Validation of a fully automated 16S/ITS tNGS solution for agnostic detection of bacterial and fungal pathogens

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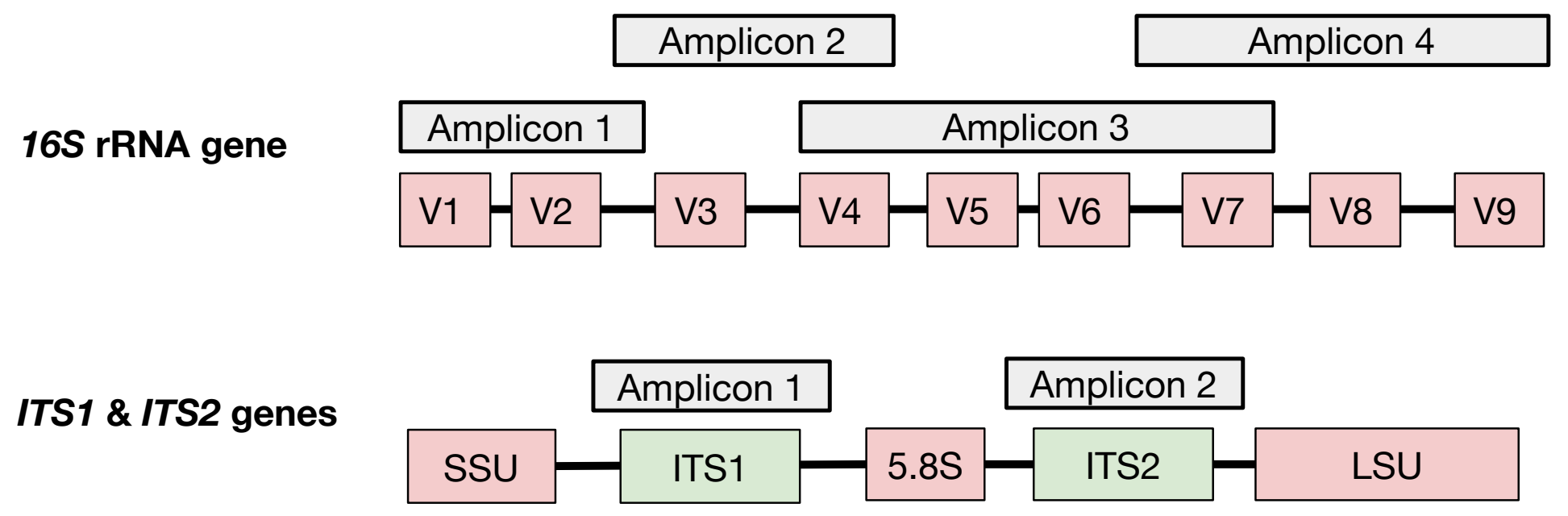
INTRODUCTION

- Agnostic detection of bacterial and fungal pathogens can be achieved via *16S* rRNA and *ITS* gene targeted next generation sequencing (tNGS), respectively.
- Complex and laborious nature of next generation sequencing (NGS) workflows create significant barriers of entry for widespread laboratory adoption.
- Integrating tNGS workflows with automation will enable laboratorians to perform complex operations with minimal training and has been proven successful for application in the food safety and infectious diseases space[1,2].
- The Clear Dx™ fully automated, cloud-integrated NGS solution has revolutionized the way laboratories in public health departments and academic medical centers approach molecular pathogen detection and testing.
- Here, we evaluated the performance of a fully automated *16S/ITS* tNGS solution across a variety of bacterial and fungal pathogens.

