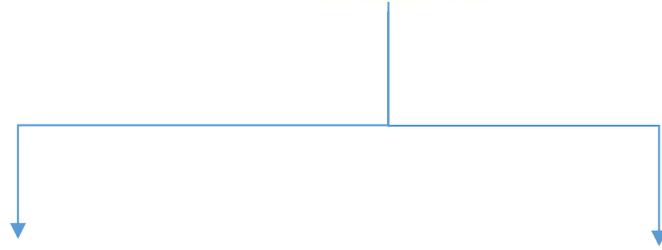






CONTENTS

1. Company Profile
2. Product Introduction.
3. Ecoguard Application Guidelines.
4. Ecoguard as Disinfectant
5. Lab trials using Ecoguard
6. Hatchery trials using Ecoguard
7. Publications.



PT. Rhea Natural Sciences

- Novel Chemical Small Molecule Design
- Chemical Synthesized Small Molecule

PT. Rhea Pharmaceutical Sciences

- Formulation of natural product for human & animal use
- Virology
- Immunology

- **CP Prima** is a leading aquaculture company in Indonesia. It has feed mills, hatcheries, & food processing plant strategically located across nation.
- **CP Prima** has strong commitment in research & development. Main R&D activities focuses on development of compounds to support its main business (i.e. natural probiotics & feed additives)
- To support its R&D drive, **CP Prima** is associated with **PT. Rhea Natural Sciences** And Rhea Pharmaceutical Sciences.
- Both are Singapore-based biopharmaceutical company under the umbrella of the Charoen Pokphand Indonesia GROUP / Central Proteina Prima Group.

OUR CREDENTIALS

Registered Products

Regulatory Body	Registration number	Product Details
 <p>Kementerian Kelautan & Perikanan</p>	<ul style="list-style-type: none"> ➤ KKP RI No D 16060285 HBC ➤ KKP RI No. D 1804350 HBS 	<ul style="list-style-type: none"> ➤ <u>PondGuard</u> ➤ <u>PondGuard Salt</u> <p>Both <u>Pondguard</u> & <u>Pondguard salt</u> are natural compound which improves overall health of aquatic animals.</p>
 <p>National Institute of Standards CJSC, Armenia</p>	<ul style="list-style-type: none"> ➤ TY AM 50106084.8409 – 2019 	<ul style="list-style-type: none"> ➤ <u>Naturalguard</u> Feed Additives <p>Natural compound to help protect livestock (<u>i.e.</u> poultry, swine, cattle) against viral infection</p> <ul style="list-style-type: none"> ➤ <u>Ecoguard</u> Natural Disinfectant (under reg process)

MILESTONES

Products Across Countries

Animal Use:

- PondGuard
- EcoGuard
- NaturalGuard



Human Use:

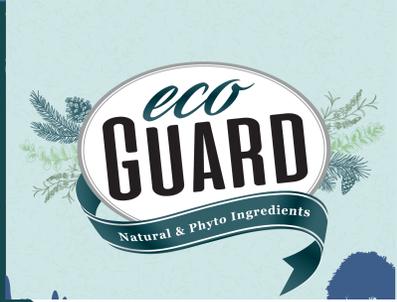
- Health Tone Oil
- Musclex



Publications and Acknowledgements

25 Publications on various fields acknowledged.





Product Introduction

- This product functions as disinfectant to deactivate and degenerate pathogens present in the aerosol, free form and attached at the surface of the floors, warehouse and storage.
- It can also serve as disinfectant when used in washing the necessary equipment.
- Unlike other disinfectant products that are usually toxic, Ecoguard is safe both for humans and animals.

**Ecoguard consist of natural oils extracts:
Jasmine Oil, Gardenia Oil and Eucalyptus oil.**

Characteristics of Ecoguard

Product Description: Yellow-transparent Liquid.

Characteristics:

It has physical properties such as pH 3.89 – 3.92, boiling point 197°C.

It is non-hazardous and user-friendly.

Packaging: Glass bottle 100 mL, 250 mL, 500 mL, and 1L.



Recommended Dose of Application

Application Method		Dose	Duration
Spraying		80 ppm	2-3 times a week
Application to the hatchery tanks	Preventive	Mysis tank: 3 ppm PLs tank: 5 ppm	Every 3 rd day
	Treatment	Mysis tank: 3 ppm PLs tank: 5 ppm	Daily



ECOGUARD SPRAYING PROTOCOL (Tanks)



Culture tank, water tanks and live-feed tank were cleaned and washed as per hatchery SOP



Clean freshwater for spray was prepared



80 ppm Ecoguard (0.8 mL for 10 liter of clean and clear water) was prepared and filled in a clean sprayer



Sprayed on the walls & floor of the tank until its completely wet



Left for at least 3 hours



The tank is ready to fill up the water



ECOGUARD SPRAYING PROTOCOL (Whole hatchery facility)



Clean freshwater for spray was prepared



80 ppm Ecoguard (0.8 mL for 10 liter of clean and clear water) was prepared and filled in a clean sprayer



Sprayed on the hatchery gates, roof, walls, office, lab, and the whole area of hatchery until it completely wet.



