

# Field Testing Protocol - Ecogreen Biosanitiser Blue

## **Aim of the test:**

To test the sanitisation effectiveness of Ecogreen Biosanitiser Blue

## **Analysis used:**

Environmental Water Analysis (wastewater) as advised by local environmental laboratory. Suitable bottles (pre-treated or preserved) for selected analysis will be supplied by the laboratory and be used to collect water samples on selected sampling area, water samples collected are to be returned to the laboratory for further chemical and microbiological testing.

### **\*Chemical Analysis**

1. Ammonia
2. Total Sulphide
3. Total Suspended Solids

### **\* Microbiological Analysis**

4. Total (Heterotrophic) Plate Count
5. Esherichia Coli
6. Enterococci (Optional)
7. Pseudomonas aeruginosa (Optional)
8. Clostridium Perfringens (Optional)

**Note:** (Optional) = Addition analysis selected for further study purposes

## **Duration required:**

55 - 60days

## **Arrangement with the laboratory:**

(2 Sampling area per week X 5 weeks) + 2 sets extra = 10 sets of bottles

\* **Note:** Each sets of bottles will contain 4-5 types of different bottle pending on the type of analysis and water samples volume requirement of the local environmental laboratory. Bottles request is to be completed a week before the planned field test to allow freight delivery. Follow the water sampling instructions as advised by the local environmental laboratory.

## **Other tools and equipment required:**

Marker pen  
Esky & ice pack  
Zip-lock bag  
Pipette  
Sterile bottle (one for each sampling area)

## **Sampling area:**

1. [Site A] Toilet Bowl/Cubicle
2. [Site B] Toilet Urinal

**Testing Schedule:**

a) [Site A] Toilet Bowl/Cubicle - 1A, 2A, 3A, 4A and 5A

	<b>Test Status:</b>	<u>Untreated</u>		<u>Treated</u>		
	<b>Test Day:</b>	<u>Day 1</u>	<u>Day 9</u>	<u>Day 17</u>	<u>Day 36</u>	<u>Day 47</u>
	<b>Test No.:</b>	<u>1<sup>st</sup> Sampling (1A)</u>	<u>2<sup>nd</sup> Sampling (2A)</u>	<u>3<sup>rd</sup> Sampling (3A)</u>	<u>4<sup>th</sup> Sampling (4A)</u>	<u>5<sup>th</sup> Sampling (5A)</u>
<b>Product Dispensed:</b>						
<b><u>Chemical Analysis</u></b>	<u>Unit</u>					
Total Suspended Solids	mg/L	√	√	√	√	√
Total Sulphide	mg/L	√	√	√	√	√
Ammonia	mg N/L	√	√	√	√	√
<b><u>Microbiological Analysis</u></b>	<u>Unit</u>					
Total (Heterotrophic) Plate Count	orgs/mL	√	√	√	√	√
Total (Heterotrophic) Plate Count	orgs/100mL	√	√	√	√	√
Escherichia Coli (Colilert)	orgs/100mL	√	√	√	√	√
Enterococci (Enterolert)	orgs/100mL	√	√	√	√	√
Pseudomonas Aeruginosa	orgs/100mL	√	√	√	√	√
Clostridium Perfringens	orgs/100mL	√	√	√	√	√
Clostridium Perfringens (Sulphite reducing Clostridia spores)	orgs/100mL	√	√	√	√	√

b) [Site B] Toilet Urinal - 1B, 2B, 3B, 4B and 5B

	<b>Test Status:</b>	<u>Untreated</u>		<u>Treated</u>		
	<b>Test Day:</b>	<u>Day 1</u>	<u>Day 9</u>	<u>Day 17</u>	<u>Day 36</u>	<u>Day 47</u>
	<b>Test No.:</b>	<u>1<sup>st</sup> Sampling (1A)</u>	<u>2<sup>nd</sup> Sampling (2A)</u>	<u>3<sup>rd</sup> Sampling (3A)</u>	<u>4<sup>th</sup> Sampling (4A)</u>	<u>5<sup>th</sup> Sampling (5A)</u>
<b>Product Dispensed:</b>						
<b><u>Chemical Analysis</u></b>	<u>Unit</u>					
Total Suspended Solids	mg/L	√	√	√	√	√
Total Sulphide	mg/L	√	√	√	√	√
Ammonia	mg N/L	√	√	√	√	√
<b><u>Microbiological Analysis</u></b>	<u>Unit</u>					
Total (Heterotrophic) Plate Count	orgs/mL	√	√	√	√	√
Total (Heterotrophic) Plate Count	orgs/100mL	√	√	√	√	√
Escherichia Coli (Colilert)	orgs/100mL	√	√	√	√	√
Enterococci (Enterolert)	orgs/100mL	√	√	√	√	√
Pseudomonas Aeruginosa	orgs/100mL	√	√	√	√	√
Clostridium Perfringens	orgs/100mL	√	√	√	√	√
Clostridium Perfringens (Sulphite reducing Clostridia spores)	orgs/100mL	√	√	√	√	√