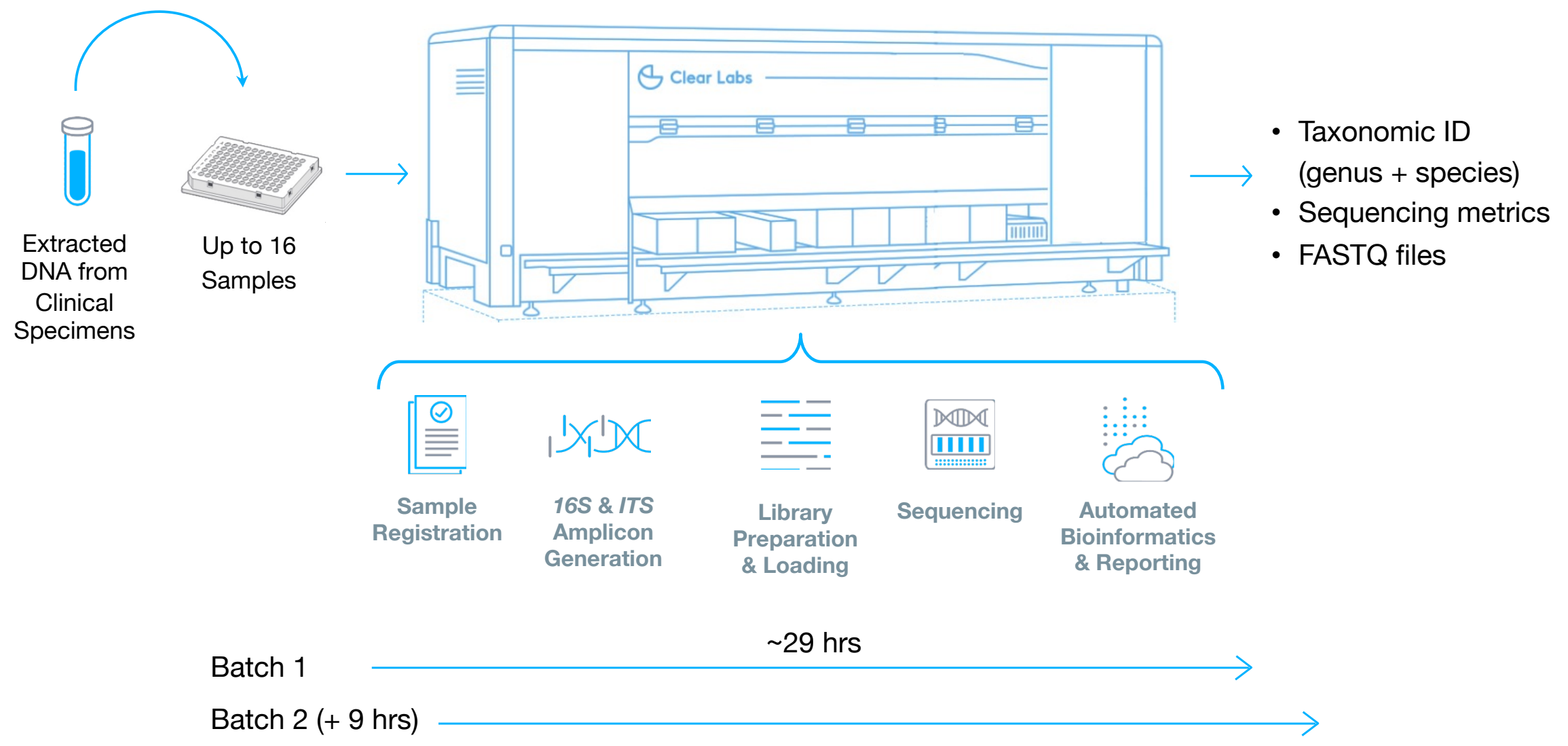
DESIGN & METHODS

The Clear Dx™ Automated Platform

The Clear Dx™ Microbial ID tNGS Assay Target



Overview of the Clear Dx™ Microbial ID tNGS Workflow



- Taxonomic ID (genus + species)
- Sequencing metrics
- FASTQ files

RESULTS

Analytical Performance with No Human Background

Organism	Concentration	# of Samples	# of Samples with Detection at the Genus Level
Bacteria	1 x 10^3 genome copies / 5 µL	10	10 (100%)
	1 x 10^2 genome copies / 5 µL	37	35 (94.6%)
	1 x 10^1 genome copies / 5 µL	10	7 (70%)
Fungi	1 x 10^3 genome copies / 5 µL	9	9 (100%)
	1 x 10^2 genome copies / 5 µL	9	9 (100%)
	1 x 10^1 genome copies / 5 µL	27	27 (100%)

Contrived Performance with Human Background

Organism	Concentration	# of Samples	# of Samples with Detection at the Genus Level
Bacteria	1 x 10^3 genome copies / 5 µL	66	61 (92.4%)
	1 x 10^2 genome copies / 5 µL	69	62 (89.9%)
	1 x 10^1 genome copies / 5 µL	18	15 (83.3%)
Fungi	1 x 10^3 genome copies / 5 µL	19	19 (100%)
	1 x 10^2 genome copies / 5 µL	16	16 (100%)
	1 x 10^1 genome copies / 5 µL	46	35 (76.1%)

