Antimicrobial Test Laboratories

Fast, Reliable Antimicrobial Efficacy Testing

Microbiology Study Report

Client Information

Company Name:	Advanced Vapor Technologies	Sponsor:	Rick Hoverson
Sponsor's Phone:	(800) 997-6584	E-mail:	rick@advap.com

Test Information

Test(s) Performed:	AVT LT Study #3 (Ultra-Low Contact Time Experiment - Endospores of *C. difficile*)		
Study ID #:	PT506	SOP Followed:	ATL SOP C-005/C-006
		Performed by:	B. Tanner

Sample Information

Test Substance ID(s):	SVS w/TANCS Technology	Unit ID Number:	55120401165

Parameters

Microorganism(s):	*C. difficile* ATCC 9689	Type of Carrier:	Unglazed Clay Coupon
Subculture Number:	3-4 (Pooled to Harvest Spores)	# of Test Carriers:	8 + 2 for Controls
Growth Medium:	Fluid Thioglycollate Medium	Carrier Dry Time:	20 minutes (Ambient Temperature)
Contact Time:	0, 5, 10, and 30 Seconds	Spores per Carrier:	171, See Chart, Worksheet on Page 2
Organic Soil Load:	5% Horse Serum	Incubation Temp.:	35.0 (+/- 1) C
Neutralizer Used:	10 mL D/E Broth (for Elution Only)	Incubation Time:	7 Days

Controls

Neutralized:	N/A	Growth Control:	Passed, Growth in Fluid Thioglycollate
Media Sterility:	Passed, Control Tubes Neg		

Test Results

# of Carriers Positive:	See chart, Page 3	Confirmation:	Appearance in Anaerobe Tube
# of Carriers Negative:	See chart, Page 3	Test(s) Valid?:	Yes

Notes: This test was done in accordance with SOP C-005, with special procedures to ensure that only *C. difficile* endospores were used and to ensure that any endospores surviving treatment were detected. Endospores for the study were harvested using a proprietary ATL protocol. After TANCS steam treatment and elution, suspensions were heat-shocked for 10 min at 80C to ensure that T = 0 coupon counts included only endospores. Endospores were enumerated using a 3-tube anaerobic MPN technique. Note: After 30 seconds of *C. difficile* endospore treatment, a section of the towel was eluted and enumerated in the same fashion. Lab contact information amended 3/31/08.

Tests Completed: 9/5/2007 **Report Sent:** 9/10/07

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Additional Study Information

C. difficile MPN Results Record/Worksheet

Date of Experiment: 8/24/2007
Date of Read: 9/5/2007

Contact Time = 30 Seconds

-5	-	-	-	-	-	-	-6	-5	-	-	-	-	-	-	-6
-4	-	-	-					-4	-	-	-				
-3	-	-	-					-3	-	-	-				
-2	-	-	-					-2	-	-	-				
-1	-	-	-	MPN: <0.03 Spores/Tube				-1	-	-	-	MPN: <0.03 Spores/Tube			
1/3 mL	-	-	-					1/3 mL	-	-	-				
	Calc = <9 Endospores/Coupon								Calc = <9 Endospores/Coupon						

Contact Time = 10 Seconds

-5	-	-	-	-	-	-	-6	-5	-	-	-	-	-	-	-6
-4	-	-	-					-4	-	-	-				
-3	-	-	-					-3	-	-	-				
-2	-	-	-					-2	-	-	-				
-1	-	-	-	MPN: <0.03 Spores/Tube				-1	-	-	-	MPN: <0.03 Spores/Tube			
1/3 mL	-	-	-					1/3 mL	-	-	-				
	Calc = <9 Endospores/Coupon								Calc = <9 Endospores/Coupon						

Contact Time = 5 Seconds

-5	-	-	-	-	-	-	-6	-5	-	-	-	-	-	-	-6
-4	-	-	-					-4	-	-	-				
-3	-	-	-					-3	-	-	-				
-2	-	-	-					-2	-	-	-				
-1	-	-	-	MPN: <0.03 Spores/Tube				-1	-	-	-	MPN: <0.03 Spores/Tube			
1/3 mL	-	-	-					1/3 mL	-	-	-				
	Calc = <9 Endospores/Coupon								Calc = <9 Endospores/Coupon						

Contact Time = 0 Seconds

-5	-	-	-	-	-	-	-6	-5	-	-	-	-	-	-	-6
-4	-	-	-					-4	-	-	-				
-3	-	-	-					-3	-	-	-				
-2	-	-	-					-2	-	-	-				
-1	+	-	+	MPN: 0.93 Spores/Tube				-1	-	+	-	MPN: 0.15 Spores/Tube			
1/3 mL	+	+	+					1/3 mL	+	-	+				
	Calc = 293 Endospores/Coupon								Calc = 45 Endospores/Coupon						

Average T = 0 Value = 171 Endospores/Coupon

Towel Section (After 30s Contact)

1/3 mL - - - Neg Control - Pos Control +

NOTE 1: All dilution factors multiplied 10X since coupon was eluted in 10 mL buffer (add'l 10-fold dilution).
NOTE 2: For MPN calculations, "1/3" = 0.0333 Coupon, "-1" = 0.0033 Coupon, "-2" = 0.0003 Coupon.
NOTE 3: All coupons/buffer heat-shocked at 80C for 10 minutes after elution, prior to enumeration

