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This report replaces Micro Report GT 94311/15

MICRO REPORT

REPORT 1

DATE: 02/03/15
DATE RECEIVED: 04/02/15
DATE TESTED: 18/02-02/03/15

REQ NO: GT 94311/15A

SAMPLE TYPE: **'ONEDROP' WATER PURIFICATION DROPS**

PROTOCOL TO DETERMINE THE EFFICACY OF 'ONEDROP' WATER PURIFICATION DROPS

Objectives

To conduct an evaluation study to determine the efficacy of 'OneDrop' Purification Drops using concentrations of 1; 2 & 4 drops per litre of water at contact times of 30; 60; 90; 120; 240 minutes & 24hrs.
The test protocol will be divided into 2 parts.

Part 1

Water samples will be artificially contaminated using the following organisms:

Escherichia coli ATCC8739
Salmonella abaetetuba SLR2954
Shigella flexneri ATCC9199
Vibrio parahaemolyticus ATCC17802
Salmonella typhimurium ATCC 29629

The efficacy will be determined by the $\log_{10}$ reduction of each organism used by calculating difference between the $\log_{10}$ value of the Pre & post treated samples.

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Part 2
Raw water will be tested; both pre & post treatment according to the microbiological specifications of SANS241-1:2011 (Edition 1).
The efficacy will be determined by the log reduction used by calculating difference between the log_{10} value of the Pre & post treated samples.
This will therefore be a real scenario test. The concentration & contact time for Part 2 will be determined by the results of Part 1; using the lowest concentration & shortest contact time of the most resistant organism tested.

Protocol
Part 1
Raw water (river/dam water) will be sourced & brought to the laboratory. 12lts of water will be required for Part 1.

- The water will be dispensed into 1lt sample sizes.
- Each organism to be tested will require 3x1lt samples. One for each concentration to be used.
- The water samples will be sterilized using an autoclave to ensure none of the natural organisms in the water are present in the sample; which could cause interference with the analysis of the specified organisms.
- The water samples will be inoculated with an inoculum level of 10^6cfu/ml of the respective organism. This will equate to an inoculum level of 10^3cfu/ml of sample.
- The samples will be tested to determine the pre-treatment count of the artificially induced contamination. The samples will be plated in triplicate and the mean count will be determined.
- The respective individual samples will be treated with the relevant product concentration.
- Following the elapse of each contact time; the sample will be tested. The samples will be plated in triplicate and the mean count will be determined.
- The samples will be plated using Plate Count Agar & incubated at 37^0C for 48hours
- The log_{10} value of the pre & post treatment mean counts will be determined and the difference will be calculated to determine the log_{10} Reduction.

Part 2
Raw water (river/dam water) will be sourced & brought to the laboratory. 2lts of water will be required for Part 2.

- The water will be dispensed into 1lt sample sizes.
- One sample will be tested to determine the pre-treatment count according to the specifications indicated in SANS241-1:2011
- One sample will be treated with 'OneDrop' at a concentration & contact time determined in Part 1.
  The concentration & contact time used will be the lowest concentration & contact time of the most limiting test organism.

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- The treated sample will be tested to determine the post-treatment count according to the specifications indicated in SANS241-1:2011
- The log_{10} value of the pre & post treatment mean counts will be determined and the difference will be calculated to determine the log_{10} Reduction.

Results – Part 1

Samples artificially contaminated with various organisms

Table 1: *Escherichia coli* ATCC8739 – Mean Counts of triplicate plates

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	11 500	1 500	490	360	190	97	<1
2 Drops	11 900	510	170	160	61	36	<1
4 Drops	10 300	310	210	36	37	11	<1

Table 2: *Escherichia coli* ATCC8739 – Log_{10} Values

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	4.0607	3.1761	2.6902	2.5563	2.2788	1.9868	<0
2 Drops	4.0755	2.7076	2.2304	2.2041	1.7853	1.5563	<0
4 Drops	4.0128	2.4914	2.3222	1.5563	1.5682	1.0414	<0