### **Testing Instructions:**

Water samples collected via the selected inspection point of the washroom into sampling bottles provided by laboratory. The results for Day 1: Untreated samples (Before product dosing) will be compared against Day 9, 17, 36, 47: Treated samples (After product dosing at pre-determined intervals).

### **Preparation for Testing - Day 0**

1. Switch off any toilet treatment - applicable if there's a washroom treatment currently in place.
2. Leave the washroom on UNTREATED status for at least 3 days before the commencement of testing.
3. Obtained the correct amount and type of sampling bottles from the laboratory.
4. Ensure the sampling bottles are new.
5. Pack sampling bottles for each site into zip-lock bag.
6. Clearly label each sampling bottle with the information as followed:
  - \* Sampling Date: (eg. 5/2/2012)
  - \* Time: (eg. 8.00am)
  - \* Sample ID: (eg. Test 2A or 2B)
7. Complete sample submission document (to be send-off together with each batch of water samples).
  - \* Company: (eg. Environmental Technologies Group)
  - \* Analysis: (eg. Heterotrophic Plate Count, Faecal Coliform or E.Coli, Ammonia and Total Suspended Solids.)
  - \* Sample ID: (eg. Test 2A & 2B)
  - \* Location: (eg. KFC Restaurant)
8. Place all items into esky (packed with ice packs) to be taken to site.

### **Untreated Samples - Day 1 (Test 1A, 1B)**

1. Collect water samples using bottles (requested from laboratory) before commencing treatment.
2. Use pipette or sterile bottle if necessary.
3. Give the water and surface a good stir and collect an even sample into sampling bottle.
4. Fill water samples evenly throughout each bottle for each set.
5. Flush toilet and collect second lot evenly throughout each sample bottles.
6. Repeat step 4. until all sampling bottles are filled up with water volume required.
7. Close the lid of the sampling bottles and place them into zip-lock bag.
8. Record the batch no. of the Ecogreen Biosanitiser Blue.
9. Check if the label details are correct and readable, replace label if necessary
10. Sample immediately following collection is to be stored in an esky (packed with ice packs) to be courier to laboratory as soon as possible.  
Note: \* Attach laboratory sample submission form together with the sample.
11. Install product dispenser and reload a new 90mL Ecogreen Biosanitiser Blue into the dispenser.  
(This vary for Qwave dispenser, request for more information if such dispenser is selected for testing)
12. Commence treatment and dosing accordingly, flush toilet to inspect product is dispensed properly, the water within toilet bow/urinal should have be light blue in colour together with formation of foam.

**Treated Samples - Day 9 (2A, 2B), Day 17 (Test 3A, 3B), Day 36 (4A, 4B), Day 47 (5A, 5B)**

1. Collect water samples using bottles (requested from laboratory) before commencing treatment.
2. Use pipette or sterile bottle if necessary.
3. Give the water and surface a good stir and collect an even sample into sampling bottle.
4. Fill water samples evenly throughout each bottle for each set.
5. Flush toilet and collect second lot evenly throughout each sample bottles.
6. Repeat step 4. until all sampling bottles are filled up with water volume required.
7. Close the lid of the sampling bottles and place them into zip-lock bag.
8. Record the batch no. of the Biosanitiser Blue.
9. Check if the label details are correct and readable, replace label if necessary
10. Sample immediately following collection is to be stored in an esky (packed with ice packs) to be courier to laboratory as soon as possible.

Dispenser and Product Inspections:

Dispenser - ensure pump working properly with product properly dosing into the treated toilet.

