

# Green Care Products Ltd.



## *A Catalogue of our Environmentally Friendly Cleaning Products*

KILLS  
99.9%  
Germs

Year  
2020

#1 Sibun Street, Belmopan  
Belize Central America  
T: 822-3518  
C: 672-3518

[william@greencleanbelize.com](mailto:william@greencleanbelize.com)  
[www.greencleanbelize.com](http://www.greencleanbelize.com)

[www.facebook.com/GreenCleanBelize](https://www.facebook.com/GreenCleanBelize)



# A Catalogue of our Environmentally Friendly Cleaning Products

## Table of Contents

<b>Who are we? .....</b>	<b>3</b>
Description of business:.....	3
Services offered: .....	4
Environmental & Social Responsibility:.....	4
<b>Greetings from our General Manager .....</b>	<b>5</b>
<b>Green Care Safeway Products.....</b>	<b>6</b>
1) GREEN CLEAN Multipurpose Cleaner: .....	6
2) KLEENA Floor & Surface Cleaner: .....	6
3) KLEENA Kitchen Cleaner (Unscented): .....	7
4) KLEENA Bathroom Cleaner:.....	7
5) KLEEN Tile, Glass & Window Cleaner (Unscented):.....	8
6) GREEN CLEAN Septic Aid: .....	8
7) KLEENA Hand Sanitizer:.....	9
<b>Product Presentations.....</b>	<b>10</b>
<b>Order Requests: .....</b>	<b>11</b>
1) Order Online: .....	11
2) Email Us: .....	11
3) WhatsApp: .....	11
4) Visit a store near you: .....	11
5) Or Call a Green Care Agent: .....	11
<b>Addendum: Green Care Products Technical Evaluation .....</b>	<b>12</b>
Germ Killing Power Efficiency Of Green Care Products.....	13
1) GREEN CLEAN & KLEENA Products Analysis against Germs (Kills 99.99% Germs) .....	13
1) EM Technology™ Products (Septic Aid etc.) Analysis against Germs .....	14
2) Influenza Virus Reduction with EM Technology™ (99.99% killing power) .....	16

# Who are we?



## **GREEN CARE PRODUCTS LTD. (GCPL)**

**Contact Person:** WILLIAM USHER

**Physical Address:** # 1 SIBUN STREET, CITY OF BELMOPAN, CAYO DISTRICT

**Mailing Address:** P.O. BOX # 352, CITY OF BELMOPAN

**City, District:** CITY OF BELMOPAN

**Telephone:** 822-3518; 672-3518   **Fax:** 822-35 18

**Email:** William@greencleanbelize.com

**Web Address:** www.greencleanbelize.com

### **Description of business:**

Green Care Products Ltd. (GCPL) is a limited liability company in existence since September 2012. GCPL is a Belizean owned company in the business of supplying sustainable and technologically innovative “Green Products” and services for the hotel, restaurant, industrial and household sectors of Belize.

GCPL is a company with a *VISION* to provide all households in Belize, the wider Caribbean and Central America with environmentally friendly cleaning products that function.

Our *MISSION* is to provide low cost and technologically innovative products and services that will allow for reduced cleaning and waste management costs to our clientele while providing a healthy and productive environment.

GCPL Technology currently includes metabolite from microorganism technologies for the purpose of daily household, hotel, restaurant and industrial cleaning, solid and liquid waste management.

GCPL is the exclusive marketer and distributor of all Belize Agro Enterprise Ltd cleaning products in Belize that currently includes the Green Clean® and Effective Microorganism™ brands.

### **Services offered:**

GCPL, through its associations, provides technical services and consultations in respect to the products being used to its clients. These services include office and residential cleaning, household and industrials' solid and liquid waste management.

### **Environmental & Social Responsibility:**

GCPL's core vision is to provide all households in Belize, the wider Caribbean and Central America with environmentally friendly cleaning products that functions on the guaranteed premise of product functionality. GCPL believes that the responsibility lies on the manufacturers and providers of cleaning products to be responsible to the environment and provide products and services to clients of all walks of life and that will be beneficial to the health of the end users and the world we live in.

GCPL's true philosophy is assuring that the technologies we provide are "Green" technologies and do not cause negative impact on the environment. GCPL's social responsibility henceforth lies in making sure our technologies are affordable and accessible to all and hence the contribution to the positive environmental foot print to be offered by our esteemed clientele.