This steps can be repeated for 2 times



Ecoguard preparation and Spraying Video





Research Trials 1

Efficacy of Ecoguard as Tank Bottom Disinfectant



TRIAL METHODS :

1. The tested tanks were cleaned and washed.
2. Before spraying, samples was taken from the tanks by swab to determined the initial number of Vibrio.
3. Ecoguard 80 ppm were prepared and sprayed on the tank's surfaces (wall and bottom).
4. After 3 hours of spray, samples were collected to determine the efficacy of Ecoguard.

Vibrio screening in Swab samples before and after spraying with 80 ppm EcoGuard

Source	Before			After		
	No. of samples	No. of +Vibrio	+Vibrio (%)	No. of samples	No. of +Vibrio	+Vibrio (%)
MNPD-Sand Filter Tank	6	0	0.0	6	0	0.0
FPD-Sand Filter Tank	20	0	0.0	20	0	0.0
MNPD-Reservoir & Water Chiller Tank	29	0	0.0	26	0	0.0
FPD-Reservoir Tank	18	0	0.0	21	0	0.0
Quarantine & Maturation Tank	26	3	11.5	26	0	0.0
Hatching Tank	30	9	30.0	30	1	3.3
Spawning Tank	24	8	33.3	9	0	0.0
Rinsing Tank	24	2	8.3	22	0	0.0
Plankton Tank	36	2	5.6	36	0	0.0
LRT Tank	105	2	1.9	105	0	0.0
Packing Tank	10	0	0.0	10	0	0.0
Artemia Tank	35	0	0.0	35	0	0.0
Polychaete (bucket&floor)	6	0	0.0	6	0	0.0
Squid (equipments & table)	9	0	0.0	9	0	0.0
Fresh water Tank	16	1	6.3	20	0	0.0