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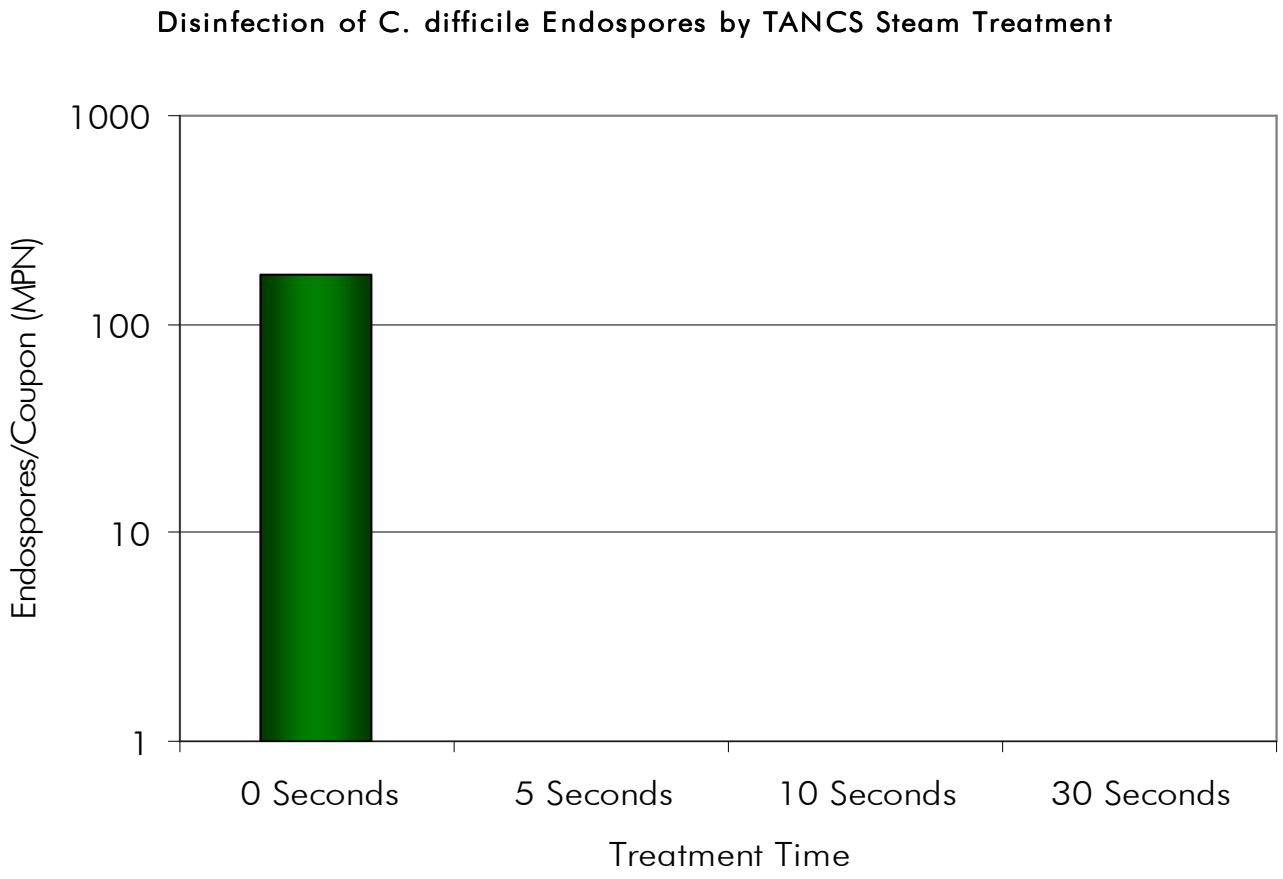

Note: Non-Detects (<9 MPN C. *difficile* Endospores) Expressed as "0" on this Chart

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Study Report Addendum – Explanation of MPN Method for Microbial Enumeration

Objective: This addendum serves to describe the Most Probable Number (MPN) Technique for the enumeration of microorganisms.

Explanation: Since microorganisms are invisible, researchers must use special procedures to count them. Three main methods are listed below:

Microorganisms can be counted directly using a microscope (**direct count**), but this is generally labor and time intensive.

Microorganisms can be counted by "**dilution and plating"** techniques, where various dilutions of microbial suspensions are spread over Petri dishes containing growth agar and incubated. During incubation, individual microorganisms or small clusters thereof grow into visible colonies. Each colony present on the agar plate after incubation corresponds to one microorganism or Colony Forming Unit (CFU) in the original suspension when the dilution factor is accounted for. This method is very common but cannot be used for microorganisms that do not grow on agar (such as viruses) and is not always practical for microorganisms that require very special growth conditions (such as anaerobic microorganisms like *C. difficile*).

The number of microorganisms in a sample can also be estimated by using the **Most Probable Number** (MPN) Technique. To estimate the number of microorganisms in a sample using MPN, the researcher prepares a series of dilutions of the sample in question. The researcher then adds a known volume from each of the dilutions into a constant number of individual tubes containing growth medium for each dilution (typically 3 or 5 tubes at each dilution). The tubes are then incubated and observed for the presence or absence of microbial growth. The researcher records the number of "positive" tubes at each dilution and then uses standard published "MPN Tables" to estimate the number of microorganisms in the sample based on the number of tubes that showed growth at each dilution. The basic premise of this method of microbial enumeration is that if a sample "A" shows more "positive" tubes at the same dilution factor than sample "B," then sample "A" most likely had a higher concentration of microorganisms to begin with. The actual numbers of microorganisms in each dilution are calculated for the MPN tables using the statistical Poisson distribution. This method of enumeration has been used successfully for years in the drinking and wastewater treatment industries and is particularly sensitive with respect to estimating concentrations of potentially injured microorganisms.

Implications: Estimations of microbial concentrations derived from MPN enumeration are accurate and reliable. Concentrations of microorganisms yielded by the MPN technique are often listed on charts as "MPN (*Organism Name*)/Unit Volume," rather than "CFU/Unit Volume."

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