

ONE Test™ PathoGenome

New Class of Targeted Metagenomic Assay for Diagnosing Infection

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Conflict Of Interest: Dr. Sepideh Alamouti have financial holdings in Fusion Genomics Corp.

Not to be used for human or animal testing. To be used only for the intended Purposes Only

ONE Test™ PathoGenome: New Class of Targeted Metagenomics

• Purpose	End-to-end assay to detect undiagnosed infections in hospitalized patients
• Design	Over 6.2 million QuantumProbes™ enrich core genomic regions (>700,000 coding genes) for 12 microbial Phyla
• Coverage	Encapsulates 255 species and hundreds of related genotype associated with lower respiratory infection – Massively multiplexed
• Sample Types	Validated for BAL with expansion planned to additional specimen types
• Testing Flexibility	Laboratories can run the test in-house, on-demand, on any desktop next-gen DNA sequencer
• Analytics Platform	FusionCloud™ uses machine learning analytics to deliver actionable results
• Turnaround Time	Sample to results in 24 hours


Expands the Range of Clinical Microbiological Testing

ONETest™: Post-enrichment and Adaptive Filtering Highlight Key Potential Pathogens

UFL103 – Culture (+), PCR (-)
Raw species panel detected by ONETest™

Abiotrophia_defectiva
 Achromobacter_xylosoxidans
 Acinetobacter_lwoffii
 Bacteroides_fragilis
 Bordetella_flabilis
 Bordetella_parapertussis
 Burkholderia_cenocepacia
 Burkholderia_cepacia
 Burkholderia_mallei
 Burkholderia_pseudomallei
 Burkholderia_singularis
 Burkholderia_stabilis
 Burkholderia_vietnamiensis
 Campylobacter_concisus
 Campylobacter_fetus
 Campylobacter_showae
 Candida_albicans
 Caulobacter_vibrioides
 Chryseobacterium_indologenes
 Citrobacter_freundii
 Citrobacter_koseri
 Corynebacterium_accolens
 Corynebacterium_jeikeium
 Corynebacterium_kroppenstedtii
 Corynebacterium_mucifaciens
 Corynebacterium_urealyticum
 Corynebacterium_xerosis
 CP028773.1_Pichia
 Cupriavidus_neocaledonicus
 Cupriavidus_sp
 Enterobacter_asburiae
 Enterobacter_bugandensis
 Enterobacter_cancerogenus
 Enterobacter_chengduensis
 Enterobacter_cloacae
 Enterobacter_hormaechei
 Enterobacter_kobei
 Enterococcus_faecalis
 Enterococcus_faecium
 Escherichia_coli
 Fretibacterium_fastidiosum
 Fusobacterium_nucleatum
 Haemophilus_painfluenzae
 Human_gammaherpesvirus
 Klebsiella_aerogenes
 Klebsiella_oxytoca
 Klebsiella_pneumoniae
 Lachnoanaerobaculum_umeaense
 Lautropia_mirabilis
 Limosilactobacillus fermentum
 Mesorhizobium_sp
 Mycobacterium_angelicum
 Mycobacterium_erosiens
 Mycobacterium_avium
 Mycobacterium_intracellulare
 Mycobacterium_kansasii
 Mycobacterium_malmoeense
 Mycobacterium_parascrofulaceum
 Mycobacterium_triplex
 Mycobacteroides_chelonae
 Mycolicibacterium_brisbanense
 Mycolicibacterium_farcinogenes
 Mycolicibacterium_fortituum
 Mycolicibacterium_mageritense
 Mycolicibacterium_smegmatis
 Mycolicibacterium_wolinskyi
 Neisseria_meningitidis
 Neisseria_sicca
 Nocardia_asteroides
 Nocardia_brasiliensis
 Nocardia_farcinica
 Parvimonas_micra
 Phocaeicola_vulgatus
 Porphyromonas_endodontalis
 Porphyromonas_gingivalis
 Prevotella_denticola
 Prevotella_histicola
 Prevotella_intermedia
 Prevotella_jejuni
 Prevotella_melaninogenica
 Prevotella_oris
 Propionibacterium_acnes
 Pseudomonas_aeruginosa
 Pseudomonas_fluorescens
 Pseudomonas_sp
 Pyramidobacter_piscolens
 Raistonia_insidirosa
 Rhodococcus_equi
 Rhodococcus_sp
 Rothia_dentocariosa
 Rothia_muclagimosa
 Serratia_marcescens
 Shigella_flexneri
 Shigella_sonnei
 Sneathia_vaginalis
 Staphylococcus_aureus
 Stenotrophomonas_maltophilia
 Streptococcus_agalactiae
 Streptococcus_intermedius
 Streptococcus_mitis
 Streptococcus_oralis
 Streptococcus_pneumoniae
 Streptococcus_pseudopneumoniae
 Streptococcus_pyogenes
 Variovorax_paradoxus
 Veillonella_atypica
 Veillonella_parvula