***GCPL IS A 100% BELIZEAN OWNED AND OPERATED COMPANY!***

# Greetings from our General Manager

Dear Client:

I am William Usher, General Manager of Green Care Products Ltd. (GCPL). It is with much pleasure that we are able to present to you the current list of environmentally friendly cleaning products that we have to offer by means of this catalogue.

This current listing is by no means exhaustive of what is in the making as we are always looking at innovative ways to satisfy the needs and requirements of our clientele. Currently, we are in the process of developing environmentally friendly hand sanitizers and liquid soaps to hopefully have before the end of the 2020 calendar year. Other cleaning products to meet hotel and restaurant needs will also be sourced or manufactured where possible and if necessary.

As an added note, our company also produces organic bio-fertilizers and natural plant base insecticides for your gardens and domestic pets as well as natural probiotics for your domestic and farm animals in order for you to reduce the need for otherwise toxic chemicals where possible and especially the tourism sector within the ambit of the 'NEW NORMAL' of the Post COVID-19 period. In this area we are also expanding our list of products.

Green Care Products Ltd offers as well solid and liquid waste management with our Effective Microorganism (EM®) Technology that includes on-site sewerage waste management, natural pond water management and other black or grey water issue such as odor control, grease trap stoppage, house fly problems, microbiome surface cleaning against pathogenic agents and more.

Please don't hesitate to contact us as all our products and services are always available upon request.

Sincerely,

A handwritten signature in black ink, appearing to read 'W. Usher', with a stylized flourish extending to the right.

William Usher  
General Manager

# Green Care Safeway Products<sup>1</sup>

## 1) GREEN CLEAN Multipurpose Cleaner: *Effective kill time of pathogens at cleaning – 5 mins*



Our multi-purpose all-natural cleaner is both a disinfectant and a sanitizer that sanitizes, deodorizes and breaks down harmful chemicals to eliminate disease causing bacteria, mold & viruses, while preventing future toxins from establishing a population in any environment giving your home or business a fresh and natural clean. The use of Green Clean reduces the presence of pathogenic bacteria, fungus, molds, viruses and mites as well as reduces bad odors while emitting a fresh fragrance.

Due to its natural composition, Green Clean does not cause harm to the environment nor to your health and is biodegradable. Scented in naturally mild Citriboom (bubble-gum), Green Apple and Lavender hypo allergenic fragrances.

Use: Dilute 3X for floor surface cleaning and use 100ml of solution in 10-15L of water

## 2) KLEENA Floor & Surface Cleaner: *Effective kill time of pathogens at cleaning – 5 mins*



KLEENA's floor & surface cleaner lifts and removes dirt while it eliminates most bacteria, fungus and virus while leaving a natural shine to the floor and surfaces where it is used. This product is a natural odor suppressant and at the same time a mild and fresh fragrance is included to improve the smell of the surroundings where it is used.

Due to its natural composition, KLEENA's floor and surface cleaner does not cause harm to the environment nor to your health. Kleena is a Biodegradable and Ecologically friendly cleaning product. Scented in naturally mild Cinnamon or Lavender hypo allergenic fragrances.

Use: Use 100ml of solution full strength for floor & surface cleaning in 10-15L of water

---

<sup>1</sup> Green Care Products can replace most cleaning products used in the hotel and restaurant industry in Belize. All cleaning products are safe for human use, biodegradable and environmentally friendly. All products are made in Belize.

3) KLEENA Kitchen Cleaner (Unscented): *Effective kill time of pathogens at cleaning – 3-5 mins*



KLEENA's kitchen & grease cleaner was designed for use in food kitchens & industrial food processing areas, health clinics & hospitals. The product eliminates food grade bacteria, fungus & some virus while suppressing bad odors.

Cleans floors, kitchen surfaces including food handling surfaces and kitchen appliances. Can be used to clean floors in common areas of hospitals and health clinics.

Due to its natural composition, KLEENA's kitchen and grease cleaner does not cause harm to the environment nor to your health. Kleena is a Biodegradable and Ecologically friendly cleaning product.

Use: Spray solution full strength for kitchen surface cleaning, leave 3-5 minutes and wipe

4) KLEENA Bathroom Cleaner: *Effective kill time of pathogens at cleaning – 5 mins*



KLEENA's bathroom cleaner removes dirt, soap scum and sanitizes by eliminating bacteria, molds and some virus while suppressing bad odors and at the same time allowing a fresh fragrance.