Trial results

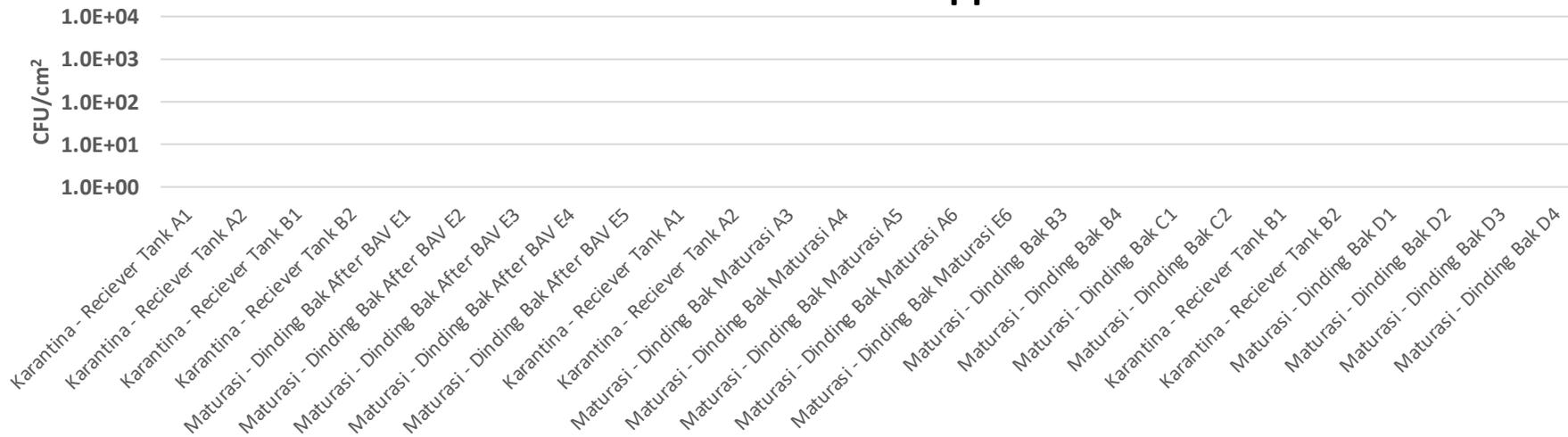
MATURATION TANK

Before EG application



After EG application

Not detected

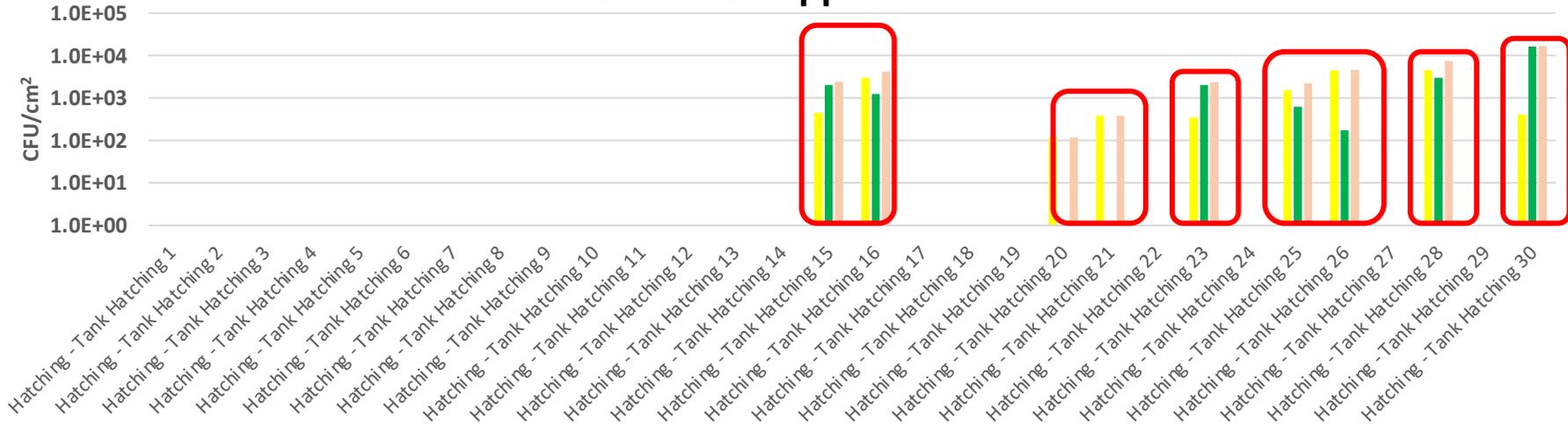


■ Swab TVC Yellow ■ Swab TVC Green ■ Swab TVC

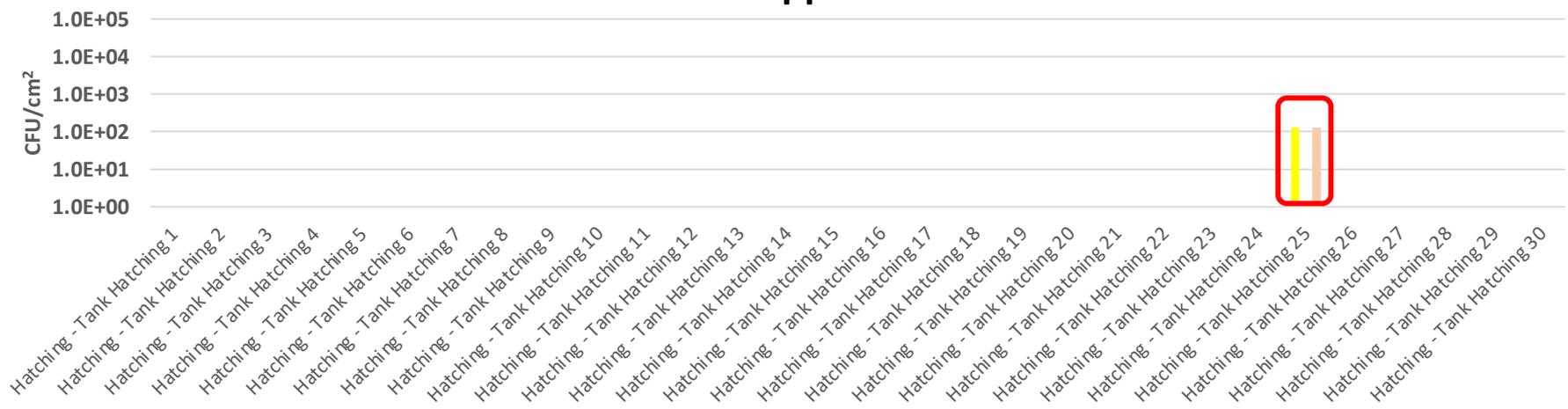
Trial results

HATCHING TANK

