P-420

ONETest Delivers Superior Target Enrichment and Improved Pathogen Detection Against Culture in BAL Samples

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Introduction

Lower respiratory tract infections remain a leading cause of morbidity and mortality[1], yet conventional microbial culture often suffers from limited sensitivity, particularly following antimicrobial therapy[2].

Whole metagenome shotgun sequencing (WmGS) has emerged as a powerful tool for broad pathogen detection to provide comprehensive unbiased microbial profiling. However, its clinical utility in host-rich matrices like bronchoalveolar lavage (BAL) is frequently constrained by overwhelming host genomic DNA, high sequencing costs, and reduced analytical sensitivity for low-abundance pathogens[3].

Targeted metagenomic sequencing circumvents these challenges by enriching for target pathogens, offering deep sequencing coverage and improved diagnostic sensitivity. In this study, we evaluated ONETest[4,5] (OT), a targeted metagenomic sequencing assay, in bronchoalveolar lavage (BAL) specimens, to assess its overall performance and pathogen detection capabilities against conventional microbiology.

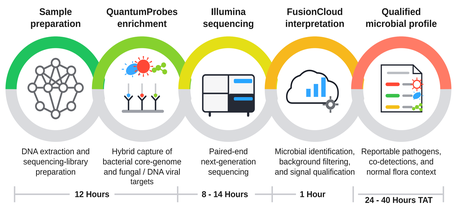
Objectives

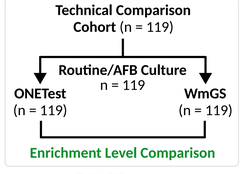
1. **Quantum Probe™ Design**
   Selection of OT panel targets
2. **Technical Performance Compared with WmGS**
   Targeted species abundance levels
3. **Assay Performance against Standard Culture**
   Species-level metrics and orthogonal PCR when available

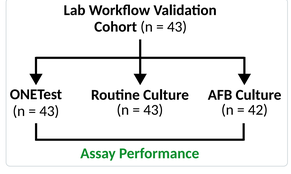
Material & Methods

How ONETest works
ONETest PathoGenome Workflow

24–40 h







More details of the ONETest methodology in our bioRxiv preprint here!

RESULTS

OneTest Panel: From Core Genome Identification to Quantum Probes Design

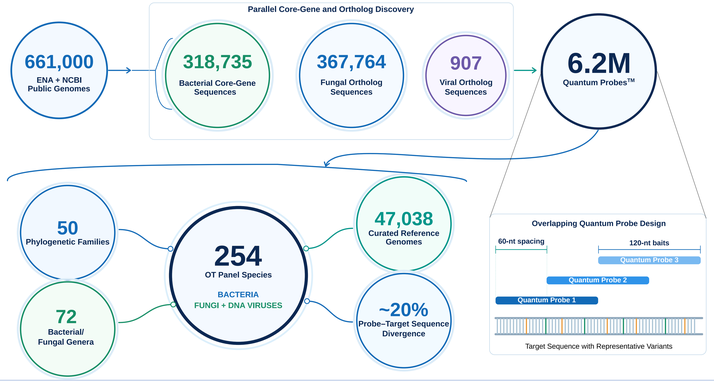


Figure. 1. A total of 687,406 target sequences, comprising bacterial core genes alongside fungal and viral orthologs, were identified from 661,000 curated genomes extracted from the European Nucleotide Archive (ENA) and NCBI public databases. These target sequences were subsequently processed to generate Quantum Probe™ (QP) designs. To enhance species- and subspecies-level resolution, QPs incorporated common nucleotide variants (frequency >1%) derived from taxon-specific multiple sequence alignments. Probes were designed with a 60-nucleotide spacing to achieve ≥2× tiling coverage for near-complete protein-coding gene recovery. Overall, 6.2 million QPs were generated, targeting core genomes and orthologs across 254 species and related lineages spanning 50 phylogenetic families.

Core-Genome Capture of *Pseudomonas aeruginosa* by OT Quantum-Probes

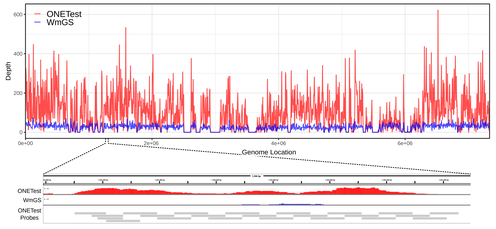


Figure. 2. Genome-wide read-depth profiles generated by OT (red) and whole-metagenome shotgun sequencing (WmGS; blue) across the P. aeruginosa genome. The OT design targets 4,171core genes using 46,287 probes, providing >2× tiling density across target regions to capture within-species genomic diversity. The upper panel shows per-base sequencing depth across the full genome, illustrating enriched coverage of targeted core-genome regions by OT compared with the lower, relatively uniform coverage obtained by WmGS. The lower panel zooms into a representative core gene, highlighting probe tiling (gray bars) and the corresponding read-depth profile. Dense, diversity-aware probe tiling supports continuous sequencing coverage across the targeted locus.