Product - ensure product is in good condition and record volume being dosed into the treated toilet.

### Testing Result (Summary):

## **Sampling Area: [Site A] Toilet Bowl / Cubicle**

**Product:** Ecogreen Biosanitiser Blue

### **Comment:**

- \* Sampling days were organised according to the availability of Pink Hygiene staff.
- \* The amount of product dispensed directly relate on amount of toilet flushing completed on weekly basis.
- \* Testing site resume daily operation throughout testing period from Day 1-47.

### **Observation:**

- 1A (Day 1) 1 X Dispenser installed, with Biosanitiser Blue (90mL bottle) being placed within it.  
2A (Day 9) 25mL product left within the 90mL bottle (approximately 75mL product being used in total from Day 1)  
3A (Day 17) 0mL product left within the 90mL bottle (approximately 25mL product being used in total from Day 9)  
New 90mL bottle of Biosanitiser Blue is placed within dispenser.  
4A (Day 36) 85mL product left within the 90mL bottle (approximately 5mL product being used from Day 17)  
Low level of flushing being completed

### **Note:**

Analysis cancellation Day 36 samples, freight company failure to deliver water samples within <24 hours (expired).

- 5A (Day 47) 55mL product left within the 90mL bottle (approximately 30mL product being used from Day 26)

	<b>Test Status:</b>	<u>Untreated</u>		<u>Treated</u>		
	<b>Test Day:</b>	<u>Day 1</u>	<u>Day 9</u>	<u>Day 17</u>	<u>Day 36</u>	<u>Day 47</u>
	<b>Test No.:</b>	<u>1<sup>st</sup> Sampling (1A)</u>	<u>2<sup>nd</sup> Sampling (2A)</u>	<u>3<sup>rd</sup> Sampling (3A)</u>	<u>4<sup>th</sup> Sampling (4A)</u>	<u>5<sup>th</sup> Sampling (5A)</u>
	<b>Product Dispensed:</b>	0mL	75mL	25mL	5mL	30mL
	<b>Laboratory Report No:</b>	300719	307429	308659	-	314787
<b><u>Chemical Analysis</u></b>	<u>Unit</u>					
Total Suspended Solids	mg/L	<2	<2	2	-	2
Total Sulphide	mg/L	<0.1	<0.1	<0.1	-	<0.1
Ammonia	mg N/L	1.4	0.3	<0.1	-	0.2
<b><u>Microbiological Analysis</u></b>	<u>Unit</u>					
Total (Heterotrophic) Plate Count	orgs/mL	>1 X 10 <sup>5</sup>	>1 X 10 <sup>4</sup>	2.3 X 10 <sup>4</sup>	-	5.5 X 10 <sup>3</sup>
Total (Heterotrophic) Plate Count	orgs/100mL	>1 X 10 <sup>7</sup>	>1 X 10 <sup>6</sup>	2.3 X 10 <sup>6</sup>	-	5.5 X 10 <sup>5</sup>
Escherichia Coli (Colilert)	orgs/100mL	1.6 X 10 <sup>5</sup>	2.4 X 10 <sup>3</sup>	2.0 X 10 <sup>3</sup>	-	730
Enterococci (Enterolert)	orgs/100mL	2.4 X 10 <sup>5</sup>	>2.4 X 10 <sup>3</sup>	870	-	0
Pseudomonas Aeruginosa	orgs/100mL	0	0	0	-	0
Clostridium Perfringens	orgs/100mL	0	0	0	-	0
Clostridium Perfringens (Sulphite reducing Clostridia spores)	orgs/100mL	0	0	1	-	0

### **Final Result:**

**↑Heterotrophic Plate Count (beneficial bacteria), ↓Ammonia (urine), ↓Escherichia Coli & Enterococci (unwanted bad bacteria)**  
Ecogreen Biosanitiser Blue (evenly dispensed throughout surface of the treated toilet bowl during flushing) has successfully demonstrated effective sanitisation activity. This is achieved via elimination of odour causing urine compound (eg. Ammonia). The exhaustion of food supply and interruption to the living environment for unwanted bad/pathogenic bacteria symbiotically led to the significant reduction of these populations (eg. Escherichia Coli and Enterococci).