Before EG application



After EG application

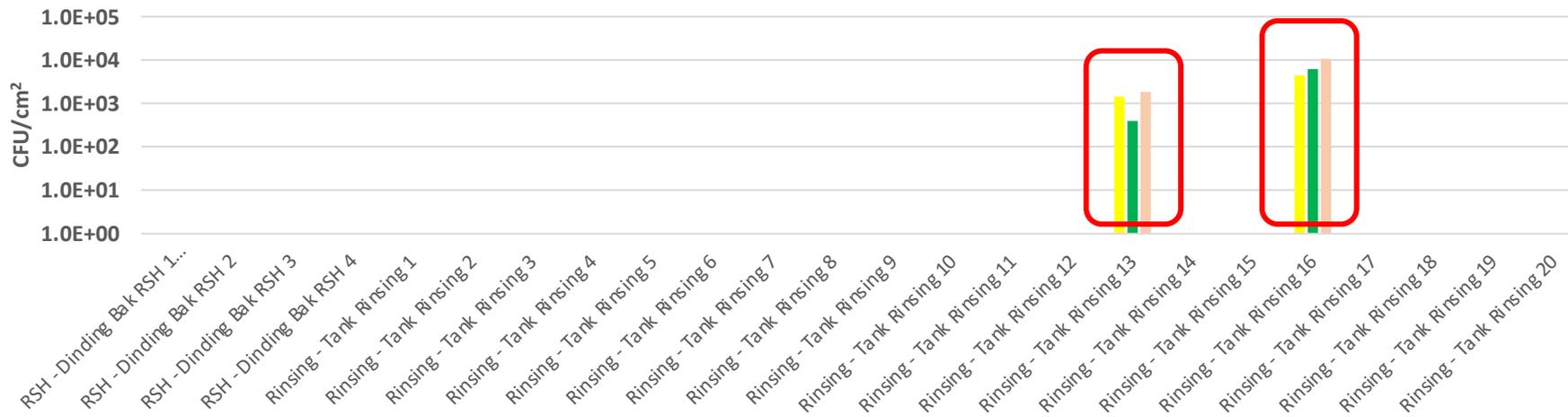


■ Swab TVC Yellow
 ■ Swab TVC Green
 ■ Swab TVC

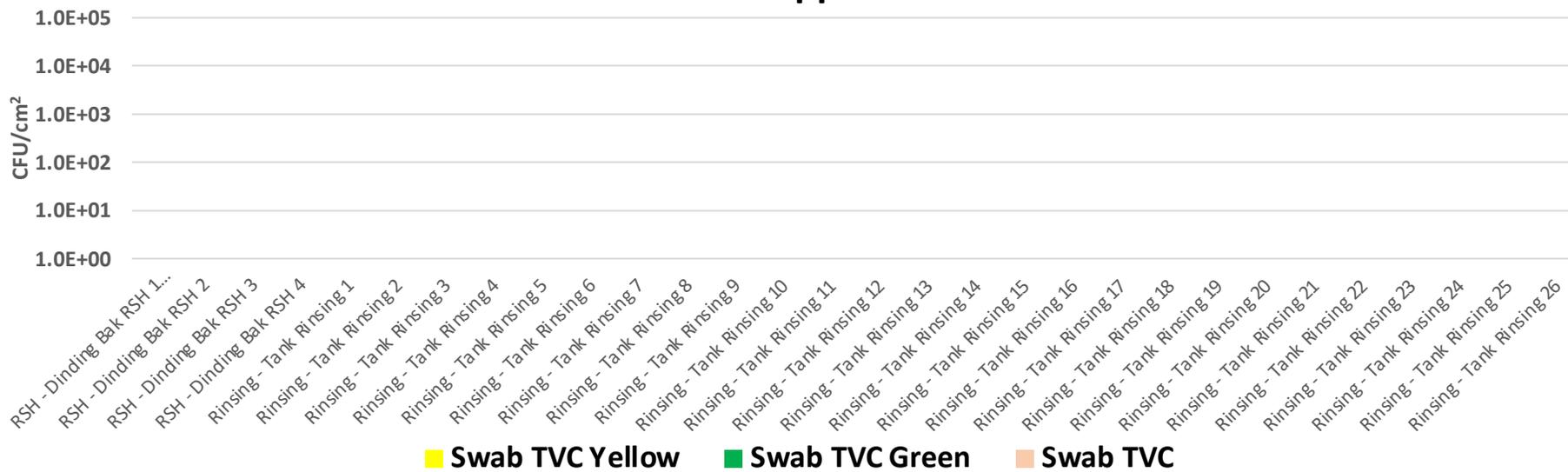
Trial results

RINSHING TANK

Before EG application



After EG application

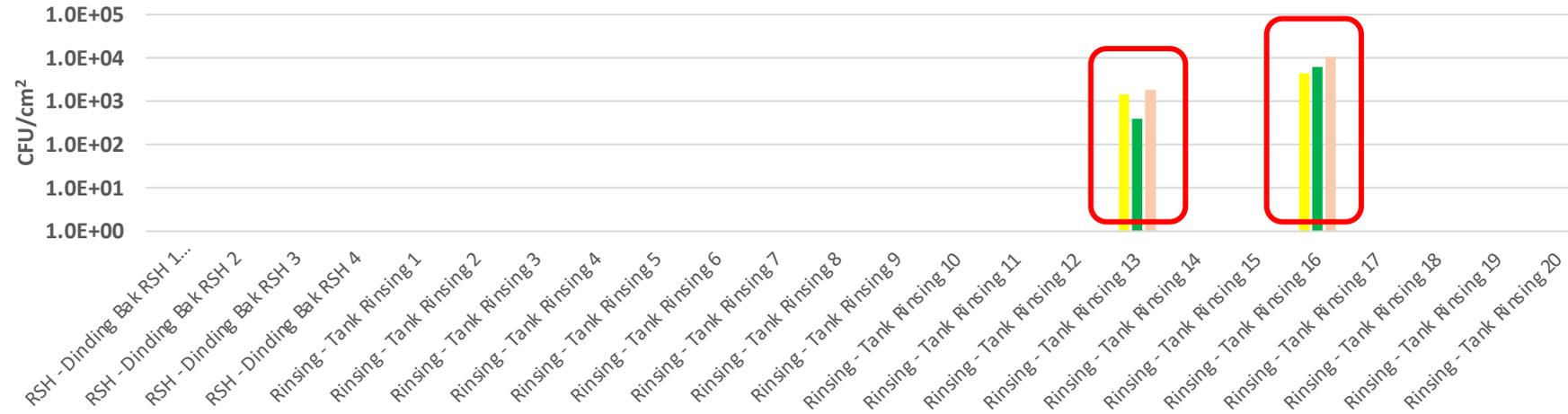


