPG, et al. Journal of Clinical Microbiology. 2026 Sept 10:

CHALLENGE 3: INTERPRETATION

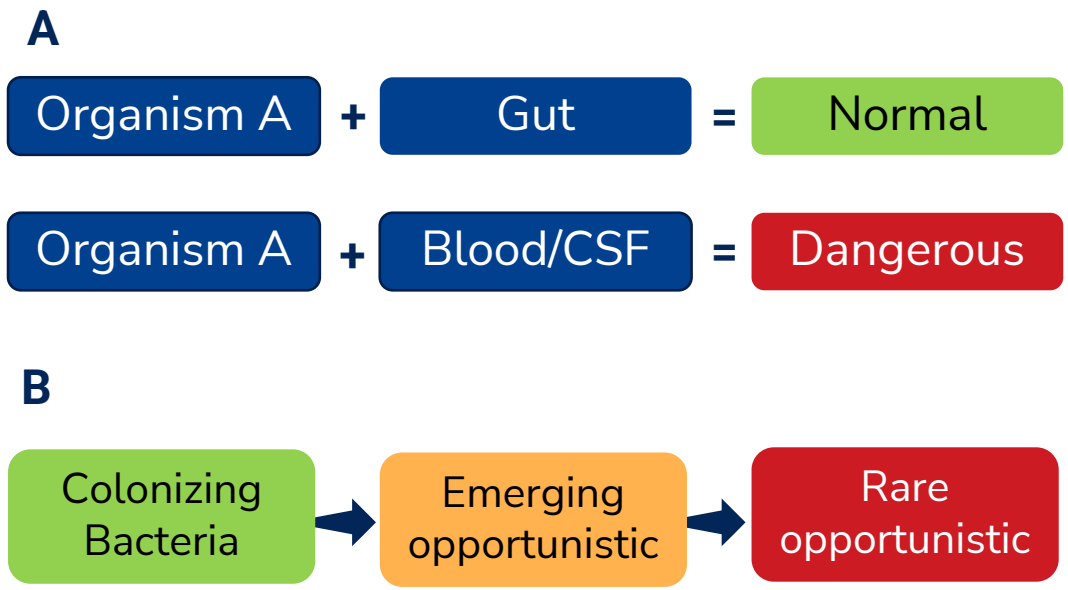
# Interpreting the Results

## Detection ≠ disease

Is it
- a causative agent?
- a bystander?
- commensal?

Metagenomics detects everything in the sample—not just what's clinically relevant.

## A True Positive Result Can Still be the Wrong Answer




**From a published lower respiratory tract infections (LRTI) cohort study:** Distinguishing between these tiers required modeling frequency + abundance + co-occurrence. Even the best tier (AUC 0.976) still misclassified approximately ~1 in 40 cases.



Heliyon. 2024 May 8;10(10):e30896. doi: 10.1016/j.heliyon.2024.e30896

# Mitigation: Five Ways to Help Interpret Metagenomic Results

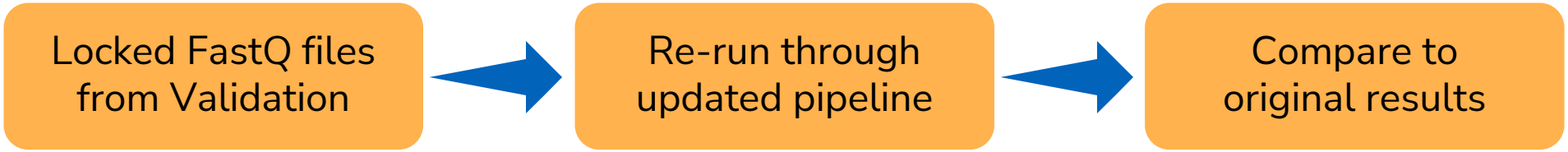
1. **Body-site-specific baselines**
   A respiratory reference panel like the LRTI tiers (prior slide) doesn't transfer to stool or CSF
2. **Abundance/burden thresholds, not presence/absence**
   Weighting by relative read abundance against expected commensal load
3. **Tiered, graduated reporting language**
   The Positive / Presumptive Positive / Inconclusive logic from Challenge 2
4. **Required clinical metadata**
   Specimen type and symptom onset paired with every result
5. **Shared public microbiome reference data**
   Human Microbiome Project baselines, akin to ClinGen/ClinVar

CHALLENGE 4: TEST UPDATES

# Updates Need a Realistic Path

Databases and pipelines evolve constantly. The test can't freeze after initial validation.