Table 3: *Escherichia coli* ATCC8739 – Log_{10} Reduction

	Concentration								
	1 Drop			2 Drops			4 Drops		
	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction
30 Min	4.0607	3.1761	**0.8846**	4.0755	2.7076	**1.3679**	4.0128	2.4914	**1.5214**
60 Min	4.0607	2.6902	**1.3705**	4.0755	2.2304	**1.8451**	4.0128	2.3222	**1.6906**
90 Min	4.0607	2.5563	**1.5044**	4.0755	2.2041	**1.8714**	4.0128	1.5563	**2.4565**
120 Min	4.0607	2.2788	**1.7819**	4.0755	1.7853	**2.2902**	4.0128	1.5682	**2.4446**
240 Min	4.0607	1.9868	**2.0739**	4.0755	1.5563	**2.5192**	4.0128	1.0414	**2.9714**
24 Hrs	4.0607	<0	**>4.0607**	4.0755	<0	**>4.0755**	4.0128	<0	**>4.0128**

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Table 4: *Salmonella abaetetuba* SLR2954 – Mean Counts of triplicate plates

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	5 300	2 800	1 800	2 000	2 300	1 700	590
2 Drops	4 500	160	62	11	8	5	<1
4 Drops	4 200	78	49	2	14	2	<1

Table 5: *Salmonella abaetetuba* SLR2954 – Log_{10} Values

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	3.7243	3.4472	3.2553	3.3010	3.3617	3.2304	2.7708
2 Drops	3.6531	2.2041	1.7924	1.0414	0.9031	0.6989	<0
4 Drops	3.6232	1.8921	1.6902	0.3010	1.1461	0.3010	<0

Table 6: *Salmonella abaetetuba* SLR2954 – Log_{10} Reduction

	Concentration								
	1 Drop			2 Drops			4 Drops		
	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction
30 Min	3.7243	3.4472	**0.2771**	3.6531	2.2041	**1.4490**	3.6232	1.8921	**1.7311**
60 Min	3.7243	3.2553	**0.4690**	3.6531	1.7924	**1.8607**	3.6232	1.6902	**1.9330**
90 Min	3.7243	3.3010	**0.4220**	3.6531	1.0414	**2.6117**	3.6232	0.3010	**3.3222**
120 Min	3.7243	3.3617	**0.3626**	3.6531	0.9031	**2.7500**	3.6232	1.1461	**2.4771**
240 Min	3.7243	3.2304	**0.4939**	3.6531	0.6989	**2.9542**	3.6232	0.3010	**3.3222**
24 Hrs	3.7243	2.7708	**0.9522**	3.6531	<0	**>3.6531**	3.6232	<0	**>3.6232**

Table 7: *Shigella flexneri* ATCC9199 – Mean Counts of triplicate plates

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	2 900	660	230	22	26	4	<1
2 Drops	2 100	150	41	7	1	<1	<1
4 Drops	1 700	59	18	1	<1	<1	<1

Table 8: *Shigella flexneri* ATCC9199 – Log_{10} Values

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	3.4624	2.8195	2.3617	1.3424	1.4149	0.6021	<0
2 Drops	3.3222	2.1761	1.6128	0.8451	0	<0	<0
4 Drops	3.2304	1.7708	1.2553	0	<0	<0	<0

Table 9: *Shigella flexneri* ATCC9199 – Log_{10} Reduction

	Concentration								
	1 Drop			2 Drops			4 Drops		
	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction
30 Min	3.4624	2.8195	**0.6429**	3.3222	2.1761	**1.1461**	3.2304	1.7708	**1.4596**
60 Min	3.4624	2.3617	**1.1007**	3.3222	1.6128	**1.7094**	3.2304	1.2553	**1.9751**
90 Min	3.4624	1.3424	**2.1200**	3.3222	0.8451	**2.4771**	3.2304	0	**3.2304**
120 Min	3.4624	1.4149	**2.0475**	3.3222	0	**3.3222**	3.2304	<0	**>3.2304**
240 Min	3.4624	0.6021	**2.8603**	3.3222	<0	**>3.3222**	3.2304	<0	**>3.2304**
24 Hrs	3.4624	<0	**>3.4624**	3.3222	<0	**>3.3222**	3.2304	<0	**>3.2304**