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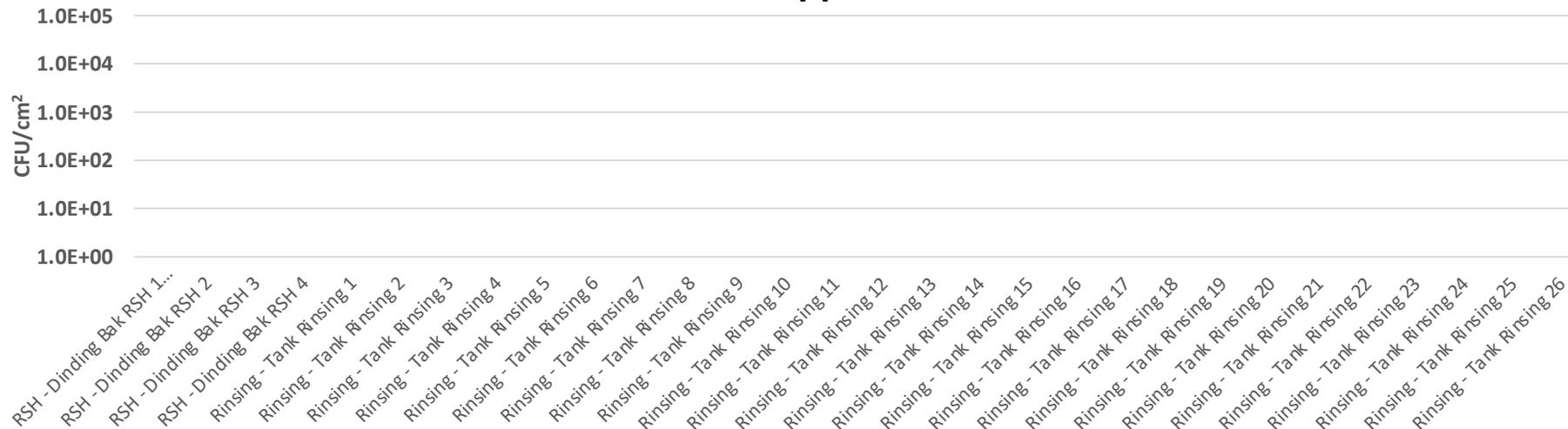
Trial Results

SPAWNING TANK

Before EG application



After EG application



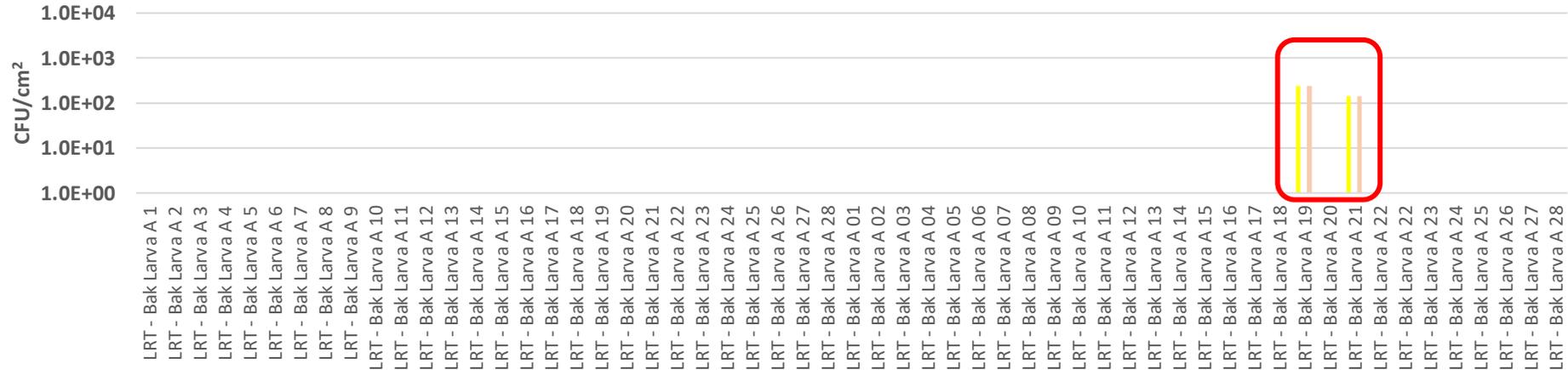
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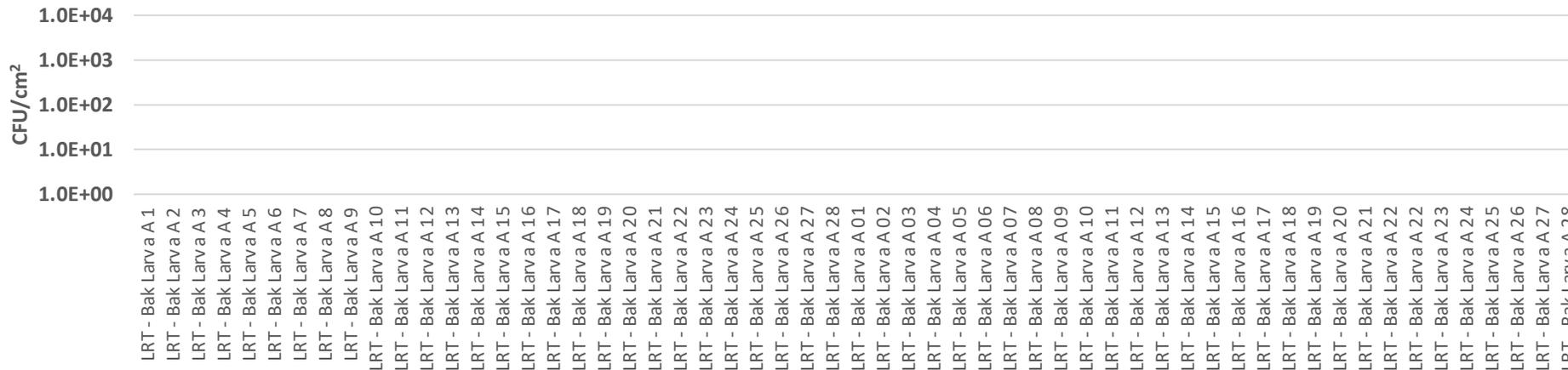
Trial results