## **Potential Solution:** A "Gold Data Set" for Revalidating without Retesting




Validate database and pipeline changes by re-analyzing a fixed set of real sequencing files. For most in silico (computational) changes, no new wet-lab testing will be needed.

CHALLENGE 4: TEST UPDATES

# In Silico Testing as Core Evidence

**Wet-lab testing for every possible pathogen is not feasible. Simulated reads, spiked variation, modeled co-infections can help fill the gaps when real specimens aren't available.**

Published studies have already used this approach to validate novel-virus detection with metagenomic next-generation sequencing (mNGS)

**Example:**

A validated respiratory mNGS assay removed all known human viruses from its reference database, then tested whether the pipeline could still recognize them based on similarities to related animal and plant viruses.

**Result:** 100% All 70 representative pathogens were correctly identified.



Nature Communications volume 15, Article number: 9016 (2024)

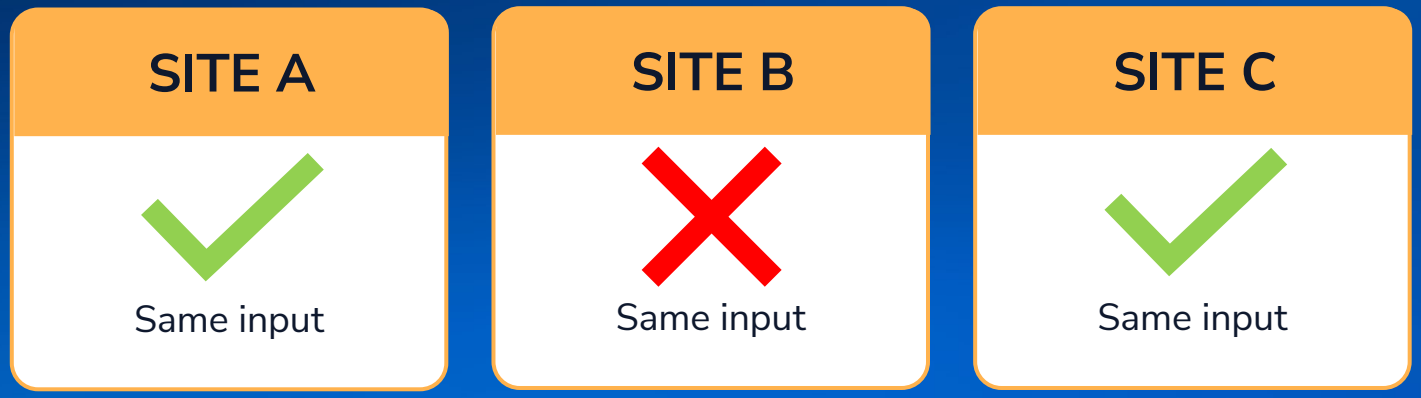
CHALLENGE 4: TEST UPDATES

# Lowering the Multi-Site Burden

Precision testing is a must across sites, operators, and instruments.

A centrally-generated synthetic challenge set could satisfy part of that burden. Matched specimens would not have to be shipped to every site. The challenge set could be reused every time the pipeline or database changes.

Identical synthetic input, different outputs.




The difference is the site's pipeline, not the sample.

CHALLENGE 5: CLINICAL TESTING

# Clinical Studies Need a Comparator — We Don't Have One

***The challenge:***

Traditional clinical studies benchmark a new process against an accepted reference method. But no single existing test covers metagenomics' open-ended target space.

