



## Microbial Inactivation Testing Summary Report

## EXPERIMENTAL DESIGN - 1

**Objective:** Testing the efficiency of PathoGene Transport Media for the inactivation of bacteria, fungi, spores and viruses.

**Approach:** PathoGene Transport Media was spiked with known concentrations of bacteria, yeast, bacterial spores, fungal spores, and viruses as follows:

- 1 x 10<sup>6</sup> CFU/mL, *E. coli*
- 1 x 10<sup>6</sup> CFU/mL, *S. aureus*
- 1 x 10<sup>6</sup> CFU/mL, *B. subtilis* Spores
- 1 x 10<sup>3</sup> CFU/mL, *Aspergillus niger* Spores
- 1 x 10<sup>3</sup> CFU/mL, Baker's Yeast
- 10<sup>4</sup> TCID<sub>50</sub>/mL, Rhinovirus 16, HRV16
- 10<sup>8</sup> TCID<sub>50</sub>/mL, Adenovirus Type 5

Each organism and virus was dispensed and suspended using the concentrations above into the PathoGene Transport Media device and incubated at room temperature. Then aliquots of 20 µL were tested for growth in several types of growth media at several time points (30 mins | 2 hrs | 6 hrs | 12 hrs | 2 days | 7 days).

- *E. coli*, *S. aureus* and *B. subtilis* Spores - LB Agar incubated at 37°C
- *Aspergillus Niger* Spores and Baker's Yeast - Sabouraud Dextrose Agar incubated at 30°C
- Rhinovirus 16, HRV16 - inoculated a 96-well plate confluent with MDCK cells
- Adenovirus Type 5 - inoculated a 96-well plate confluent with A549 Cells

	INCUBATION TIME						
	0 Mins/Positive Control	30 mins	2 hrs	6 hrs	12 hrs	2 Days	7 Days
<i>E. coli</i>	1 x 10 <sup>6</sup> CFU/mL	10 CFUs	NG	NG	NG	NG	NG
<i>S. aureus</i>	1 x 10 <sup>6</sup> CFU/mL	20 CFUs	NG	NG	NG	NG	NG
<i>B. subtilis</i> Spores	1 x 10 <sup>6</sup> CFU/mL*	32 CFUs	NG	NG	NG	NG	NG
<i>Aspergillus niger</i> Spores	1 x 10 <sup>3</sup> CFU/mL**	NG	NG	NG	NG	NG	NG
Baker's Yeast	1 x 10 <sup>3</sup> CFU/mL**	NG	NG	NG	NG	NG	NG
Rhinovirus 16, HRV-16	10 <sup>4</sup> TCID <sub>50</sub> /mL	ND	ND	ND	ND	ND	ND
Adenovirus Type 5	10 <sup>8</sup> TCID <sub>50</sub> /mL	ND	ND	ND	ND	ND	ND

\**B. subtilis* Spores were incubated to allow for growth and confirm viability for proper count.

\*\**Aspergillus niger* Spores and Baker's Yeast were incubated to allow for growth and confirm viability for proper count.

NG = No growth detected.

ND = No cytopathic effect detected.

### CONCLUSION

PathoGene Transport Media stabilizing buffer effectively deactivated bacteria, fungi, spores, and both DNA and RNA viruses.

Experiment 2 on the next page.

#### CONTACT CYGNUS MEDICAL

965 West Main Street, Branford, CT 06405 | Fax: 203.764.1858 | Email: sales@cygnusmedical.com  
www.cygnusmedical.com | 800.990.7489

# Microbial Inactivation Testing Summary Report

## EXPERIMENTAL DESIGN - 2

**Objective:** Testing the efficiency of PathoGene Transport Media in stabilizing microbial species in a pooled sample, including fungi, spores, and viruses.

**Approach:** Pool fecal samples from 10 individuals, then collect the pooled fecal material with PathoGene Transport Media as per product IFU.

**Spike the collected sample with 5  $\mu$ L from the following:**

- 1 x 10<sup>2</sup> CFU/mL, *Aspergillus niger* Spores
- 1 x 10<sup>2</sup> CFU/mL, Baker's Yeast
- 10<sup>4</sup> TCID<sub>50</sub>/mL, Rhinovirus 16, HRV16
- 10<sup>4</sup> TCID<sub>50</sub>/mL, Adenovirus Type 5

Extract DNA and RNA from the same pooled sample with PowerSoil DNA<sup>®</sup>, and PowerSoil RNA<sup>®</sup> Extraction Kits. The DNA and RNA was analyzed by qPCR as per: Gorzelak et. al. *Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool*. PLOS ONE 10(8): 1-14, 2015.