PLANKTON TANK

Before EG application



After EG application



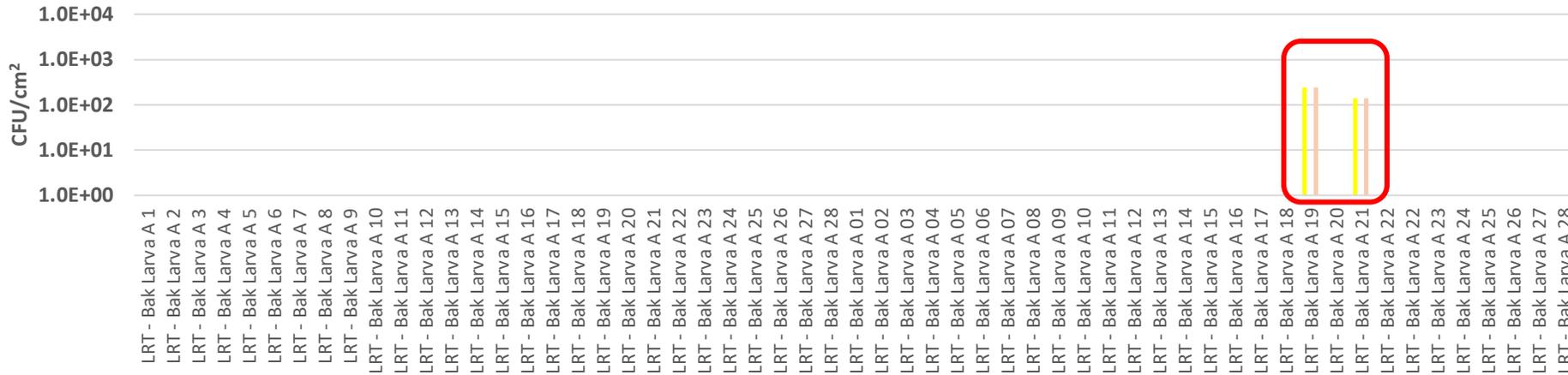
Not detected

■ Swab TVC Yellow
 ■ Swab TVC Green
 ■ Swab TVC

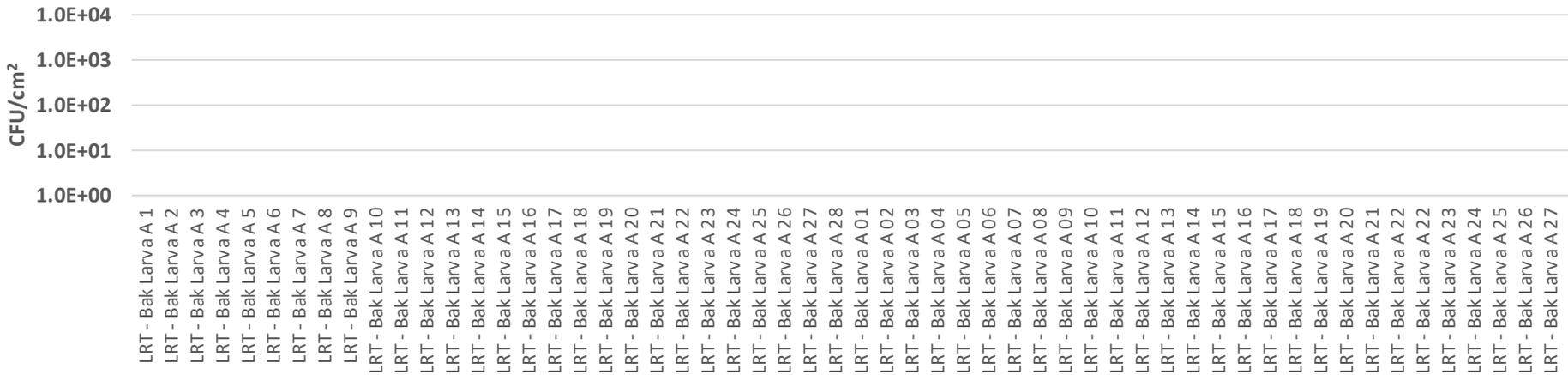
Trial results

LARVAE REARING TANK

Before EG application



After EG application



Not detected

■ Swab TVC Yellow ■ Swab TVC Green ■ Swab TVC

Conclusions and Recommendations

Conclusions:

Ecoguard can minimize the load of total Vibrio and Green Vibrio from the culture tanks' bottom and surface.