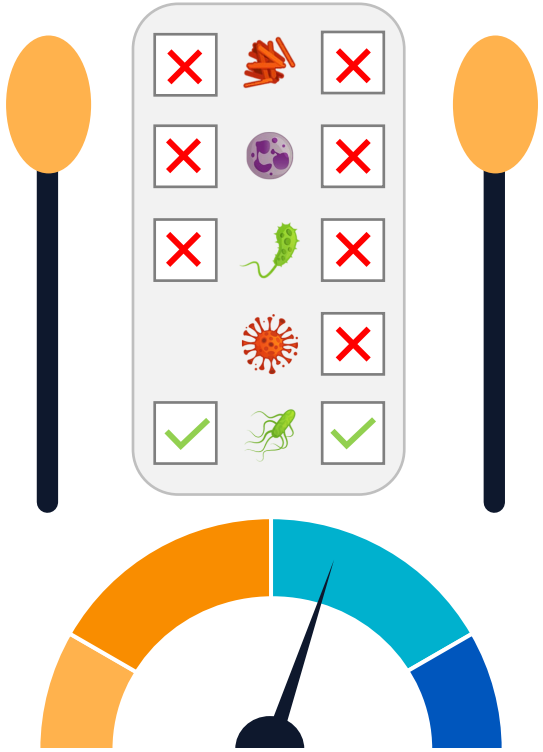
Combining culture, other test results, and clinical review can help—but the comparison becomes complicated if the sequencing result itself influences the final diagnosis.

# Clinical Studies Need a Comparator — We Don't Have One

***A Potential Partial Fix: Stage the Comparator***

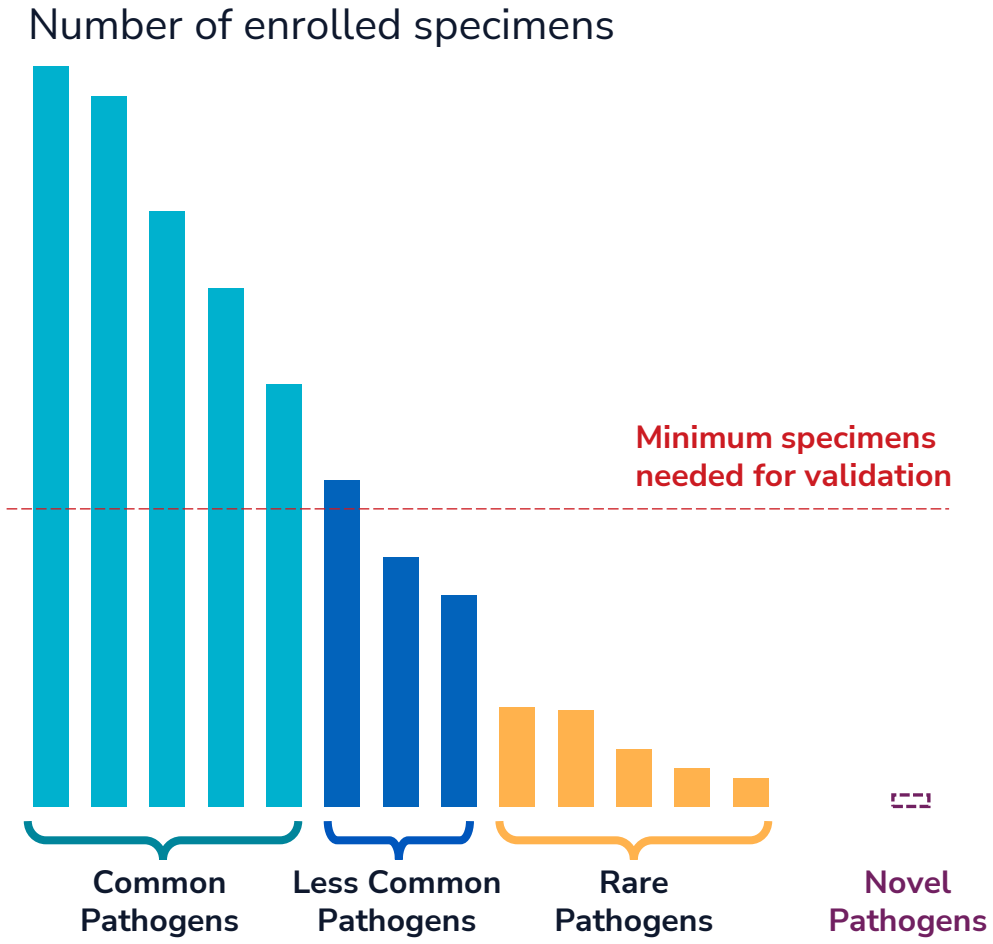
**Stage 1:**
Run sequencing data through a restricted, curated database first, matching only what the existing FDA-cleared panel can also detect.
This gives a clean, apples-to-apples agreement comparison against an accepted comparator.

