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46-48 Banksia Rd, Welshpool,
(08) 6253 4444

Client:

Quenton Leach
Natural Water Solutions

CleanOxide Efficacy Testing Methodology

EcoDiagnostics conducted testing for Natural Water solutions between the 13/06/2018 – 29/06/2018, on the efficacy of CleanOxide tablets at two concentrations (Chlorine dioxide at 25 and 50ppm). This testing aimed to examine the effect CleanOxide (tablets) have on general bacterial counts in salad, and its effects on bacteria of interest; *Escherichia coli* and Salmonella.

Methodology

13/06/2018 – ED project number: 7343

Testing using CleanOxide (0.5g) tablets at 25ppm.

A homogenous salad was prepared using lettuce, bean sprouts, radish, carrots, broccoli and spinach. A working solution of CleanOxide was prepared at a concentration of 25ppm Chlorine dioxide by laboratory staff. There were three different treatment groups used:

1. Salad that was unwashed and untreated (No CleanOxide treatment).
2. Salad that was washed in potable water twice, and untreated.
3. Salad that was washed in potable water once and soaked in the prepared CleanOxide solution at 25ppm Chlorine dioxide for 5 minutes.

Following the appropriate treatment, the salads were further homogenized using a bag mixer, and 10 grams of salad was diluted in 90mL of Buffered Peptone Water. This mixture was homogenized, and analysed following NATA accredited EDP-403 (3M Aerobic Plate Count Petrifilm method) to obtain a total aerobic bacterial count.

Results:

Total aerobic bacterial counts are presented in Table 1, obtained from EDR007343.0, in colony forming units (CFU) per 10g of salad.



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Table 1: Total Aerobic Bacterial count (CFU/10g)

Analytical Results						
ED SAMPLE NUMBER	Your Sample ID / Description / Number	Collection Information (Date)	Method Type	Target	Result	Comments
34616	1 Salad - Unwashed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	2,900,000 CFU / 10g	
34617	2 Salad - Unwashed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	7,100,000 CFU / 10g	
34618	3 Salad - Unwashed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	4,600,000 CFU / 10g	
34619	4 Salad - Unwashed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	6,200,000 CFU / 10g	
34620	5 Salad - Unwashed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	8,200,000 CFU / 10g	
34621	1 Salad - Washed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	720,000 CFU / 10g	
34622	2 Salad - Washed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	660,000 CFU / 10g	
34623	3 Salad - Washed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	700,000 CFU / 10g	
34624	4 Salad - Washed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	880,000 CFU / 10g	
34625	5 Salad - Washed, Untreated	2018-06-13	EDP-403	Aerobic Plate Count	780,000 CFU / 10g	
34626	1 Salad - Washed, CleanOxide Salad Wash (Chlorine dioxide) treated	2018-06-13	EDP-403	Aerobic Plate Count	282,000 CFU / 10g	
34627	2 Salad - Washed, CleanOxide Salad Wash (Chlorine dioxide) treated	2018-06-13	EDP-403	Aerobic Plate Count	264,000 CFU / 10g	
34628	3 Salad - Washed, CleanOxide Salad Wash (Chlorine dioxide) treated	2018-06-13	EDP-403	Aerobic Plate Count	249,000 CFU / 10g	
34629	4 Salad - Washed, CleanOxide Salad Wash (Chlorine dioxide) treated	2018-06-13	EDP-403	Aerobic Plate Count	278,000 CFU / 10g	
34630	5 Salad - Washed, CleanOxide Salad Wash (Chlorine dioxide) treated	2018-06-13	EDP-403	Aerobic Plate Count	294,000 CFU / 10g	



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27/06/2018 – ED project number: 7471

Testing using CleanOxide (1.0g) tablets at 50ppm

A homogenous salad was prepared using lettuce, bean sprouts, radish, carrots and spinach. A working solution of CleanOxide (1g tablets) was prepared at a concentration of 50ppm Chlorine dioxide by laboratory staff. There were two different treatment groups used:

1. Salad that was washed in potable water twice, and untreated.
2. Salad that was washed in potable water once and soaked in the prepared CleanOxide solution at 50ppm Chlorine dioxide for 5 minutes.

Following the appropriate treatment, the salads were further homogenized using a bag mixer, and 10 grams of salad was placed in 90mL of Buffered Peptone Water. This mixture was homogenized, and analysed following NATA accredited EDP-403 (3M Aerobic Plate Count Petrifilm method) to obtain a total Aerobic Bacterial count.

Results:

Bacterial counts are presented in Table 2, obtained from EDR007471.0, in colony forming units (CFU) per 10g of salad.