Recommendations:

- 1.Ecoguard can function as a disinfectant in a shrimp hatchery.
- 2.Ecoguard has proven to inhibit and minimize a load of pathogens in the culture tanks.



Research Trials 2

Efficacy of Ecoguard against *Vibrio parahaemolyticus* – AHPND using Spraying method of disinfection

TRIAL METHODS :

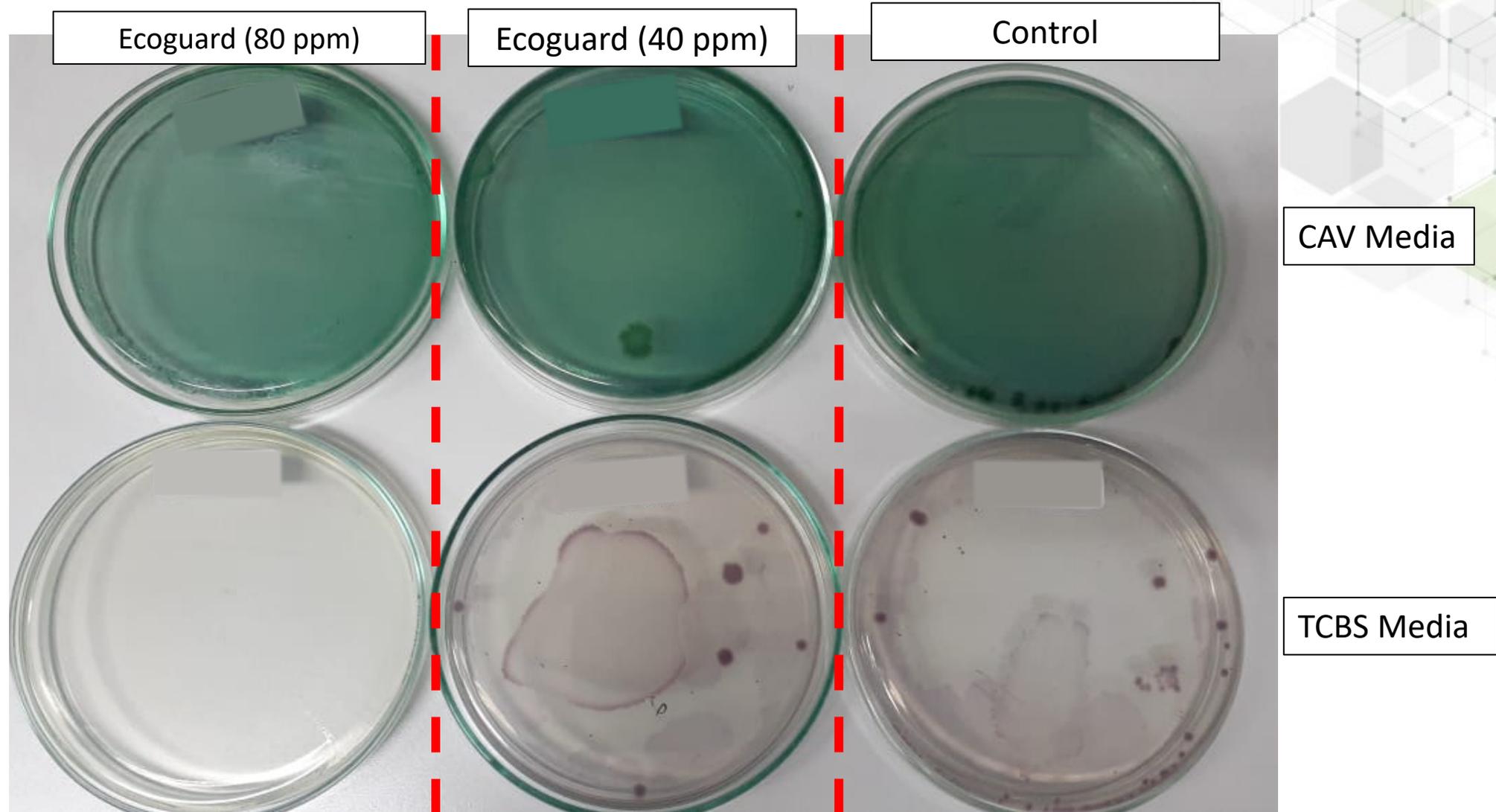
1. Swab the VP_(AHPND) on the sterile Petri dish; approximately 10^3 CFU/cm².
2. Let it dry for 3 hours or more
3. After drying, spray the Ecoguard doses (40 ppm and 80 ppm, three replicates per group) on the Petri dish surface (with dose 400 ml/m²)
4. After 3 hours of spray, pour the TCBS and CAV media on the Petridis. Incubate for 24 hours at 30°C

The trial was conducted at DRC, CeFAS, Vietnam

Number of Vibrio colony before and after Ecoguard application

Group	R	Growth of VP after Spray with Ecoguard			
		TCBS media		CAV media	
		Growth	Number of Colony	Growth	Number of Colony
Control	1	+	13	+	21
	2	+		+	
	3	+		+	
40 ppm of Ecoguard	1	+	1	+	5
	2	+		+	
	3	+		+	
80 ppm of Ecoguard	1	-	0	-	0
	2	-		-	
	3	-		-	