**Stage 2:**
Re-analyze the same data against the full, expanded database, capturing open-ended detections separately.



This doesn't fully solve the problem but shows how to get a valid comparator for the subset of targets that overlap with existing tests.

# You Can't Power a Study for a Pathogen You Haven't Seen Yet




Real world enrollment reflects what shows up in clinical practice. A real-world study may not enroll enough patients with rare pathogens to evaluate every pathogen family.

Enriching the study with rare cases can help but may make the study less representative.

And truly novel pathogens can’t be enrolled in advance.

# You Can't Power a Study for a Pathogen You Haven't Seen Yet

## A Practical Fallback: Set the minimum and plan for gaps

1. **Set a minimum in advance**
   Pre-set a **lower bound** of specimens needed per pathogen family during the prospective trial
2. **Fill remaining gaps**
   If a family doesn't reach that bound, supplement with a **banked or surrogate specimen evaluation** (CLSI EP39-compliant) for that family specifically

Have a concrete answer to "what happens when real-world enrollment can’t cover every target”. In advance, agree on the floor **and** the fallback plan if the minimum is not met.

# Multi-Site Clinical Studies Carry a Second Variable

**A false negative at one site could mean:**

- assay limitation
- pipeline issue
- atypical patient

This is hard to disentangle without the synthetic cross-site checks running alongside the clinical study.

(Discussed in Challenge 4)

The turnaround time and real-time clinical action further blur whether the study is measuring accuracy, clinical impact, or both.

*There's an opportunity to work toward a hybrid clinical study framework-composite standards, mixed enrollment, and parallel synthetic cross-site checks-fit for an open-ended diagnostic.*

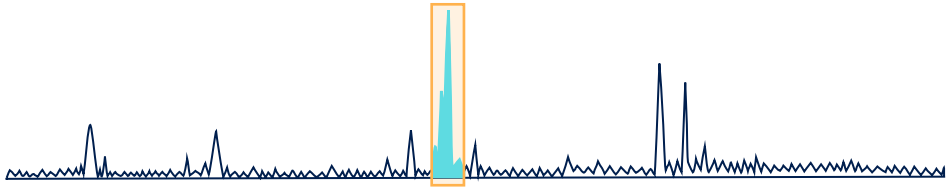
# Precedent for expandable ID Technology

MALDI-TOF is an example of a test that identifies organisms against an evolving reference library, with a mechanism for expanding that library over time.

**But the MALDI-TOF validation model doesn't scale directly to mNGS.** Its library expansion relied on a 2,695-isolate multicenter study. mNGS targets may be rare, newly characterized, or not culturable, so comparable isolate banks may not exist.

**2017 FDA Cleared MADLI –TOF test**
identifies bacteria, yeasts, molds, mycobacteria

**Initial Clearance** → **Library Update** → **Library Update**



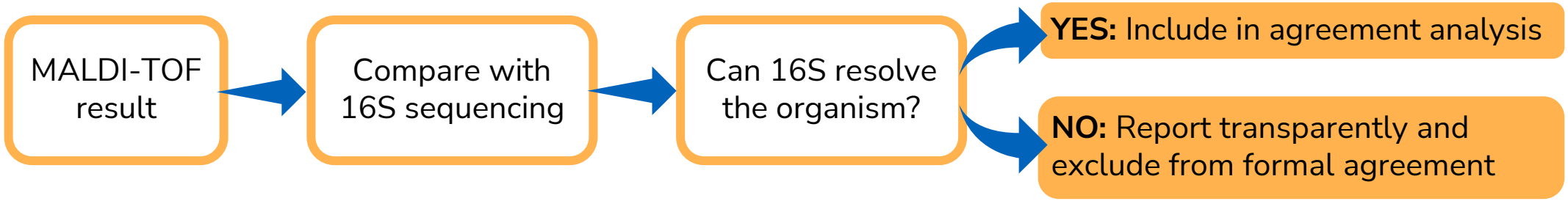
Unlike MALDI-TOF's spectral pattern...



...mNGS uses multiple sequence features to distinguish and confirm an identification.

# Working With an Imperfect Comparator

MALDI-TOF validation typically uses 16S rRNA gene sequencing as the reference comparator, but **some isolates could not be resolved to genus level by 16S itself.** These cases were reported but excluded from analysis for lack of a valid gold standard.