Paired Comparison of Human-Reads/ Total-Clean-Reads Ratio between OT and WmGS

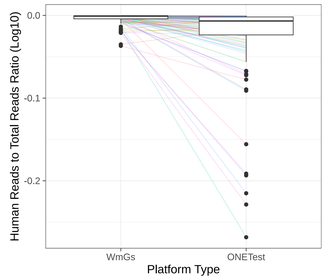


Figure. 3.Values are displayed on a log10 scale. Points represent individual samples connected by lines. Box plots indicate medians and interquartile ranges.

With WmGS sequencing depth matched to the corresponding OT library size for each specimen, OT reduced the human-read fraction by a mean of 4.2% (median 0.89%, p<0.001).

Comparative Evaluation of OT and WmGS in BAL Specimens

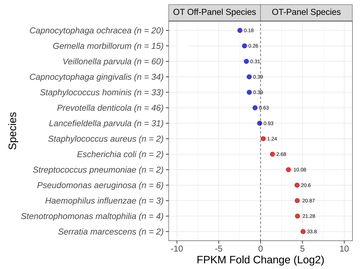


Figure. 4. Log2 fold-change in FPKM for culture-positive species, comparing OT to full-depth WmGS data across 119 BAL samples. Points represent species-specific fold-changes. n values represent the number of culture-positive samples for each species. Distinct shades denote OT panel species and species not included in the OT panel (off-panel). Species are listed in descending order of fold-change.

Species-Level performance of OT against Routine Culture

Species	Culture (+)	Agreement	Specificity	sensitivity	Additional Detection confirmed by PN Panel
Mycobacterium avium complex	10	0.60	0.97	0.60	
Pseudomonas aeruginosa	9	0.89	0.85	0.89	4/5
Achromobacter xylosoxidans	4	1.00	0.95	1.00	
Burkholderia cepacia complex	4	0.75	1.00	0.75	
Chryseobacterium indologenes	3	0.67	1.00	0.67	
Aspergillus fumigatus	2	0.50	1.00	0.50	
Enterobacter cloacae complex	2	1.00	1.00	1.00	
Klebsiella oxytoca complex	2	1.00	1.00	1.00	
Moraxella catarrhalis	2	1.00	1.00	1.00	
Mycobacteroides abscessus	2	1.00	1.00	1.00	
Paecilomyces species	2	0.00	1.00	0.00	
Staphylococcus aureus	2	1.00	0.95	1.00	1/1
Stenotrophomonas maltophilia	2	1.00	0.95	1.00	
Aspergillus niger	1	0.00	1.00	0.00	
Aspergillus species	1	0.00	1.00	0.00	
Coccidioides posadasii	1	1.00	1.00	1.00	
Haemophilus influenzae	1	1.00	0.93	1.00	3/3
Mycobacterium gordonae	1	0.00	1.00	0.00	
Mycobacterium tuberculosis complex	1	1.00	1.00	1.00	
Pasteurella multocida	1	1.00	1.00	1.00	
Pseudomonas stutzeri	1	1.00	1.00	1.00	
Streptococcus pneumoniae	1	0.00	1.00	0.00	
Micro-Average		Agreement **74.5%**	Specificity **98%**	Sensitivity **75%**	

Table. 1. Species level performance of OT against routine culture. Among the validation cohort (n = 43), ONETest achieved 74.5% agreement with culture, 98% specificity, and 75% sensitivity across recurrent species. PN Panel confirmed the presence of OT additional detections in *Pseudomonas aeruginosa* (4/5), *Staphylococcus aureus* (1/1) and *Haemophilus influenzae* (3/3). Escherichia coli and Human mastadenovirus D not identified by standard bacterial culture were also confirmed by PCR for 1/1 and 1/1 isolates, respectively.

Conclusion

Compared to wMGS, the ONETest targeted metagenomics assay demonstrated significantly higher abundance levels for culture-recoverable species, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Serratia marcescens*. Overall targeted microbial abundance increased 26-fold.

ONETest performance was evaluated against bacterial standard and AFB cultures using the laboratory workflow validation cohort (n = 43). ONETest achieved an an overall accuracy of 97%, 75% sensitivity, and 98% specificity. In addition, PN PCR panel confirmed positivity for 10/11 isolates identified by ONETest but not routine culture.

Overall, by providing enriched coverage for target pathogens at significantly lower sequencing costs and host background interference, ONETest presents a scalable and superior clinical alternative to WmGs. Furthermore, ONETest represents a highly effective complementary diagnostic approach for BAL specimens, uncovering actionable microbiological insights missed by standard culture.

Perspectives

Commensal and normal colonizing species can dilute true pathogenic signals, even in targeted metagenomic assays subject to non-specific capture. To maximize the clinical utility of ONETest, gathering larger prospective datasets will be essential to establish better dynamic metric cutoffs and calibrated clinical reporting thresholds that reliably differentiate true pathogens from background commensals.

Although ONETest significantly enhanced signal-to-noise ratio over WmGs, orthogonal PCR confirmed the presence of culture-discordant calls that were true positives. Addressing the trade-off between high sensitivity and moderate specificity will require larger prospective studies that pair molecular detection with host biomarker profiles and clinical treatment responses to refine diagnostic stewardship.

References

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Acknowledgements

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