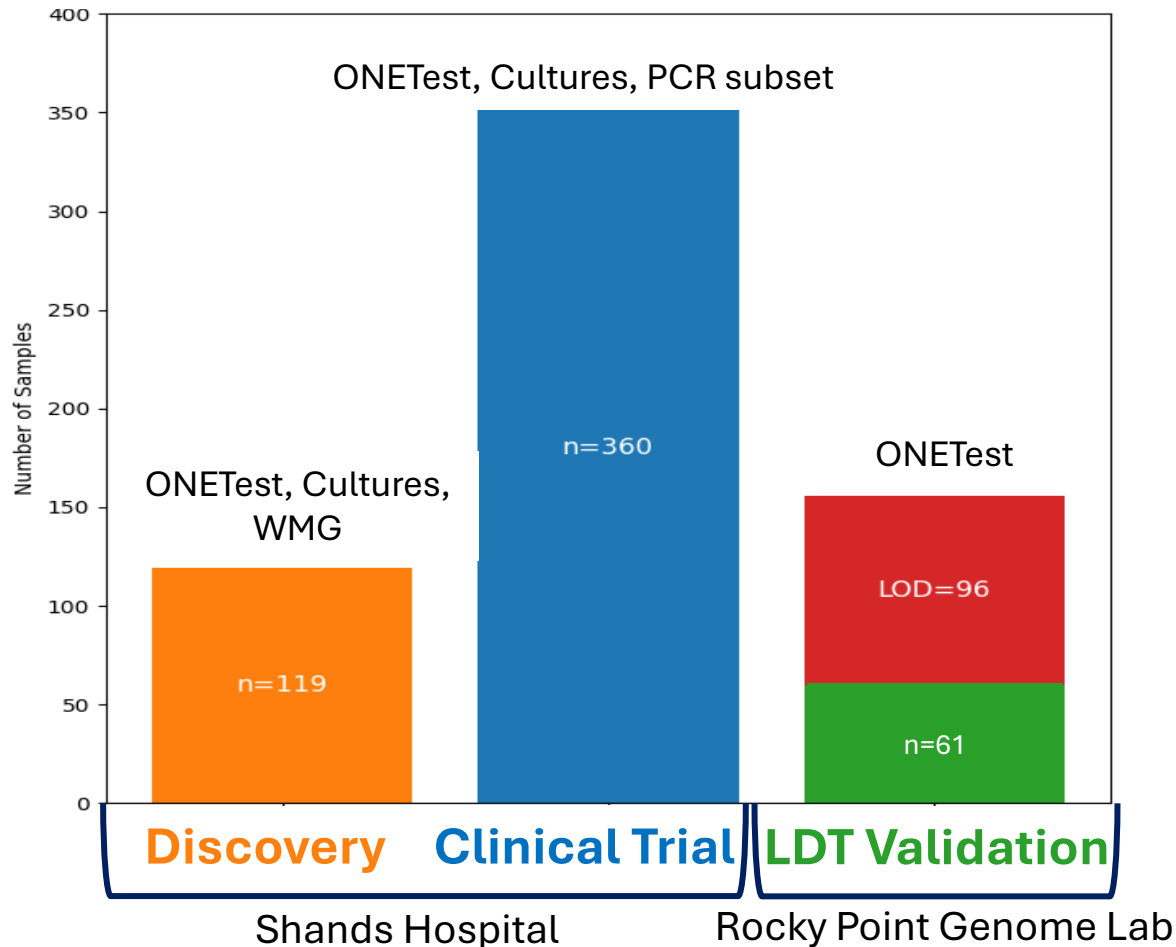
Final species panel detected by ONETest™ (Post-Enrichment and AI Processing)

Sample ID	Species	Categorization	FPKM*	Assembly Length (BoC)	Relative abundance
UFL103 	<i>Klebsiella pneumoniae</i>	Strong	0.30	31,9309 (0.007)	11.9
	<i>Enterobacter hormaechei</i>	Strong	0.18	327,898 (0.0002)	12.9
	<i>Prevotella denticola</i>	Moderate	0.11	129,600 (0.1)	7.85
	<i>Prevotella oris</i>	Moderate	0.14	161,690 (0.2)	9.89
	<i>Streptococcus oralis</i>	Weak	0.07	51,343 (0.0001)	5.19

