

III. RESULTS.

***S. aureus* (MRSA) suspension:**

Titer.

Dilution:	<u>1:1x10⁷</u>	<u>1:1x10⁸</u>
Number of colonies:	242	29
	257	34
	225	43

Solution A:

(Received 05/16/14)

Exposure	Dilution of spore/Solution suspension:			
<u>Time</u>	<u>1:1x10¹</u>	<u>1:1x10²</u>	<u>1:1x10³</u>	<u>1:1x10⁴</u>
2 min	TNC	~520	63	6
	TNC	~420	50	6
	TNC	~520	49	3

Neutralization Control

<u>Undiluted</u>	<u>1:10</u>
207	25
215	27
217	22

Expected Counts:

<u>Undiluted</u>	<u>1:10</u>
350	35

Percent of Expected:

78.2

Solution B:

(Received 05/16/14)

Exposure	Dilution of spore/Solution suspension:			
<u>Time</u>	<u>1:1x10¹</u>	<u>1:1x10²</u>	<u>1:1x10³</u>	<u>1:1x10⁴</u>
2 min	Lawn	Lawn	TNC	~1240
	Lawn	Lawn	TNC	~1720
	Lawn	Lawn	TNC	~1740

Neutralization Control

<u>Undiluted</u>	<u>1:10</u>
239	28
246	22
238	28

Expected Counts:

<u>Undiluted</u>	<u>1:10</u>
350	35

Percent of Expected:

85.4

Solution C:

(Received 05/16/14)

Exposure	Dilution of spore/Solution suspension:			
<u>Time</u>	<u>1:1x10¹</u>	<u>1:1x10²</u>	<u>1:1x10³</u>	<u>1:1x10⁴</u>
2 min	2	0	0	0
	0	0	0	0
	2	1	0	0

Neutralization Control

<u>Undiluted</u>	<u>1:10</u>
227	22
260	26
249	27

Expected Counts:

<u>Undiluted</u>	<u>1:10</u>
350	35

Percent of Expected:

84.4

Solution D:

(Received 05/16/14)

Exposure	Dilution of spore/Solution suspension:			
<u>Time</u>	<u>1:1x10¹</u>	<u>1:1x10²</u>	<u>1:1x10³</u>	<u>1:1x10⁴</u>
2 min	Lawn	Lawn	TNC	~1180
	Lawn	Lawn	TNC	~1260
	Lawn	Lawn	TNC	~1280

Neutralization Control

<u>Undiluted</u>	<u>1:10</u>
225	30
221	34
238	30

Expected Counts:

<u>Undiluted</u>	<u>1:10</u>
350	35

Percent of Expected:

92.2

Solution E:

(Received 05/16/14)

Exposure	Dilution of spore/Solution suspension:			
<u>Time</u>	<u>1:1x10¹</u>	<u>1:1x10²</u>	<u>1:1x10³</u>	<u>1:1x10⁴</u>
2 min	Lawn	Lawn	TNC	~1880
	Lawn	Lawn	TNC	~1680
	Lawn	Lawn	TNC	~1820

Neutralization Control

<u>Undiluted</u>	<u>1:10</u>
266	26
235	35
223	28

Expected Counts:

<u>Undiluted</u>	<u>1:10</u>
350	35

Percent of Expected:

91.5

Sterility Controls:

<u>Material</u>	<u>Counts</u>
PSS-1	0, 0, 0
PSS-2	0, 0, 0
PSS-3	0, 0, 0
PSS-4	0, 0, 0
Neutralizer	0, 0, 0
Solution A	0, 0, 0
Solution B	0, 0, 0
Solution C	0, 0, 0
Solution D	0, 0, 0
Solution E	0, 0, 0
Media	0, 0, 0

IV. DISCUSSION.

Results of the titer showed a viable *S. aureus* (MRSA) concentration of 2.97×10^9 organisms per ml in the original suspension. Inoculation of 9.9 ml of a solution with 0.1 ml of this suspension produced an initial concentration of 2.97×10^7 CFU per ml in the assay tube.

Results from these procedures allowed log reduction (LR) and percent kill (PK) values to be calculated using the formulas: 1) $LR = -\log(S/S_0)$; where S = concentration of viable organisms after the specified contact time; and S_0 = the initial concentration of viable organisms at time zero. 2) $PK = (1 - (S/S_0)) \times 100$. These values are shown below.

<u>Test Solution</u>	<u>Contact Time</u>	<u>Log Reduction (LR)</u>	<u>Percent Kill (PK)</u>
Solution A	2 min	2.74	99.82%
Solution B	2 min	~0.28	~47.1%
Solution C	2 min	6.35	99.999955%
Solution D	2 min	~0.38	~58.3%
Solution E	2 min	~0.22	~39.7%

Neutralization control data revealed that the neutralizer was able to adequately neutralize the test solutions. Observed counts were 78.2-92.2% of those expected.

A wide disparity in antimicrobial activity between the five test solutions was observed. Solution C had the highest antimicrobial activity, producing a 6.35 log reduction of MRSA in 2 minutes. Solution A had the next highest activity, producing a 2.74 log reduction in 2 minutes. It should be kept in mind that these values are log reductions, and thus, the antimicrobial activity of Solution C was roughly 4,000 times greater than that of Solution A. Solutions B, D, and E all displayed little or no antimicrobial activity against MRSA in 2 minutes. Counts were so high, that the number of CFU had to be estimated on the 1:10,000 dilution of the reaction mixture. Thus, the log reduction and percent kill values are also estimates. That said, all log reduction estimates for Solutions B, D, and E were less than 0.4, indicating relatively low antimicrobial activity against MRSA in 2 minutes.

Test Dates: May 16 – 22, 2014

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