Due to its natural composition, KLEENA's bathroom cleaner does not cause harm to the environment nor to your health. Kleena is a Biodegradable and Ecologically friendly cleaning product. Scented in a naturally mild citrus apple bloom blend of hypo allergenic fragrances.

Use: Spray solution full strength on all bathroom surface & fixtures; leave 5 minutes and wipe



- 5) **KLEEN Tile, Glass & Window Cleaner (Unscented)**: *Effective kill time of pathogens at cleaning – 3-5 mins*



KLEENA's Glass, Tile & Window cleaner was designed for cleaning glass, windows and tiles. The product removes grease, mild hard water deposits and smears leaving a crystal clear and streak free shine after cleaning. This product will also eliminate bacteria, fungus and some virus upon contact.

Due to its natural composition, KLEENA does not cause harm to the environment nor to your health. Kleena is a Biodegradable and Ecologically friendly cleaning product.

**Use:** Spray solution full strength for kitchen surface cleaning, leave 3-5 minutes and wipe

- 6) **GREEN CLEAN Septic Aid**: *Effective kill time of pathogens while surface cleaning – 1-3 mins; When use for black & gray water treatments the time of pathogen suppression depends on the degree of problem that exist during treatment time.*



Septic Aid is a biological product that is formulated naturally of aerobic and anaerobic microorganisms. Septic Aid is made using Effective Microorganism™ Technology, which is a combination of non-toxic and beneficial fermenting microorganisms that include lactic acid bacteria, phototrophic bacteria and yeast. The product is used to control pathogenic processes in both grey and sewage black water and on surface areas.

The product can also be used as a biological surface cleaner within any household or businesses including hotels and restaurants; important note is that since the product is biological in nature, any Adenosine Tri-Phosphate (ATP) monitoring equipment will detect the presence of microbes within the system being used.

Septic Aid was developed to address three critical components of sewerage waste within septic and septic-like structures that include: odor control, bacterial equilibrium and physicochemical integrity of the solid and liquid waste within. The product is 100% organic, naturally biodegradable, environmentally friendly and safe on human and animal contact.

**Use 1 (Gray & Black water management):**

Flush 200ml directly into sewerage, urinals & kitchen sink daily

Use 2 (Biological Surface Cleaner & Mister):

- a) As a surface cleaner dilute 1 part of solution in 30-40 parts of water. Spray diluted solution on surfaces or mop floor surface.
- b) As a mister dilute 1 part of solution to 50 parts of water and apply within an automated mister where clients can be free to walk in order to decontaminate.

Use 3 (Outdoor Microbiome protector against pathogenic microbes and viruses):

Dilute 1 part of solution to 50 parts water and spray daily within the areas outside the establishments where human and vehicular traffic are constant including walkways, parking lots and the general open spaces outdoor (i.e. Public spaces & Communal area).

- 7) KLEENA Hand Sanitizer: *Effective kill time of pathogens with surface cleaning use – 1min.*

**COMING SOON!**



# Product Presentations

Products are currently available in the following sizes:

a) 1L at ( 12 x 1L) or case presentations → All products



Kleena Products



Multipurpose Cleaner & Septic Aid Products

b) 5L at ( 1 x 15L) presentations → Multipurpose Cleaners only



3 Fragrances (Green Apple, Citri-boom, Lavender)

c) 15L at ( 1 x 15L) presentations → Septic Aid Product only



## \*Upon Request

- All products can be made available in 15L presentations.
- 5L presentations can be possible for the Green Clean and Kleena products only.

**Note:** All 15L containers are reusable and hence these products are returnable.

# Order Requests:

There Are Several Options To Get Your Hands On Some Green Clean Products:

1) Order Online:

Green Care Cleaning Products NOW AVAILABLE online at:



2) Email Us:

[info@GreenCleanBelize.com](mailto:info@GreenCleanBelize.com)

3) WhatsApp:

[CLICK TO SEND US A WHATSAPP MESSAGE](#)

4) Visit a store near you:

[CLICK TO VIEW STORES LOCATION](#)

5) Or Call a Green Care Agent:

Green Care Products Ltd.