Analytical LoD of the assay is 10^3 genome copies / 5 µL for bacteria and 10^2 genome copies / 5 µL for fungi

Positive Control and No Template Control Performance

Controls	Performance
Positive Control	12 / 12 Detected for both Bacterial & Fungal DNA
No Template Control	11 / 14* Not Detected for Bacterial or Fungal DNA

* Three NTCs returned low-level positive results for environmental organisms commonly associated with contamination (e.g., *Methylobacterium*, *Bradyrhizobium*, *Mesorhizobium terrae*, *Lawsonella clevlander*, *Sphingomonas*). These results are presumed to be due to environmental contamination during sample handling or processing.

Bacteria and Fungi Validated to Date

Bacteria			Fungi
Acinetobacter	*Escherichia*	*Neisseria*	*Aspergillus*
Bacillus	*Francisella*	*Pluralibacter*	*Candida*
Bacteroides	*Klebsiella*	*Proteus*	*Cryptococcus*
Borreliella	*Legionella*	*Pseudomonas*	*Encephalitozoon*
Brucella	*Listeria*	*Salmonella*	*Fusarium*
Burkholderia	*Micrococcus*	*Serratia*	*Mucor*
Campylobacter	*Moraxella*	*Shigella*	*Nakaseomyces*
Citrobacter	*Morganella*	*Staphylococcus*	*Rhizopus*
Clostridioides	*Mycobacterium*	*Streptococcus*	*Sporothrix*
Clostridium	*Mycobacteroides*	*Ureaplasma*	
Corynebacterium	*Mycolicibacterium*	*Vibrio*	
Enterobacter	*Mycoplasma*	*Yersinia*	
Enterococcus	*Mycoplasmoides*		

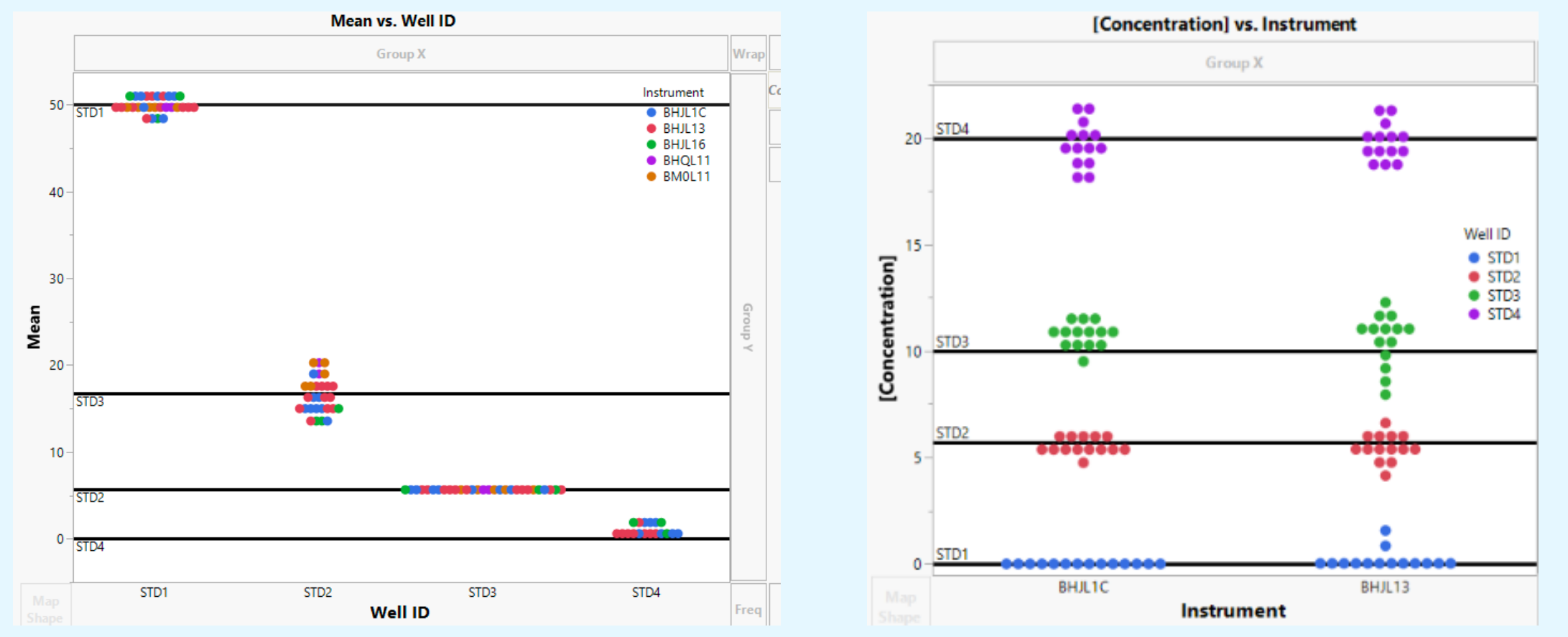
Internal Validation Studies – Sequencing Run Results