* Normalized Depth of Coverage (Fragments Per Kilobase per Million mapped reads)

ONETest™: Validation across 540 Blinded BAL Specimens

Samples Breakdown (Clinical BAL 540 + Level of Detection 96)



360 Clinical Trial BAL Samples (Florida Shands Hospital)

- **Inclusion Criteria** Samples from patients for any reason, hematology excess
- **Gold-standard test** Bacterial and Acid-Fast Bacillus (AFB) Cultures
- **FDA-approved test** BioFire™ Pneumonia-PCR Panel (Subset 259/360)
- **Age range** 1–90 years (median: 61)
- **Primary Diagnoses** 128 transplant patients (heart, lung, kidney, liver BMT), Various respiratory and systemic conditions

ONETest™ vs. Culture: High Concordance and Balanced Performance in 360 BAL Challenges

Sample performance based on 22 species detected by culture in BAL samples

Culture Positives Cases	TP	FP	FN	TN	Accuracy	Recall	Specificity	PPV	F1
123/360	109	42	14	195	0.84	0.84	0.89	0.72	0.8

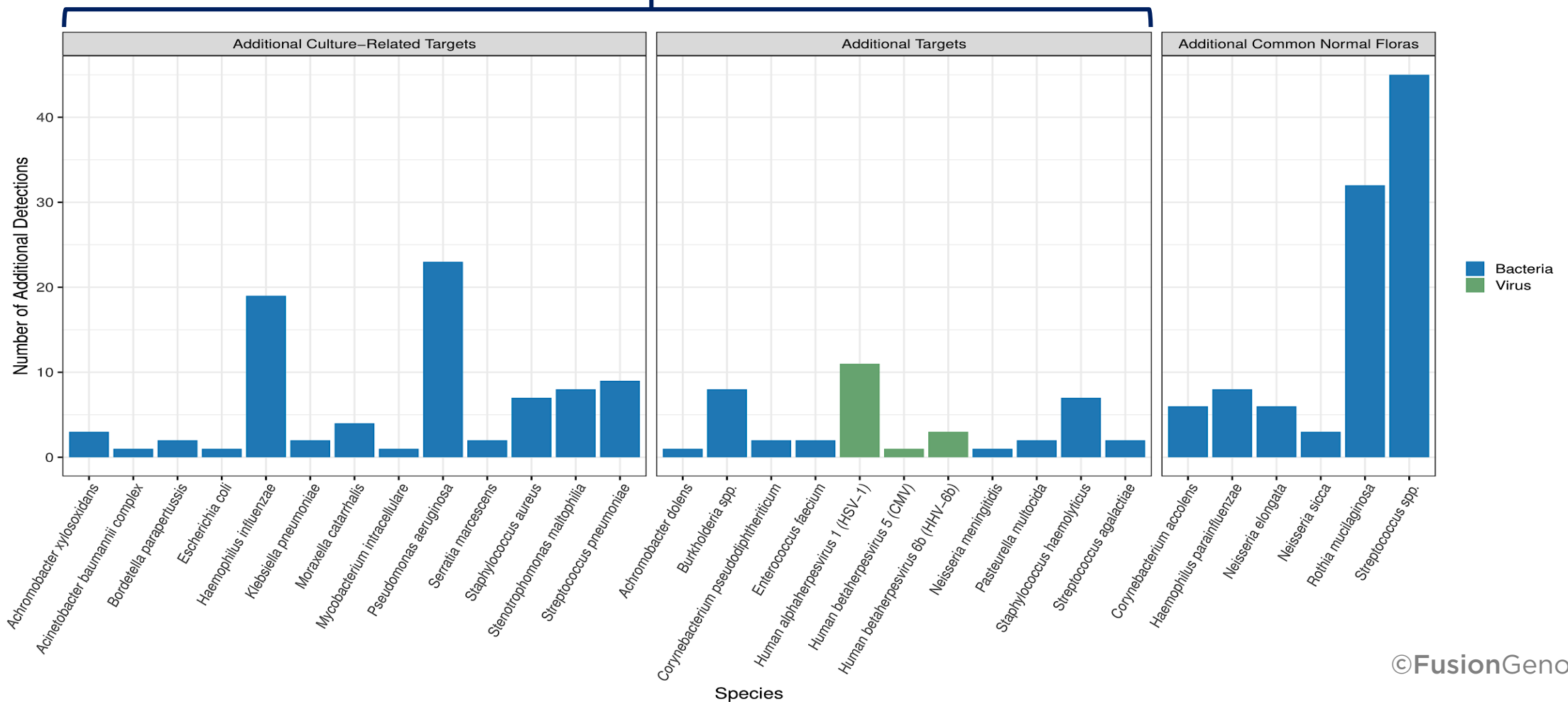
Species performance for most prevalent species 7/22 in BAL samples