## Multi-site performance can be measures.

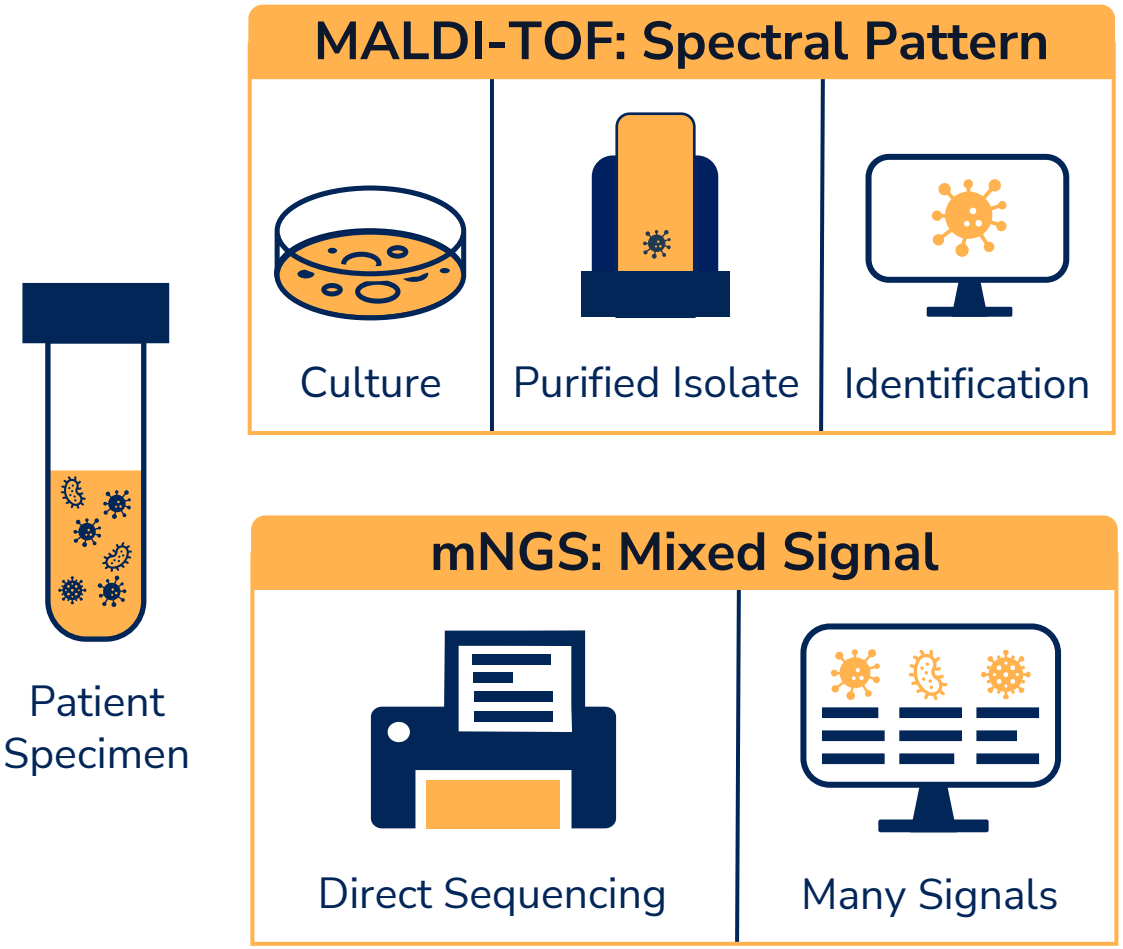
In a blind-coded study, eight laboratories were able to identify 480 samples with 98.75% reproducibility. This demonstrates that multi-site performance can be rigorously evaluated. But the specific number reflects MALDI-TOF's simpler, spectral pattern and **is not comparable enough to be used as the bar for mNGS.**

# Closer, But Not a Perfect Match

MALDI-TOF identifies **cultured, purified isolates** rather than a mixed, low-biomass clinical specimen. It largely avoids challenges such as low pathogen levels, background from commensal organisms, and discriminating closely-related organisms.