	% R1 ≥ Q30	% R2 ≥ Q30	% Occupancy	% PhiX Detected	Total Yield (Gb)
Acceptance Criteria	*≥ 80.0%*	*≥ 80.0%*	*≥ 77.0%*	*≤ 1.80%*	*≥ 0.71*
Run 1	91.3	89.2	90.5	1.04	1.7
Run 2	89.2	87.3	84.5	1.42	1.58
Run 3	89.1	87.1	85.9	1.70	1.61
Run 4	87.5	85.1	84.1	1.25	1.45
Run 5	91.1	90.1	88.6	0.80	1.64
Run 6	91.1	88.8	95.5	0.55	1.64
Run 7	88.4	86.3	90.9	1.21	1.6
Run 8	87.9	86.7	96.4	0.36	1.53
Run 9	88.7	86.1	89.3	1.16	1.54
Run 10	89.7	88.0	91.0	0.73	1.6
Run 11	88.9	86.0	89.5	0.89	1.54
Run 12	89.5	86.7	83.0	0.69	1.51

Internal Validation Studies – Sample Level Results

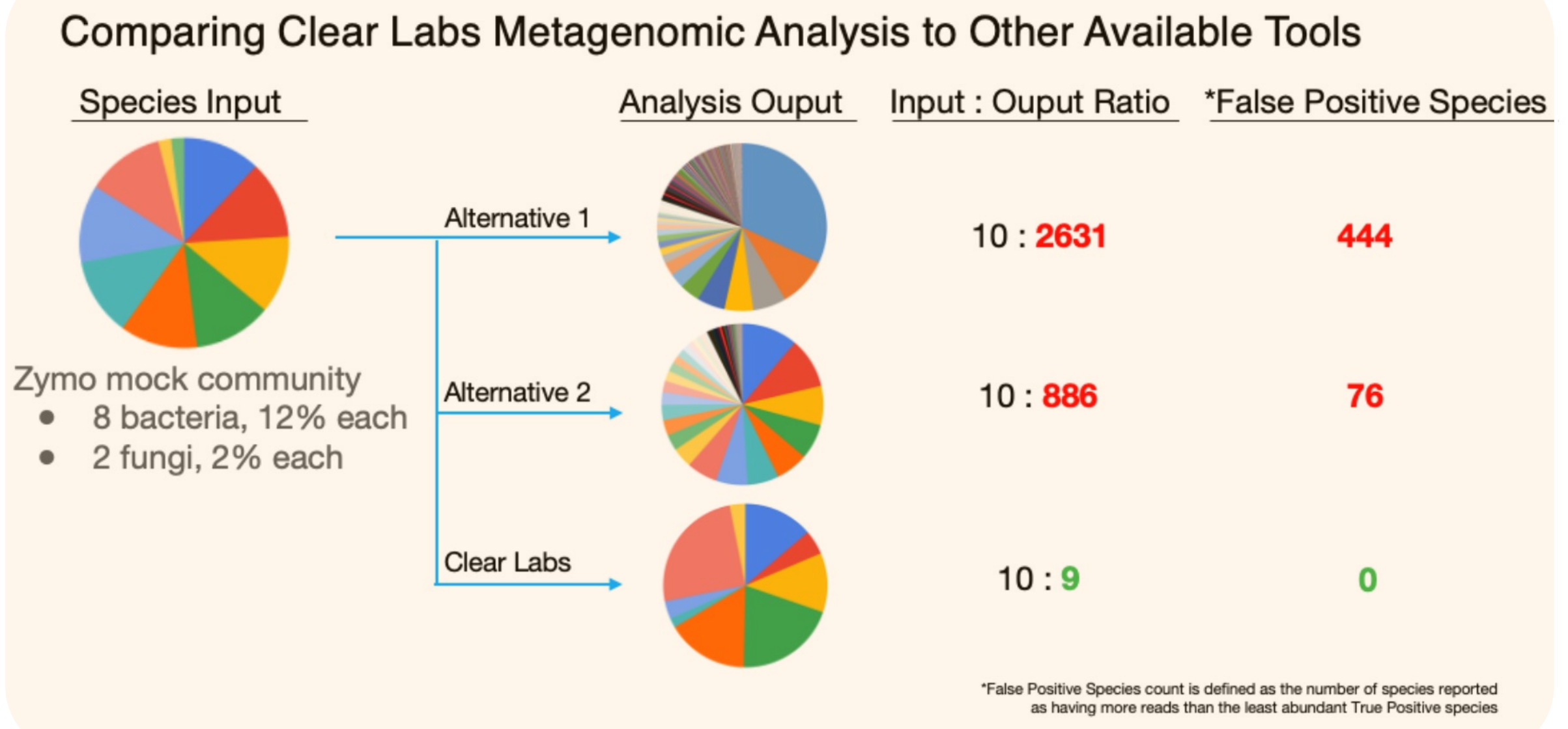
Study	Metric	Sample Acceptance criteria	Overall Sample Level Performance
Analytical LoD for Bacteria	Single infection detection rate to at least the genus level	**≥70%** of samples with no background at ≥10^2 cp/uL should be detected	93.0% (25/27 Samples Detected)
Analytical LoD for Fungi	Single infection detection rate to at least the genus level	**≥45%** of samples with no background at ≥10^1 cp/uL should be detected	100% (18/18 samples detected)
Contrived Bacterial Sample Performance	Single infection detection rate to at least the genus level	**≥75%** of samples in 20ng/uL blood at ≥10^2 cp/uL should be detected	83.0% (40/48 samples detected)
Contrived Fungal Sample Performance	Single infection detection rate to at least the genus level	**≥75%** of samples in 20ng/uL blood at ≥10^1 cp/uL should be detected	77.8% (11/27 samples detected)
NTC (No Background)	Not Detected	**≥60%** of NTCs with no background should be called Not Detected for both Bacteria AND Fungi	78.5% (11/14 samples not detected)
Contrived Co-infection Sample Performance	≥3 pathogens detected in co-infection samples to at least the genus level	**≥40%** of co-infection samples should detect at least 3 organisms	77.7% (14/18 co-infection samples detected)