#1 Sibun Street, Belmopan, Cayo District

T - Company Office (Belmopan): **822-3518**

C - Cayo (Belmopan) & Toledo: **672-3518**

C - Cayo (San Ignacio & Santa Elena): **667-5032** // 672-3518

C - Belize City: **602-6823** // 672-3518

C - Stann Creek: **671-2737** // 672-3518

C - Corozal & Orange Walk: **673-1683** // 672-3518

- **Note:** Numbers in **bold** are Primary Agent Contacts

# Addendum: Green Care Products

## Technical Evaluation

### Addendum 1: Germ Killing Power

1	Laboratorio Chaso San Jose, Costa Rica	Analysis against germs with Green Care (i.e. Green Clean and KLEENA)
2a	<b>Title:</b> Establishment of human and environmentally friendly hospital with consideration for human and environmental microbiome	<b>Authors:</b> <i>Isa Saito, Masaki Shintani, Nami Murakami, Yutaro Aoki and Teruo Higa</i> Scientific research against germs in hospital (i.e. Activated EM/ Septic Aid)
2b	EMRO Presentation. Okinawa Japan	Influenza virus inactivation with EM.1 (i.e. Septic Aid)
3	Japan Food Research Laboratories Tokyo, Japan	Influenza virus inactivation with EM.1 (i.e. Septic Aid)
4	Product Developer/Manufacturer	Biography: William Usher

# Germ Killing Power Efficiency Of Green Care Products

## 1) GREEN CLEAN & KLEENA Products Analysis against Germs (Kills 99.99% Germs)



114 - Laboratorio Chaso - Contacto: Gestión de Calidad - Teléfono +901-5915 - Correo: [asesor tecnico1@chaso.com](mailto:asesor tecnico1@chaso.com) - Dirección: Sabana Sur, San José

### INFORME DE ENSAYO N5MTA



<b>Tipo de muestra y descripción:</b> A1, B1 y C1 son eslive x 10 cm2	<b>Temperatura de recolección:</b> 25°C	<b>Fecha de toma de muestra:</b> 30/04/2020	<b>Fecha de ingreso:</b> 30/04/2020
A2: Desinfectante Green Care 1:10 x 10 cm2	<b>Propósito del ensayo:</b> Monitoreo	<b>Responsable de la custodia:</b> Andrea Chavarria	<b>Lote #:</b> N/A
B2: Desinfectante Green Care 1:16 x 10 cm2		<b>Temperatura de recolección:</b> 25°C	<b>Fecha de ejecución del ensayo:</b> 30/04/2020
C0: Desinfectante Green Care 1:20 x 10 cm2			
<b>Responsable de toma de muestra:</b> Francisco Soto			
<b>Toma de muestra por:</b> Laboratorio			

Análisis #	Análisis realizado	Resultado	Observaciones / Límites máximos	Metodología
N5MTA-1	* Recuento Total Aerobio	3.5 x 10 exp 4 UFC/10 cm2	A1	A01 - Microbiological Examination Methods of Food and Water CRC Press 2013-APHA (PTL01-MT05)
N5MTA-2	* Recuento Total Aerobio	4.1 x 10 exp 4 UFC/10 cm2	B1	A01 - Microbiological Examination Methods of Food and Water CRC Press 2013-APHA (PTL01-MT05)
N5MTA-3	* Recuento Total Aerobio	3.5 x 10 exp 4 UFC/10 cm2	C1	A01 - Microbiological Examination Methods of Food and Water CRC Press 2013-APHA (PTL01-MT05)
N5MTA-4	* Recuento Total Aerobio	< 10 UFC/10cm2	A2	A01 - Microbiological Examination Methods of Food and Water CRC Press 2013-APHA (PTL01-MT05)
N5MTA-5	* Recuento Total Aerobio	< 10 UFC/10 cm2	B2	A01 - Microbiological Examination Methods of Food and Water CRC Press 2013-APHA (PTL01-MT05)
N5MTA-6	* Recuento Total Aerobio	< 10 UFC/10 cm2	C2	A01 - Microbiological Examination Methods of Food and Water CRC Press 2013-APHA (PTL01-MT05)

**Nota 1: \* Ensayo acreditado / \*\* Ensayo no acreditado, ver alcance [www.eca.or.cr](http://www.eca.or.cr)**

Nota 2: El muestreo acreditado en el alcance solo aplica para las muestras tomadas por el personal de Chaso del Valle S.A. Este informe tiene validez únicamente con la firma digital o física del microbiólogo, los resultados son válidos únicamente para la muestra analizada Laboratorio de segunda parte. Está prohibida la reproducción parcial o total de este informe sin autorización de Chaso del Valle S.A.