### **Recommendation:**

Routine toilet flushing after daily toilet usage with 30mL or more product accumulatively flushed/dispensed apply on treated toilet throughout the week is recommended to ensure surface is constantly repopulated Ecogreen Biosanitiser Blue beneficial bacteria for long term optimum sanitisation effect.

## Sampling Area: [Site B] Toilet Urinal

**Product:** Ecogreen Biosanitiser Blue

**Comment:**  
 \* Sampling days were organised according to the availability of Pink Hygiene staff.  
 \* The amount of product dispensed directly relate on amount of toilet flushing completed on weekly basis.  
 \* Testing site resume daily operation throughout testing period from Day 1-47.

**Observation:**  
 1B (Day 1) 2 X Dispensers installed, with Biosanitiser Blue (90mL bottle) being placed within each dispenser.  
 2B (Day 9) 85mL product left within each 90mL bottle (approximately 10mL product being used in total from Day 1)  
 3B (Day 17) 80mL product left within each 90mL bottles (approximately 10mL product being used in total from Day 9)  
     Low level of flushing/product dispensed.  
     One of the dispenser has been disconnected intentionally - random act of vandalism)  
 4B (Day 36) 80mL product left each 90mL bottles (0mL product being used from Day 17)  
     Zero level of flushing/product dispensed.  
     One of the dispenser has been disconnected intentionally - repeat act of vandalism)  
**Note:**  
 Analysis cancellation Day 36 samples, freight company failure to deliver water samples within <24 hours (expired)  
 5B (Day 47) 80mL product left each 90mL bottles (0mL product being used from Day 17)  
     Zero level of flushing/product dispensed.  
     One of the dispenser has been disconnected intentionally - repeat act of vandalism)  
**Note:**  
 Analysis termination and field test ended on Day 17 (Test 3B) due to the fact that dispenser equipment being disconnected intentionally (vandalisms) and no product has been dispensed into the urinal after Day 17

	Test Status:	Treated				
	Test Day:	Day 1	Day 9	Day 17	Day 36	Day 47
	Test No.:	1 <sup>st</sup> Sampling (1B)	2 <sup>nd</sup> Sampling (2B)	3 <sup>rd</sup> Sampling (3B)	4 <sup>th</sup> Sampling (4B)	5 <sup>th</sup> Sampling (5B)
<b>Product Dispensed:</b>		0mL	10mL	10mL	0mL	0mL
<b>Laboratory Report No:</b>		300719	307429	308659	-	-

<b>Chemical Analysis</b>	<u>Unit</u>					
Total Suspended Solids	mg/L	90	10	3	-	-
Total Sulphide	mg/L	<0.1	<0.1	<0.1	-	-
Ammonia	mg N/L	110	8.7	5.5	-	-
<b>Microbiological Analysis</b>	<u>Unit</u>					
Total (Heterotrophic) Plate Count	orgs/mL	4.1 X 10 <sup>3</sup>	>1 X 10 <sup>4</sup>	1.1 X 10 <sup>4</sup>	-	-
Total (Heterotrophic) Plate Count	orgs/100mL	4.1 X 10 <sup>5</sup>	>1 X 10 <sup>6</sup>	1.1 X 10 <sup>6</sup>	-	-
Escherichia Coli (Colilert)	orgs/100mL	15	28	520	-	-
Enterococci (Enterolert)	orgs/100mL	10	9	10	-	-
Pseudomonas Aeruginosa	orgs/100mL	<10	0	0	-	-
Clostridium Perfringens	orgs/100mL	0	0	0	-	-
Clostridium Perfringens (Sulphites reducing Clostridia spores)	orgs/100mL	0	0	1	-	-

**Final Result:**  
**↑Heterotrophic Plate Count (beneficial bacteria), ↓Ammonia & Total Suspended Solid (complex urine & faecal built-up)**  
 Ecogreen Biosanitiser Blue (evenly dispensed throughout surface of the treated toilet urinal during flushing) has successfully demonstrated effective sanitisation activity. This is achieved via elimination odour causing complex urine and faecal built-up (eg. Ammonia and Total Suspended Solids) on wall of the urinal and further down the drainage.

**Recommendation:**  
 Routine toilet flushing after daily toilet usage with 30mL or more product accumulatively flushed/dispensed apply on treated toilet throughout the week is recommended to ensure surface is constantly repopulated Ecogreen Biosanitiser Blue beneficial bacteria for long term optimum sanitisation effect.