Internal Validation Studies – On-Deck Quantification



Automated quantification steps were highly reproducible on the Clear Dx™ platform

Validation of Clear Labs' Bioinformatics Analytical Pipeline



*False Positive Species count is defined as the number of species reported as having more reads than the least abundant True Positive species

Time and Motion Study

	Clear Dx™	Manual tNGS
Time to results	~29 Hrs	2-10 Days*
Approximate hands-on time	~30 Mins	9-10 Hrs
Human touchpoints	1	>20

* Internal or send-out testing, including specimen shipping

CONCLUSION

- The Clear Dx™ Microbial Microbial ID tNGS is a fully automated, end-to-end solution for agnostic bacterial and fungal detection in a single assay from clinical specimens.
- Ability to detect down to 10^3 genome copies/5 µL for bacteria and 10^2 genome copies/5 µL for fungi in the presence of host DNA background
- Robust and easy-to-use application with integrated bioinformatics analysis for cleaner, more actionable results.
- Empower laboratories to effectively detect bacterial and fungal pathogens in-house with minimal technical expertise and hands-on time.

Come chat with us at **Booth #811**!

Check out **Poster ID057** for the performance of this assay on FFPE specimens in collaboration with Dr. Rebecca Yee from GWU!

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[1] Abdullah, K. *et al.* Applications of Clear Dx whole genome sequencing system in SARS-CoV-2 diagnostics. *J. Infect. Public Health.* 2022, **15**(8), 894-895.
[2] Holland, I. & Davies, J.A. Automation in the Life Science Research Laboratory. *Front. Bioeng. Biotechnol.* 2020, **8**, 571777.

For more information regarding Clear Dx™ products, please contact inquiries@clearlabs.com
For specific information regarding the poster, please contact justin.ng@clearlabs.com

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Disclaimer:
The Clear Dx™ instrument is for **Research Use Only (RUO)** and is not intended for diagnostic purposes.