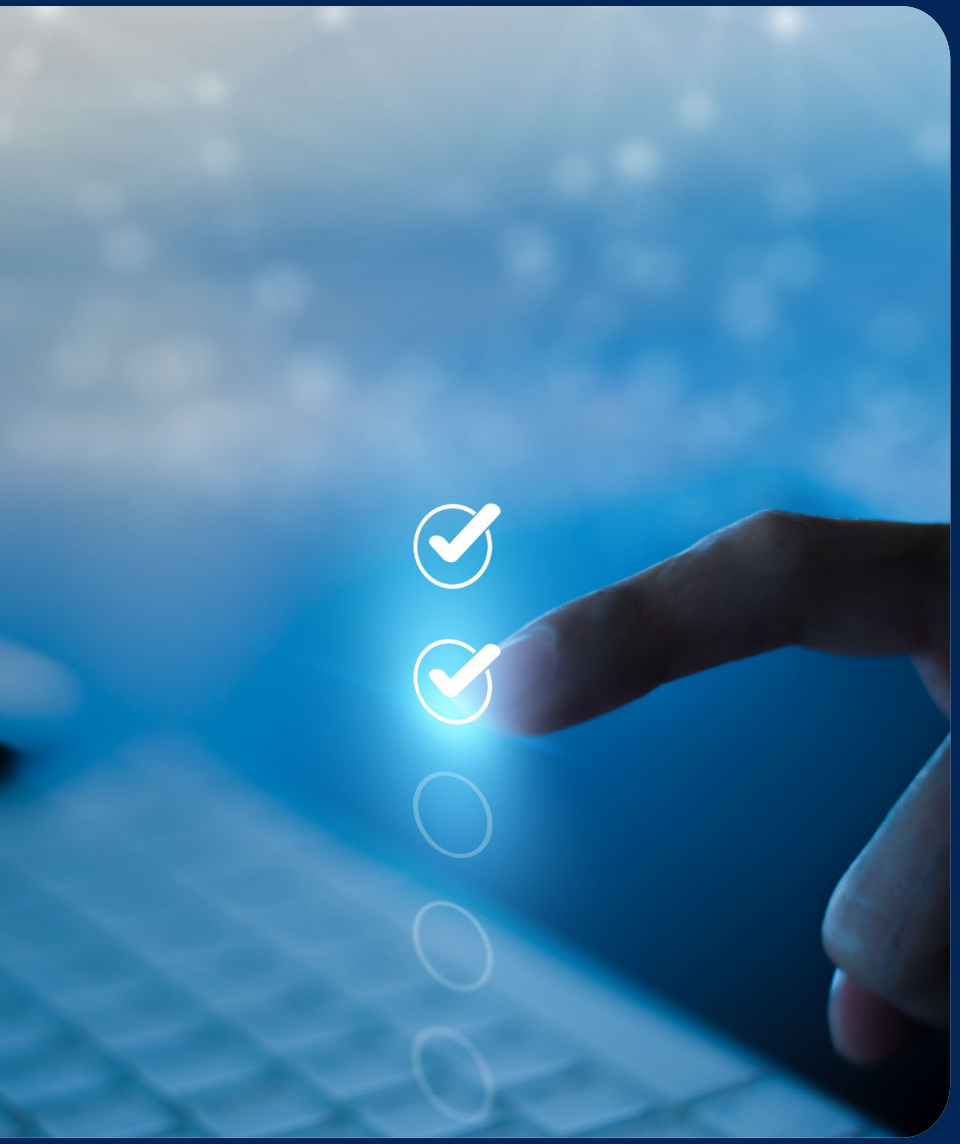
**MALDI-TOF provides useful precedent, but it only takes us part of the way.**

It shows that expandable identification and multi-site reproducibility across sites can be evaluated successfully. The remaining challenge for mNGS is to demonstrate the **complexity of direct-from-specimen, mixed-signal detection**.



# Oncology NGS Adds One More Piece

| ONCOLOGY NGS PRECEDENT | RELEVANCE HERE |
|---|---|
| **Device-level QC (2018 guidance)** | Validates method capability but not every variant<br>*Precedent for Challenge 2* |
| **Recognized public databases (ClinGen)** | External, living evidence source<br>*Precedent for ongoing updates* |



Between MALDI-TOF and oncology NGS, there are precedents established for every major tool this technology needs—open-ended ID, database growth, device-level QC, and recognized evidence sources.

A successful approach could be to combine them rather than inventing something new.

# Thank you.

For more information, contact CDC
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