		Room Temperatures (15°C - 45°C) <sup>§</sup>			4°C	***(-20°C)				****(-80°C)	
		DAYS >	0	7 $\diamond$	14 $\diamond$	180	60	90	180	360	180
PATHOGENE TRANSPORT MEDIA	Stool Real-Time Stability Testing (pooled fecal and spiked.)	DNA Yield* ( $\mu$ g)	3.22	3.51	3.27	3.05	2.99	3.12	3.23	3.09	3.54
		DNA A260/280	2.07	2.04	2.11	2.00	1.85	1.93	2.00	1.88	1.89
		DNA A260/230	1.78	1.92	2.07	1.70	1.78	1.75	1.83	1.85	1.90
		RNA Yield** ( $\mu$ g)	4.60	4.70	4.30	3.90	4.50	4.11	4.20	4.35	4.25
		RNA A260/280	1.95	1.59	1.85	1.82	1.79	1.89	1.69	1.81	1.92
		RNA A260/230	2.19	1.98	1.79	2.02	1.91	2.05	2.10	1.99	1.95
		Bacteroidetes (Ct)	32.18	32.9	32.68	31.99	32.12	32.31	32.01	32.55	33.01
		Bifidobacteria spp (Ct)	21.91	20.9	22.80	22.10	20.85	21.64	21.52	20.98	22.10
		Enterobacteriaceae (Ct)	16.95	16.38	17.10	16.50	17.10	16.80	17.20	16.89	17.03
		Firmicutes (Ct)	13.77	13.66	13.64	12.83	12.66	13.21	13.25	12.77	12.71
		Lactobacillus spp (Ct)	25.07	23.97	25.85	24.88	25.06	24.33	24.98	24.82	23.99
		Universal Eubacteria (Ct)	13.88	13.24	14.91	13.65	13.59	14.11	13.96	13.61	16.89
SPIKED	1 x 10 <sup>2</sup> CFU/mL	<i>Aspergillus niger</i> Spores	27.00	26.30	27.10	26.40	26.70	26.70	27.20	26.10	26.80
SPIKED	1 x 10 <sup>2</sup> CFU/mL	Baker's Yeast	26.30	26.40	26.40	26.30	26.40	26.50	26.00	26.50	26.20
SPIKED	10 <sup>4</sup> TCID <sub>50</sub> /mL	Rhinovirus 16, HRV-16	25.20	24.90	24.80	25.00	25.30	25.30	25.30	25.10	26.10
SPIKED	10 <sup>8</sup> TCID <sub>50</sub> /mL	Adenovirus Type 5	22.90	22.00	22.30	21.00	22.00	21.90	20.90	21.80	22.50

\*DNA Isolation - PowerMicrobiome

\*\*RNA Isolation - PowerMicrobiome

\*\*\* Samples stored at -20°C post 4 weeks room temperature storage

\*\*\*\* Samples stored at -80°C post 4 weeks room temperature storage

**$\diamond$  Pooled & spiked samples:** Subjected to actual ambient temperature transport cycle across the US which was designed to take 7 days, 14 days, or 4 weeks in transit times respectively, using standard USPS mailing services. The samples were then shipped back and forth between locations until the desired testing point had been achieved, and processed in the lab upon arrival.

**$\S$  Room temperature shipping and storage tolerance range determination process:** Collected samples were shipped with LogTag TRIX-8 Temperature Data Recorder (part #TRIX-8, MicroDAQ, Contoocook, NH). The data was then extracted using LogTag LTI-HID USB Docking Station (part # LTI-HIDLogTag) and Analyzer Software, Version 2.9.8. Based on the transit temperature recordings the samples were subjected to temperatures ranging from 15°C to 45°C during the shipping process. Shipping the samples did not involve any refrigeration or cold shipping enhancers such as cold packs or dry ice. Based on this data the room temperature tolerance range of the collected samples was determined to be between 15°C to 45°C as indicated in the table.

### CONTACT CYGNUS MEDICAL

965 West Main Street, Branford, CT 06405 | Fax: 203.764.1858 | Email: sales@cygnusmedical.com  
www.cygnusmedical.com | 800.990.7489