PTL07-0001 - Versión 1

Sabana Sur, 108 avda y 175 sur de McDavid  
Teléfono: 4001-9915 Fax: 4001-4493 [www.chaso.com](http://www.chaso.com) [asesor tecnico1@chaso.com](mailto:asesor tecnico1@chaso.com)

Página 1 de 1



# 1) EM Technology™ Products (Septic Aid etc.) Analysis against Germs

## a) EM Technology™ reduction of Germs in Hospital (99.9% effective on most germs)

found that foul odor in toilets and particular smell of hospital disinfectant were remarkably reduced after introduction of EM for cleaning. Sanitation workers reported that their rough hands were improved after stopping the use of bleaching agents and bactericidal agents.

### 3.2. Effect of cleaning with EM on *E. coli* & coliform group, MRSA and general viable bacteria

Results at Asaka Kosei Hospital are shown in Table 1, 2 and 3. Before cleaning with EM, less than 10 colonies per 10 cm<sup>2</sup> of *E. coli* & coliform group were detected in garbage bin in the sanitary room and sink faucet. After the introduction of cleaning with EM, no colonies were detected except in garbage bin in the sanitary room (Table 1). Colonies of MRSA were detected before cleaning with EM, at 5 out of 10 sampling points. Namely, 30 colonies of MRSA were detected on the floor of room 305. After introducing EM, colonies were still detected at 1 to 2 sampling points until Oct. 2015. No colonies were detected from Dec 2015 to Feb 2016 (Table 2).

Our data suggest that growth of *E. coli* & coliform group and MRSA is efficiently suppressed by cleaning with EM. Growth of other general viable bacteria, however, was not suppressed by cleaning with EM (Table 3).

Table.1 Effect of cleaning with EM on *E. coli* & coliform group

Sampling point	Contamination level									
	No EM	Cleaning with EM								
		Nov. 17 2014	Nov. 20 2014	Dec. 1 2014	Jan. 27 2015	Aug. 27 2015	Oct. 27 2015	Dec. 24 2015	Feb. 26 2016	Apr. 28 2016
1 Toilet stoolink, 2nd floor	—	—	—	—	—	—	—	—	—	—
2 Toilet seat, 2nd floor	—	—	—	—	—	—	—	—	—	—
3 Garbage bin in the sanitary room, 2nd floor	+	—	—	—	+	—	—	—	—	—
4 Disinfectant, 1st floor west side	—	—	—	—	—	—	—	—	—	—
5 Rail in front of the endoscope room, 1st floor	—	—	—	—	—	—	—	—	—	—
6 Floor of Room 305	—	—	—	—	—	—	—	—	—	—
7 Wall of Room 305	—	—	—	—	—	—	—	—	—	—
8 Sink faucet, 2nd floor	+	—	—	—	—	—	—	—	—	—
9 Washing trough faucet, 1st floor	—	—	—	—	—	—	—	—	—	—
10 Stoolbin in front of the toilet, 2nd floor	—	—	—	—	—	—	—	—	—	—

Table.2 Effect of cleaning with EM on Methicillin-resistant *Staphylococcus aureus* (MRSA)

Sampling point	Contamination level									
	No EM	Cleaning with EM								
		Nov. 17 2014	Nov. 20 2014	Dec. 1 2014	Jan. 27 2015	Aug. 27 2015	Oct. 27 2015	Dec. 24 2015	Feb. 26 2016	Apr. 28 2016
1 Toilet stoolink, 2nd floor	—	—	—	—	—	—	—	—	—	—
2 Toilet seat, 2nd floor	+	—	—	—	—	—	—	—	—	—
3 Garbage bin in the sanitary room, 2nd floor	+	—	—	—	+	+	—	—	—	—
4 Disinfectant, 1st floor west side	—	—	—	—	—	—	—	—	—	—
5 Rail in front of the endoscope room, 1st floor	—	—	—	—	—	—	—	—	—	—
6 Floor of Room 305	+++	+	—	—	—	—	—	—	—	—
7 Wall of Room 305	+	—	—	+	—	—	—	—	—	—
8 Sink faucet, 2nd floor	+	—	—	—	—	—	—	—	—	—
9 Washing trough faucet, 1st floor	—	—	—	—	—	—	—	—	—	—
10 Stoolbin in front of the toilet, 2nd floor	—	+	—	—	—	—	—	—	—	—

