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Antimicrobial Efficacy Testing of a Peroxyacetic acid based Disinfectant - Hyper San - Against microbial biofilm of *Pseudomonas aeruginosa* and *Listeria monocytogenes* species using the MBEC™ Assay following ASTM E2799-11 Standard.

FINAL REPORT

Contract Research Manager:
Amin Omar, Ph.D., B. Pharma
Innovotech Inc.
Suite 101 -- 2011 94 St.
Edmonton, Alberta
Canada T6N 1H1
Phone: (780) 448-0585 ext 228
Fax: (780) 424-0941
Email: amin.omar@innovotech.ca

Laboratory Supervisor:
Nicole Butler, B.Sc.
Innovotech Inc.
Phone: (780) 448-0585 ext 234
Fax: (780) 424-0941
Email: nicole.butler@innovotech.ca

Study Director:
Amin Omar, Ph.D., B. Pharma
Study Director, Innovotech Inc.

Customer:
Pierre Stewart
Manager of Technical Services / Directeur Technique
West Penetone Inc.
514-355-6060, Ex. 3007
Fax: 514-355-2319
Email: pstewart@westpenetoneinc.com

Approved By:

_____ _____

Judy Yeung, Quality Officer
Innovotech Inc.

Date

_____ _____

Amin Omar, Study Director
Innovotech Inc.

Date

1. Scope

1.1 This test method specifies the operational parameters required to grow and treat a different bacterial biofilms in a high throughput screening assay known as the MBEC™[1] (Minimum Biofilm Eradication Concentration) Physiology and Genetics Assay. The assay device consists of a plastic lid with ninety-six (96) pegs and a corresponding receiver plate with ninety-six (96) individual wells that have a maximum 200 µL working volume. Biofilm is established on the pegs under batch conditions (i.e., no flow of nutrients into or out of an individual well) with gentle mixing. The established biofilm is transferred to a new receiver plate for disinfectant efficacy testing.[1,2] The reactor design allows for the simultaneous testing of multiple disinfectants or one disinfectant with multiple concentrations, and replicate samples, making the assay an efficient screening tool.

5. Significance and Use

5.1 Vegetative biofilm bacteria are phenotypically different from suspended planktonic cells of the same genotype. Biofilm growth reactors are engineered to produce biofilms with specific characteristics. Altering either the engineered system or operating conditions will modify those characteristics. The goal in biofilm research and efficacy testing is to choose the growth reactor that generates the most relevant biofilm for the particular study.

5.2 The purpose of this test method is to direct a user in how to grow, treat, sample and analyze different bacterial biofilms using the MBEC™ Assay. Microscopically, the biofilm is sheet-like with few architectural details as seen in Harrison et al (**6**). The MBEC™ Assay was originally designed as a rapid and reproducible assay for evaluating biofilm susceptibility to antibiotics (**2**). The engineering design allows for the simultaneous evaluation of multiple test conditions, making it an efficient method for screening multiple disinfectants or multiple concentrations of the same disinfectant. Additional efficiency is added by including the neutralizer controls within the assay device. The small well volume is advantageous for testing expensive disinfectants, or when only small volumes of the disinfectant are available.

Table 1: List of Tested Disinfectants.

Lot Code	Lot Number	Disinfectant	Dilution Range	Source	Class
1	14-190712	Hyper San	1.6%v/v	West Penetone	Oxidizing agent
2	14-230512	Hyper San	1.6%v/v	West Penetone	Oxidizing agent
3	201059	Hyper San	1.6%v/v	West Penetone	Oxidizing agent

Table 2: Disinfectant Content Description.

CODE	Disinfectant	Active Ingredients	Biocide Class	Description
A	Hyper San	Peroxyacetic acid based	Oxidizing agent	Test compound

[1] The MBEC™ trademark is held by Innovotech, Inc., Edmonton, Alberta, Canada.