Species	Culture+	TP	FP	FN	Recall	Specificity	PPV	F1
<i>Pseudomonas aeruginosa</i>	37	34	23	3	0.92	0.93	0.60	0.72
<i>Staphylococcus aureus</i>	19	14	7	5	0.74	0.98	0.67	0.70
<i>Stenotrophomonas maltophilia</i>	12	11	8	1	0.92	0.98	0.58	0.71
<i>Mycobacterium avium complex</i>	11	7	1	4	0.64	0.99	0.88	0.74
<i>Haemophilus influenzae</i>	7	7	19	0	1.00	0.95	0.27	0.42
<i>Achromobacter xylosoxidans</i>	6	5	3	1	0.83	0.99	0.63	0.71
<i>Moraxella catarrhalis</i>	6	5	4	1	0.83	0.99	0.56	0.67

Total positive signals for all 22 detected pathogens: Culture (+) 130 calls from 123 patients , ONETest only (+) 82 calls from 42 patients

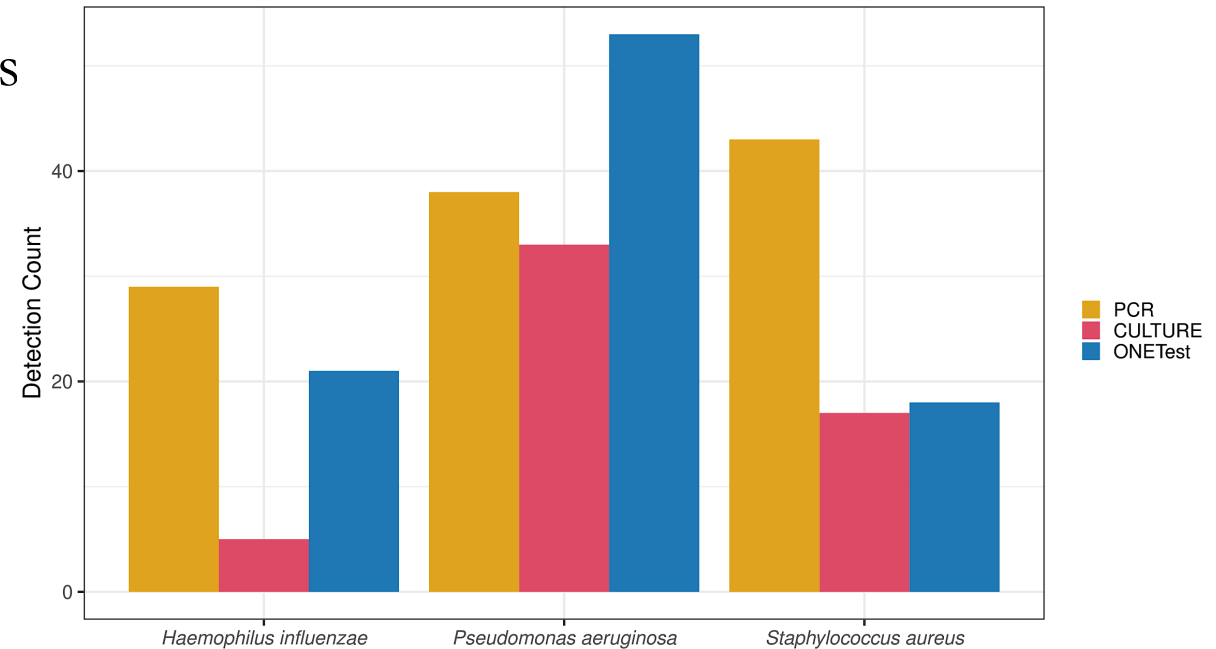
ONE Test™ Additional Detections Beyond Culture: 17.8% Additive Diagnostic Values

26 Clinically-Relevant Species (n=64 positive cases)



Detection Variability in Top 3 Respiratory Pathogens : Insights from PCR *vs.* Culture with **ONE**Test™ Context

- **Key insights:** PCR can over-detect some species while culture can suffer from sensitivity limits
- **Performance highlights:** ONE Test shows higher concordance and F1-score than PCR – each with trade-offs in precision/recall
- **Implication: ONE**Test (metagenomic) testing provides **balance** between sensitivity/precision