Table.3 Effect of cleaning with EM on general viable bacteria

Sampling point	Contamination level									
	No EM	Cleaning with EM								
		Nov. 17 2014	Nov. 20 2014	Dec. 1 2014	Jan. 27 2015	Aug. 27 2015	Oct. 27 2015	Dec. 24 2015	Feb. 26 2016	Apr. 28 2016
1 Toilet stoolink, 2nd floor	+	—	—	—	—	—	—	—	—	—
2 Toilet seat, 2nd floor	+	+	+	+	+	+	+	+	+	+
3 Garbage bin in the sanitary room, 2nd floor	+++	+++	+	+	+	+	+	+	+	+
4 Disinfectant, 1st floor west side	+++	++	—	—	—	—	—	—	—	—
5 Rail in front of the endoscope room, 1st floor	+	++	+	+	+	+	+	+	+	+
6 Floor of Room 305	+++	+++	+	+	+	+	+	+	+	+
7 Wall of Room 305	+	++	+	+	+	+	+	+	+	+
8 Sink faucet, 2nd floor	+	+	+++	+++	+++	+++	+++	+++	+++	+++
9 Washing trough faucet, 1st floor	+	++	+	+	—	—	+	+	+	+
10 Stoolbin in front of the toilet, 2nd floor	+	++	+++	+++	+	+	+	+	+	++

Criteria method based on L. Ten Cate

Number of colonies/10cm <sup>2</sup>	Rating
No colony was formed	—
<10	+
10 - 30	++
30 - 100	+++
> 100	++++
Too numerous to count	+++++

This finding may suggest that growth of *E. coli* & coliform group and MRSA is suppressed by microbes such as lactic acid bacteria in EM. It was previously shown that the direct interaction of lactic acid bacteria and MRSA in such a mixture led to the elimination of 99% of the MRSA cells within 24 hours. [10]

Detection test for MRSA was also performed at Noda hospital, and no colonies were detected from all sampling points (n=10). Re-infection of MRSA was not reported after introducing EM at both Asaka Kosei Hospital and Noda Hospital.

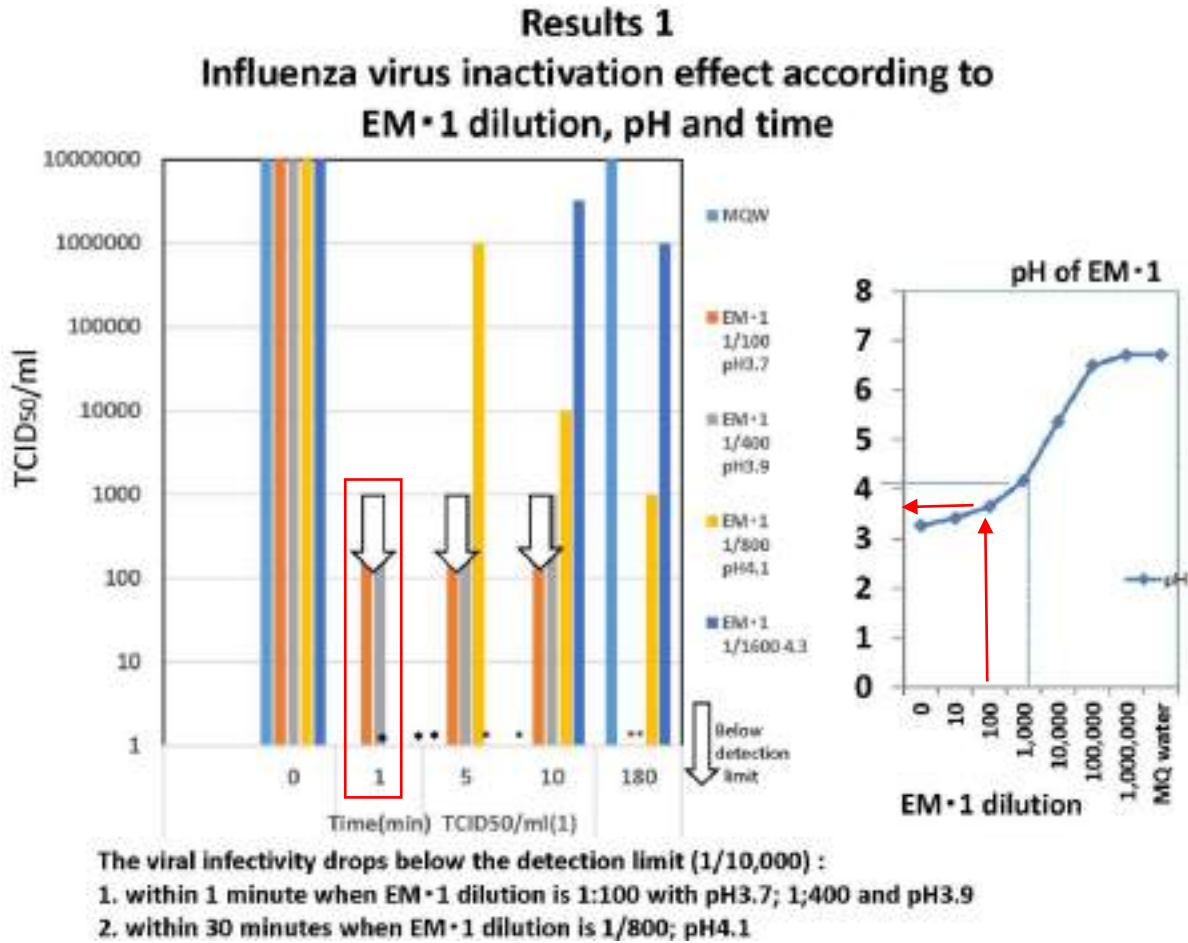
Our observations and findings show that active use of beneficial microorganisms such as EM in hospital environment turn into reality the kitchen garbage recycling, improvement of hospital diet, improvement of gardening and greening, reduction of foul odor in wastewater and toilets, reduction of use bleaching agents and bactericidal agents, and suppression of pathogen such as coliform group and MRSA in hospital.