ECOGUARD Dose 40 and 80 ppm – after 48 Hours Incubation



Conclusions

Ecoguard at 80 ppm dose can deactivate the growth of *Vibrio parahaemolyticus* –
AHPND bacteria in vitro medium



Research Trials 3



Efficacy of Ecoguard against *Vibrio parahaemolyticus* – AHPND

Challenge Method

- VP(AHPND) culture was added into 5 L of seawater with a final density of approximately log 3 CFU/mL
- Challenge was conducted on the treatment after product application on day 3

Product Application

- Ecoguard Dose 5 ppm was applied daily, starting from day 1

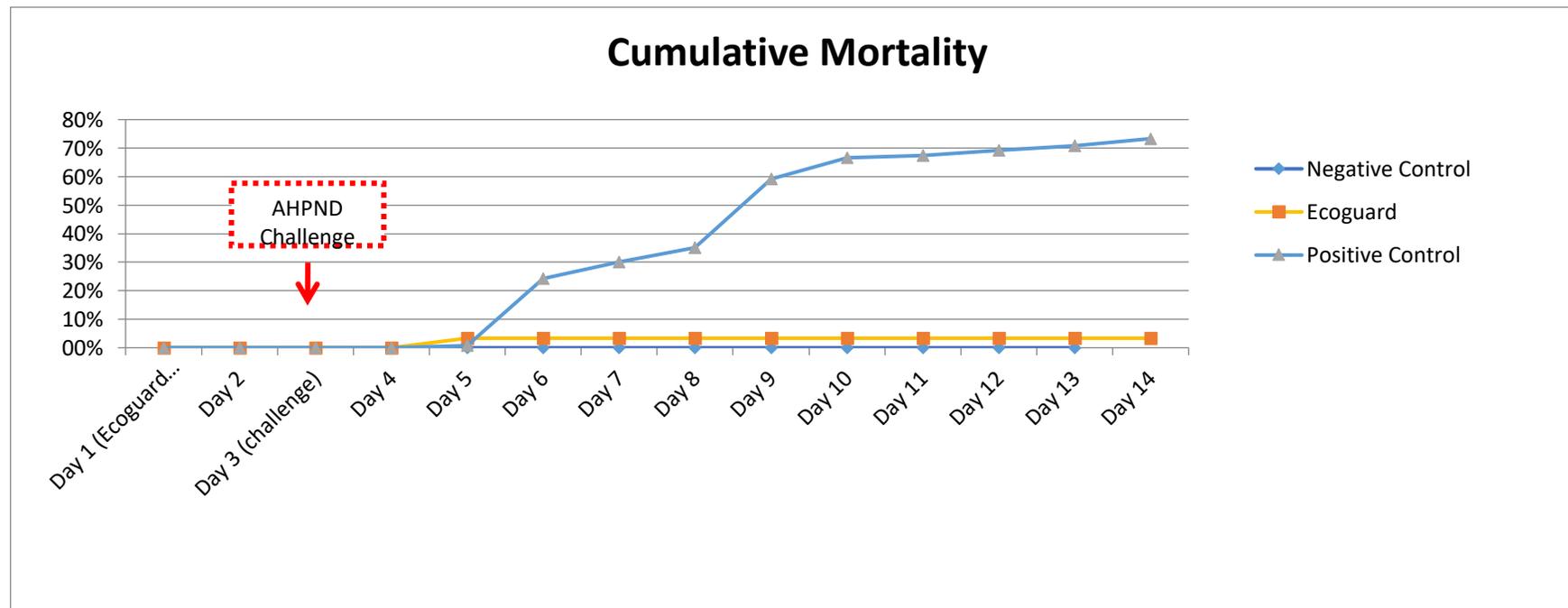
Observation

- Mortality of PL's : 3 times per day. Take out the dead shrimps and check for RT PCR from the first mortality
- PCR Analysis: every three days

The trial was conducted at DRC, CeFAS, Vietnam

Cumulative Mortality

Group	Replicates	Shrimp per tanks	Cumulative Mortality													
			Day 1 (Ecoguard application started)	Day 2	Day 3 (challenge)	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Negative Control	3	40	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Positive Control			0.0%	0.0%	0.0%	0.0%	0.8%	24.2%	30.0%	35.0%	59.2%	66.7%	67.5%	69.2%	70.8%	73.3%
Ecoguard			0.0%	0.0%	0.0%	0.0%	3.3%	3.3%	3.3%	3.3%	3.3%	3.3%	3.3%	3.3%	3.3%	3.3%





Trial Results



PCR Results

Group	Replicate	Day 1 (Ecoguard application started)	Day 2	Day 3 (challenge)	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Negative Control	1	Negative			Negative			Negative			Negative
	2	Negative			Negative			Negative			Negative
	3	Negative			Negative			Negative			Negative
Positive Control	1	Negative			Positive			Positive			Positive
	2	Negative			Positive			Positive			Positive
	3	Negative			Negative			Positive			Positive
Ecoguard	1	Negative			Positive			Negative			Negative
	2	Negative			Negative			Negative			Negative
	3	Negative			Negative			Negative			Negative

Conclusion

- Ecoguard can protect shrimp from *Vibrio parahaemolyticus* – AHPND bacteria.

THANK YOU

RHEA
natural