Table 10: *Vibrio parahaemolyticus* ATCC17802 – Mean Counts of triplicate plates

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	320	<1	<1	<1	<1	<1	<1
2 Drops	740	<1	<1	<1	<1	<1	<1
4 Drops	5 400	<1	<1	<1	<1	<1	<1

Table 11: *Vibrio parahaemolyticus* ATCC17802 – Log_{10} Values

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	2.5051	<0	<0	<0	<0	<0	<0
2 Drops	2.8692	<0	<0	<0	<0	<0	<0
4 Drops	3.7324	<0	<0	<0	<0	<0	<0

Table 12: *Vibrio parahaemolyticus* ATCC17802 – Log_{10} Reduction

	Concentration								
	1 Drop			2 Drops			4 Drops		
	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction
30 Min	2.5051	<0	**>2.5051**	2.8692	<0	**>2.8692**	3.7324	<0	**>3.7324**
60 Min	2.5051	<0	**>2.5051**	2.8692	<0	**>2.8692**	3.7324	<0	**>3.7324**
90 Min	2.5051	<0	**>2.5051**	2.8692	<0	**>2.8692**	3.7324	<0	**>3.7324**
120 Min	2.5051	<0	**>2.5051**	2.8692	<0	**>2.8692**	3.7324	<0	**>3.7324**
240 Min	2.5051	<0	**>2.5051**	2.8692	<0	**>2.8692**	3.7324	<0	**>3.7324**
24 Hrs	2.5051	<0	**>2.5051**	2.8692	<0	**>2.8692**	3.7324	<0	**>3.7324**

Table 13: *Salmonella typhimurium* ATCC 29629 – Mean Counts of triplicate plates

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	5 100	1 300	4 800	3 500	3 800	300	<1
2 Drops	4 700	140	4 400	1 800	390	13	<1
4 Drops	5 400	3600	3 900	1 700	360	9	<1

Table 14: *Salmonella typhimurium* ATCC 29629 – Log_{10} Values

Concentration	Contact Time						
	Control	30 Minutes	60 Minutes	90 Minutes	120 Minutes	240 Minutes	24 Hours
1 Drop	3.7076	3.1139	3.6812	3.5441	3.5798	2.4771	<0
2 Drops	3.6721	2.1461	3.6434	3.2553	2.5911	1.1139	<0
4 Drops	3.7324	3.5563	3.5911	3.2304	2.5563	0.9542	<0

Table 15: *Salmonella typhimurium* ATCC 29629 – Log_{10} Reduction

	Concentration								
	1 Drop			2 Drops			4 Drops		
	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction	Log_{10} Control	Log_{10} Test	Log_{10} Reduction
30 Min	3.7076	3.1139	**0.5937**	3.6721	2.1461	**1.5260**	3.7324	3.5563	**0.1761**
60 Min	3.7076	3.6812	**0.0264**	3.6721	3.6434	**0.0287**	3.7324	3.5911	**0.1413**
90 Min	3.7076	3.5441	**0.1635**	3.6721	3.2553	**0.4168**	3.7324	3.2304	**0.5020**
120 Min	3.7076	3.5798	**0.1278**	3.6721	2.5911	**1.0810**	3.7324	2.5563	**1.1761**
240 Min	3.7076	2.4771	**1.2305**	3.6721	1.1139	**2.5582**	3.7324	0.9542	**2.7782**
24 Hrs	3.7076	<0	**>3.7076**	3.6721	<0	**>3.6721**	3.7324	<0	**>3.7324**

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To understand the relationship between log reduction & percentage reduction; the following pattern is followed:

- 1log Reduction = 90% Reduction
- 2log Reduction = 99% Reduction
- 3log Reduction = 99.9% Reduction
- 4log Reduction = 99.99% Reduction
- 5log Reduction = 99.999% Reduction
- 6log Reduction = 99.9999% Reduction

The log reduction is determined by the number of organisms introduced into the sample before treatment. The higher the number of organisms introduced; the greater potential for a higher log reduction.
During this trial the greatest log reduction was achieved using the *Escherichia coli*; as the inoculum level was the highest. A $\log_{10}$ reduction of >4.0 was achieved for all concentrations after a 24 hour contact time

The inoculum levels introduced into the samples during this trial were in a range to give a 3log reduction.

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