Intentional use of microorganisms in hospitals could conceptually be regarded as opposite to the conventional approach from the point of hospital hygiene. Therefore, studies are needed to further understand the effect of EM application on human and hospital microbiome.

### 4. CONCLUSION

Our studies in two hospitals in Japan suggest that improving the human and hospital microbiome utilizing effective microorganisms (EM) in hospital environment represents an alternative approach to control hospital acquired infection (HAI) and to create human and environmentally friendly hospital.

b) Influenza Virus Reduction with EM Technology™ (99.99% killing power)<sup>2</sup>





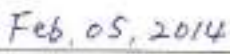

**Note:**

- a) TCID<sub>50</sub>/ml = Median Tissue Culture Infectious Dose per milliliter.
- b) This measurement tells the virus counts necessary for infection. Above 10,000 TCID<sub>50</sub>/ml means the virus will gain the possibility of infection hence the limit of detection.
- c) At 1:100 dilution of EM.1® the detection was found to be less than 1000 TCID<sub>50</sub>/ml after 1 minute of association with the virus at pH 3.7.

<sup>2</sup>A complete reference of the results a) and b) can be provided upon request.



## 2) Influenza Virus Reduction with EM Technology™ (99.99% killing power)

	<b>Japan Food Research Laboratories</b>	<b>Japan Food Research Laboratories</b> Authorized by the Japanese Government 52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan <a href="http://www.jfri.or.jp/">http://www.jfri.or.jp/</a>
		No. 13100704001-02 Page 1 of 4 December 16, 2013
<b>REPORT</b>		
Client: EM Research Organization, Inc. 1478 Kishaba, Kitanakagusuku-Soe, Nakagami-Gun, Okinawa 901-2311, Japan		
Sample(s): EM-1		
Title: Virus Inactivation Test		
Received date of sample(s): October 16, 2013		
This report has been translated into English from the Japanese report No. 13100704001-01 (December 16, 2013).		
 _____ Takeko Arai Principal Investigator		 _____ Date
		
—000221— 日本食品分析センター		



## Virus Inactivation Test

1. Client

EM Research Organization, Inc.

2. Sample

EM-I

3. Purpose

This test aims to evaluate the virus inactivation (*Influenza virus*) of the sample.

4. Outline of methods

The sample dilution was mixed with *Influenza virus* suspension and stored at room temperature (20 °C to 25 °C). After 30 minutes, the virus infectivity titer of the mixture was determined.

The method for determining virus infectivity titers was validated by a preliminary test.

5. Results

1) Preliminary test

The preliminary test indicated that the effects of the sample were removed by diluting the mixture of the sample solution and the virus suspension to 10-fold with MOPS cell support medium.

2) Virus infectivity assay

Table 1 shows the results.

The virus infectivity titer (log TCID<sub>50</sub>/mL) of 100-fold sample dilution containing the virus suspension decreased more than 4.3 when compared to the initial time and after 30 minutes.