Table 2: Total Aerobic Bacterial count (CFU/10g)

Analytical Results						
ED SAMPLE NUMBER	Your Sample ID / Description / Number	Collection Information (Date)	Method Type	Target	Result	Comments
35272	1 Salad - Washed, Untreated	2018-06-27	EDP-403	Aerobic Plate Count	920,000 CFU / 10g	
35273	1 Salad - Washed, CleanOxide Salad Wash (Chlorine Dioxide, 50ppm) treated	2018-06-27	EDP-403	Aerobic Plate Count	320,000 CFU / 10g	



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28/06/2018 – ED project number: 7477

Testing efficacy of CleanOxide (1.0g) tablets against viable *Escherichia coli* (E.coli) bacteria at 50ppm.

A working solution of CleanOxide (1g tablets) was prepared at a concentration of 50ppm Chlorine dioxide using distilled water by laboratory staff. A pure suspension of *Escherichia coli* was prepared at a concentration of 8×10^4 CFU/mL using sterile 0.1% Peptone water. Two bags were set up, one with 50mL of distilled water, another with 50mL of the CleanOxide working solution. A calculated volume of the E.coli suspension was spiked into each bag, to obtain a working solution between 10-100 CFU/mL. Samples were taken at 3 intervals: Immediately post spiking, 5 minutes, and 10 minutes after spiking. These samples were analysed following EDP-404 (E.coli/coliform Petrifilm method).

Results:

Escherichia coli counts are represented in Table 3, obtained from EDR007477.0, in CFU/mL.

Table 3: E.coli counts (CFU/mL)

Analytical Results						
ED SAMPLE NUMBER	Your Sample ID / Description / Number	Collection Information (Date)	Method Type	Target	Result	Comments
35283	Water- Spiked E.coli – Time 0	2018-06-28	EDP-404	E.coli	6 CFU / 1 mL	
35284	Water- Spiked E.coli – Time 5 minutes	2018-06-28	EDP-404	E.coli	4 CFU / 1 mL	
35285	Water- Spiked E.coli- Time 10 minutes	2018-06-28	EDP-404	E.coli	5 CFU / 1 mL	
35286	CleanOxide Tablet Solution (Chlorine dioxide, 50ppm) – E.coli spiked – Time 0	2018-06-28	EDP-404	E.coli	<1 CFU / 1 mL	
35287	CleanOxide Tablet Solution (Chlorine dioxide, 50ppm) - E.coli spiked – Time 5 minutes	2018-06-28	EDP-404	E.coli	<1 CFU / 1 mL	
35288	CleanOxide Tablet Solution (Chlorine dioxide, 50ppm) - E.coli spiked – Time 10 minutes	2018-06-28	EDP-404	E.coli	<1 CFU / 1 mL	



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28/06/2018 – ED project number: 7478

Testing efficacy of CleanOxide (1.0g) tablets against viable Salmonella bacteria at 50ppm.

A working solution of CleanOxide (1g tablets) was prepared at a concentration of 50ppm Chlorine dioxide using distilled water by laboratory staff. A pure suspension of Salmonella bacteria was prepared at a concentration of 6.3×10^4 CFU/mL using sterile 0.1% Peptone water. Two bags were set up, one with 100mL of distilled water, another with 100mL of the working solution of CleanOxide. A calculated volume of the Salmonella suspension was spiked into each bag, to obtain a working solution between 10-100 CFU/mL. Samples were taken at 3 intervals: Immediately post spiking, 5 minutes, and 10 minutes after spiking. These samples were analysed using EDP-401 (Salmonella qPCR method). This method involves an enrichment stage, where the samples are incubated at $37 \pm 1^\circ\text{C}$ for $18 \text{ hrs} \pm 2$.

Results:

The results are shown in Table 4, obtained from EDR007478.0, stating whether Salmonella was detected/not detected in 25mL post treatment and post incubation.

Table 4: qPCR detection of Salmonella in 25mL of sample, obtained from EDR007478.

Analytical Results						
ED SAMPLE NUMBER	Your Sample ID / Description / Number	Collection Information (Date)	Method Type	Target	Result	Comments
35289	Water - Salmonella spike – Time 0	2018-06-28	qPCR	Salmonella spp	Detected / 25 mL	
35290	Water - Salmonella spike – Time 5 minutes	2018-06-28	qPCR	Salmonella spp	Detected / 25 mL	
35291	Water - Salmonella spike – Time 10 minutes	2018-06-28	qPCR	Salmonella spp	Detected / 25mL	
35292	CleanOxide tablet solution (Chlorine Dioxide, 50ppm) - Salmonella spike – Time 0	2018-06-28	qPCR	Salmonella spp	Not Detected / 25 mL	
35293	CleanOxide tablet solution (Chlorine Dioxide, 50ppm) - Salmonella spike – Time 5 minutes	2018-06-28	qPCR	Salmonella spp	Not Detected / 25 mL	
35294	CleanOxide tablet solution (Chlorine Dioxide, 50ppm) - Salmonella spike – Time 10 minutes	2018-06-28	qPCR	Salmonella spp	Not Detected / 25 mL	

Authorised by:

Hannah Ashbil – Diagnostics Assistant
on 3/07/2018