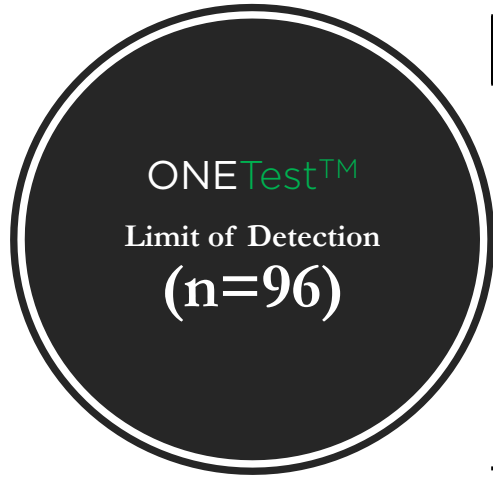
BioFire™ PCR performance *vs.* **Culture** (n=259/360)

Accuracy	76%
Precision/PPV	0.59
Recall	0.89
F1-Score	0.71

ONETest™ performance *vs.* **Culture** (n=259/360)

Accuracy	86%
Precision/PPV	0.76
Recall	0.93
F1-Score	0.84

ONETest™ LDT Validation: LoD and BAL Performance



Bacterial and Fungal spp.	960K*	96K	9.6k	960	96	10	1
<i>Aspergillus flavus</i>	+	+	+	+	+	-	-
<i>Escherichia coli</i>	+	+	+	+	+	-	-
<i>Mycobacterium kansasii</i>	+	+	+	+	+	+	-
<i>Nocardia brasiliensis</i>	+	+	+	+	+	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	-	-	-

* Colony Forming Unit (CFU)/Sample

LoD – Six species with varying genome **GC content/size** including **fastidious pathogens**

Mycobacterium detection performance against AFB on blinded BAL samples (n=61)

Species	TP	FP	FN	TN	Accuracy	Recall	Specificity	PPV	F1
<i>M. avium complex</i>	7	0	6	48	0.90	0.54	1	1	0.68
<i>Mycobacteroides abscessus</i>	2	0	1	58	0.98	0.67	1	1	0.80
<i>M. tuberculosis</i>	1	0	0	60	1.00	1.00	1	1	1.00
<i>M. goodii</i>	0	0	1	60	0.98	0.00	1	0	0.00

ONETest™ Beyond Agreement

Cases	Culture	AFB* Culture	BioFire™ PCR	ONETest™
100075579	1+ <i>Escherichia coli</i>	NA	<i>E. coli</i> 10 ^{e7} <i>Staphylococcus aureus</i> 10 ^{e4}	<i>E. Coli</i> : Strong <i>Simplex virus</i> (HSV-1): Strong
100075904	1+ <i>Achromobacter xylosoxidans</i>	NA	<i>Pseudomonas aeruginosa</i> 10 ^{e6}	<i>A. xylosoxidans</i> : Strong <i>Rothia mucilaginosa</i> : Strong
100075669	Few <i>P. aeruginosa</i>	<i>Mycobacterium intracellulare</i>	NA	<i>M. intracellulare</i> : Strong <i>P. aeruginosa</i> : Moderate
100075751	2+ <i>S. aureus</i> (MRSA)	<i>M. chimaera</i> , <i>M. avium</i> in 2020 and in 2019	<i>S. aureus</i> 10 ^{e6} MRSA <i>P. aeruginosa</i> 10 ^{e4}	<i>Mycobacterium intracellulare subsp. chimaera</i> : Strong <i>S. aureus</i> : Moderate
100156670	<i>Burkholderia cepacia-complex</i>	–	NA	<i>Burkholderia ambifaria</i> : Strong
04-494	No Growth	<i>Mycobacteroides abscessus</i> 3/27 <i>M. intracellulare</i> 8/13 (subsequent culture)	NA	<i>M. abscessus</i> : Strong <i>M. intracellulare</i> : Strong <i>A. xylosoxidans</i> : Strong

*Acid-Fast Bacillus (AFB)

Conclusions

- **Impact:** ONETest™ redefines pathogen detection with adaptive technology, empowering clinicians with actionable insights when used as part of a composite diagnostic framework.
- **Clinical Synergy:** Integrates seamlessly with existing methods, enhancing diagnostic accuracy in complex cases/specimens.
- **Path Forward:** Sets the stage for broader applications, driving next-generation healthcare solutions.
- **Deployment:** Full walk-away automation using Hamilton STAR liquid handling station & analysis by FusionCloud™

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