Table 1. Virus infectivity titers of the mixtures

Test organism	Object	Concentration	log TCID <sub>50</sub> /mL <sup>*1</sup>	
			Initial	After 30 minutes
<i>Influenza virus</i>	Sample	100-fold dilution <sup>*2</sup>	5.8	<1.5
		1000-fold dilution <sup>*2</sup>	5.8	5.7
	Control	—	5.8	6.0

TCID<sub>50</sub>: Median tissue culture infectious dose

Initial: The TCID<sub>50</sub> of the control was measured immediately after mixing the solutions, and designated as the result at the initial time.

Control: Purified water

Storage temperature: room temperature (20 °C to 25 °C)

<1.5: Not detected

\*1 Logarithm of TCID<sub>50</sub> per 1 mL of the mixture

\*2 The mixture was diluted with purified water just before testing.

## 6. Methods in detail

### 1) Test virus

*Influenza A virus* (H1N1) A/PR/8/34 ATCC VR-1469

### 2) Test cell

MDCK (NBL-2) cells ATCC CCL-34 strain (Dainippon Pharmaceutical Co., Ltd.)

### 3) Culture media

#### a) Cell culture medium

Eagle's MEM "Nissui"<sup>(1)</sup> (Nissui Pharmaceutical Co., Ltd.) including 10 % of fetal bovine serum

#### b) Cell support medium

Eagle's MEM "Nissui" <sup>(1)</sup>	1000 mL
10 % NaHCO <sub>3</sub>	14 mL
L-glutamine (30 g/L)	9.8 mL
100 × Vitamins for MEM	30 mL
10 % albumin	20 mL
0.25 % trypsin	20 mL

#### c) MOPS cell support medium (for pH buffer)

Eagle's MEM "Nissui" <sup>(1)</sup>	1000 mL*
L-glutamine (30 g/L)	9.8 mL
100 × Vitamins for MEM	30 mL
10 % albumin	20 mL
0.25 % trypsin	20 mL

\* Prepared with 20 mM MOPS (Dojindo Laboratories).



4) Preparation of virus suspension

a) Cell incubation

The test cell was monolayer-cultured in a tissue culture flask using the cell culture medium.

b) Virus inoculation

After monolayer culture, the culture medium was removed from the flask, and the test virus was inoculated. Next, the cell support medium was added, and the virus was cultured in a CO<sub>2</sub> incubator (CO<sub>2</sub>: 5 %) at 37 °C ± 1 °C for 1 to 5 days.

c) Preparation of virus suspension

The cell form was observed with an inverted phase-contrast microscope. After morphological changes (cytopathic effects) were confirmed, the culture solution was centrifuged (3000 r/min, 10 minutes). The supernatant was used as a virus suspension.

5) Test procedure

The sample was diluted to 100-fold and 1000-fold with purified water. Then, 1 mL of each sample dilution was mixed with 0.1 mL of the virus suspension and stored at room temperature (20 °C to 25 °C). After 30 minutes, the mixtures were diluted to 10-fold with MOPS cell support medium, and the virus infectivity titers of the mixtures were measured.

As a control, purified water was prepared in the same manner as described above. The virus infectivity titer of the control was measured at the initial time and after 30 minutes.

6) Virus infectivity assay

The test cell was monolayer-cultured in a tissue culture microplate (96-well, Asahi Glass Co., Ltd.) using the cell culture medium. After the culture medium was removed, 0.1 mL of MOPS cell support medium was added. Next, 10-fold dilution of the mixtures of the sample and the virus suspension, and the control were separately diluted in 10-fold series with MOPS cell support medium. Then, 0.1 mL of each dilution was poured into four wells each and cultured in a CO<sub>2</sub> incubator (CO<sub>2</sub>: 5 %) at 37 °C ± 1 °C for 4 to 7 days. After culture, the cells were observed by inverted phase-contrast microscope to observe if any morphological changes (cytopathic effects) had occurred. Then, median tissue culture infectious dose (TCID<sub>50</sub>) was calculated by Reed-Muench method and converted into virus infectivity titer per 1 mL of the mixture.

\*\*End of Report\*\*

# *A Catalogue of our Environmentally Friendly Cleaning Products*



#1 Sibun Street, Belmopan

Belize Central America

T: 822-3518

C: 672-3518

[william@greencleanbelize.com](mailto:william@greencleanbelize.com)

[www.greencleanbelize.com](http://www.greencleanbelize.com)

[www.facebook.com/GreenCleanBelize](https://www.facebook.com/GreenCleanBelize)