Table 3: Challenge Time for tested disinfectant

Test Compound	Contact time
Hyper San	10 min, 20 min and 30 min

Table 4: List of tested Microorganisms:

Microorganisms	Source	Dilution	Growth Conditions
Listeria monocytogenes	ATCC 19114	1,000X	BHIB+20% human serum/BHIA, 18-24 hours, aerobic at 37±1ºC
Pseudomonas aeruginosa	ATCC 27853	1,000X	TSB+20% human serum /TSA, 18-24 Hours, aerobic at 37±1ºC

15.0 RESULTS

Calculations:

CFU/mL = (CFU/10µL)/0.01mL
CFU/peg = (Raw Data/0.01mL)*0.2
Log_{10} CFU/peg = Log_{10}(CFU/Peg+1)
Log Reduction= Average Log10 (Growth Control) - Average Log10 (Test Compound)
Statistics - Non-pairwise, two-tailed Student's T-test (for statistical significance, $p \leq 0.05$)
GC = Growth Control
SC = Sterility Control
BGC = Biofilm Growth Check

NOTE: The Hyper San lot codes used in the following tables correspond to the assigned West Penetone lot numbers (Lot 1 = 14-190712, Lot 2 = 14-230512 and Lot 3 = 201059).

15.1 10 Minute Data

15.1.1 Average Log Reduction for each strain and Hyper San lot tested at the 10 minute time point.

		10 Minute					
		L. monocytogenes ATCC 19114			*P. aeruginosa* ATCC 27853		
Test Compound	Lot	Log Reduction	P Value	S/NS	Log Reduction	P Value	S/NS
Hyper San (A)	1	5.25	0.00	S	7.12	0.00	S
	2	5.25	0.00	S	7.12	0.00	S
	3	5.25	0.00	S	7.12	0.00	S

15.2 20 Minute Data

15.2.1 Average Log Reduction for each strain and Hyper San lot tested at the 20 minute time point.

		20 Minute					
		L. monocytogenes ATCC 19114			*P. aeruginosa* ATCC 27853		
Test Compound	Lot	Log Reduction	P Value	S/NS	Log Reduction	P Value	S/NS
Hyper San (A)	1	5.07	0.00	S	7.24	0.00	S
	2	5.07	0.00	S	7.24	0.00	S
	3	5.07	0.00	S	7.24	0.00	S

15.3 30 Minute Data

15.3.1 Average Log Reduction for each Hyper San lot and strain tested at the 30 minute time point.

		30 Minute					
		L. monocytogenes ATCC 19114			*P. aeruginosa* ATCC 27853		
Test Compound	Lot	Log Reduction	P Value	S/NS	Log Reduction	P Value	S/NS
Hyper San (A)	1	4.26	0.00	S	6.97	0.00	S
	2	4.26	0.00	S	6.97	0.00	S
	3	4.26	0.00	S	6.97	0.00	S

16.0 DISCUSSION AND CONCLUSION

16.1 Log Reduction summary table for the 10, 20 and 30 minute time points which shows the average log reduction values for each organism and Hyper San lot tested when the test sample was compared to the average growth control value**.** Yellow shading indicates an average Log reduction value greater than or equal to 4.

			Average Log Reduction		
Organism	Lot Code	Hyper San Lot	10 Minute	20 Minute	30 Minute
L. monocytogenes ATCC 19114	1	14-190712	5.25	5.07	4.26
	2	14-230512	5.25	5.07	4.26
	3	201059	5.25	5.07	4.26
P. aeruginosa ATCC 27853	1	14-190712	7.12	7.24	6.97
	2	14-230512	7.12	7.24	6.97
	3	201059	7.12	7.24	6.97

16.2 The 3 Hyper San lots tested showed significant antimicrobial activity against *L. monocytogenes* ATCC 19114 and *P. aeruginosa* ATCC 27853 at the 10, 20 and 30 minute time points consistently demonstrating a > 4 average Log reduction.

16.3 At the 10 minute time point, the 3 lots of Hyper San were most effective against the *L. monocytogenes* strain (average Log reduction values > 5.2), whereas the 20 minute time point proved to be most efficacious against the *P. aeruginos*a ATCC 27853 strain (average Log reduction values >7.2).

17.0 NOTES

17.1 This study was initiated on August 07, 2012 and was completed on August 15, 2012.