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FINAL R&D REPORT:

VIRUS CLEARANCE STUDY FOR LEDpac

Transmissible Gastroenteritis Virus (TGEV)
21-045466-262813 – Report.01

Prepared for:

LEDpac LLC
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Escondido, CA 92026

LL STUDY CODE: STUQL21AA0059-1
LL STUDY ACTIVITY CODE: STUQL21AA0059-1:k

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ABSTRACT

This study was performed to evaluate the inactivation Transmissible Gastroenteritis Virus (TGEV) by UVC Inactivation steps. Empty polystyrene petri dishes were spiked with virus and processed by Eurofins Lancaster Laboratories, Inc. (ELLI). Samples were assayed according to the analysis as listed in Table 2.

1. INTRODUCTION

The purpose of this study was to evaluate the ability of the client's UVC devices to inactivate TGEV spiked onto an empty polystyrene petri dish surface. UVC processing regimes are outlined in Figure 1. Prior to the viral clearance portion of the study, spike recovery processes were performed to evaluate the ability to recover TGEV in both wet and dry states from the polystyrene petri dish surface. Process steps that were tested and the samples collected are identified in Table 1. A description of the virus and assay is shown in Table 2.

Virus infection of cells in vitro often results in characteristic cytopathic effects (CPE) which are morphological changes in the cell monolayer often resulting in cell death. Plaques are areas of localized cell death on a tissue culture monolayer and result from advanced CPE. Plaque assays are a quantitative assay used to measure the number of infectious virus particles in a sample that contains virus. Plaques can be counted and the number of infective virus particles in the original solution estimated based upon the number of plaques. The unit of measure for a single infectious virus particle is a Plaque Forming Unit (PFU). In a plaque assay, tissue culture cells inoculated with sample(s) are compared to positive control cells infected with known concentrations of virus and negative control cultures inoculated with media only (no virus). After the initial infection of the culture and a brief incubation to allow host cell infection by any virus present, convective spread of virus is prevented by overlaying the cells with a viscous medium.

Figure 1: LEDpac UVC Device Processing Regimes

POSITION	A	B	C	D
LED	XBT-1313	XBT-3535	XST-3535	XFM-5050
PCB	XLP-60.5.1/4J.4P	XLP-13.5.1J/4P	XLPi-13.5.1J/4P	LP-4.12.1J/4P
VIEWING ANGLE	150	130	60	130
DOSE	3 Seconds	2 Seconds	2 Seconds	1.5 Seconds
KILL ZONE @ 1.0 IN	8.015 IN SQ [203.59]	4.964 IN SQ [126.01]	1.827 IN SQ [46.40]	4.024 IN SQ [102.21]
KILL ZONE @0.5 IN	4.283 IN SQ [108.79]	2.817 IN SQ [71.74]	1.249 IN SQ [31.73]	2.292 IN SQ [58.21]
KILL ZONE @0.25 IN	2.417 IN SQ [61.4]	1.744 IN SQ [44.31]	.961 IN SQ [24.4]	1.426 IN SQ [36.22]

Table 1: Process steps and samples

Process Step	Samples
UVC Spike Recovery (Wet)	Untreated, Treated
UVC Spike Recovery (Dry)	Untreated, Treated

UVC Inactivation Step	Untreated at 0.25 Inches, Position A at 0.25 Inches, Position B at 0.25 Inches, Position C at 0.25 Inches, Position D a 0.25 Inches
UVC Inactivation Step	Untreated at 1 Inch, Position A at 1 Inch, Position B at 1 Inch, Position C at 1 Inch, Position D at 1 Inch

Table 2: TGEV

Family	Genome	Envelope	Size (nm)	Physico-Chemical Resistance	Assay Type	Analysis #
Corona	RNA	Yes	100-150	Low	Standard	1-P-QM-WI-9089624

2. METHODS AND MATERIALS

The sample numbers are related to the associated tests as listed in Table 3.

Table 3: Samples

Sample Number	Test
STUQL21AA0059-1:a	Spike Recovery – Wet – TGEV
STUQL21AA0059-1:b	Spike Recovery – Dry – TGEV
STUQL21AA0059-1:c	Inactivation – Height 1 – TGEV
STUQL21AA0059-1:d	Inactivation – Height 2 – TGEV

2.1 Spike Recovery Assay

The spike recovery assays were performed according to client Protocol 21-045466-262813. The spike recovery assays were evaluated under both dry and wet conditions in order to determine the most appropriate method of virus recovery after UVC exposure. The dry test was designed to be representative of the most likely scenario in which virus has been exposed on a dry surface. As the TGEV virus is an enveloped virus, lack of recovery in the dry test could be indicative of inactivation due to drying. The wet test evaluated the scenario in which a droplet, containing virus, has been exposed on a dry surface. A lack of recovery in the wet test could be indicative of a lack of inactivation due to UVC refracting through the liquid. For evaluation of spike recovery, an aliquot of generated sample was serially diluted, then inoculated according to the analysis for TGEV (1-P-QM-WI-9089624 (Table 2)).

2.1.1 Dry Spike Recovery Testing for Plaque Assay

For dry spike recovery testing, two dry surfaces were prepared for testing. Virus was spiked on one surface and allowed to dry. The Untreated sample was harvested from the dry surface, suspended in media and immediately assayed. The second surface was then exposed to UVC light. After exposure, the surface was spiked with virus and allowed to dry. The Treated sample was then harvested from the surface, suspended in media and immediately assayed.

2.1.2 Wet Spike Recovery Testing for Plaque Assay

For wet spike recovery, an aliquot of media was prepared. The Untreated sample (media) was removed from the aliquot, spiked with virus and immediately assayed. The Treated sample (media) will be removed from the aliquot and exposed to UVC light. After exposure, the Treated sample will be spiked with virus and immediately assayed.

At the end of the assay incubation, the cultures, including positive controls, were observed for plaque formation. Plaque formation results from the Treated samples were compared with the results from the Untreated samples. A decrease in plaque recovery of 1 Log_{10} PFU or more was interpreted as incomplete spike recovery.

If either testing modality, dry or wet spike, showed incomplete spike recovery, that testing modality would not be used for the inactivation study. The UVC Inactivation Step was dependent upon the results of recovery from the wet and dry spike recovery testing. The results of both dry and wet spike recovery testing showed complete virus recovery. The dry testing modality was chosen by the client for the inactivation study. Each inactivation step was performed independently of each other.

2.2 Inactivation study

UVC Inactivation Step

Ten (10) dry surfaces were prepared for the samples. The two (2) Untreated samples were spiked with virus, allowed to dry and then immediately assayed. Four of the samples were then spiked with virus, allowed to dry and exposed to one dose of UVC light at Positions A, B, C and D at 0.25 inches. After exposure, these four samples were immediately assayed. Then, four additional samples were spiked with virus, allowed to dry and exposed to three doses of UVC light at Positions A, B, C and D at 1 inch. After exposure, these four samples were immediately assayed.

2.3 Analysis of Results

Virus titers and viral clearance factors were calculated according to 1-P-QM-PRO-9018295 using a validated Excel spreadsheet. Calculations were performed using full precision, and are reported rounded to 2 decimal places. The virus clearance factor (VCF) for each step was calculated as follows:

$$VCF = \log_{10}\left(\frac{\text{Volume * titer before processing}}{\text{Volume * titer after processing}}\right)$$

3. RESULTS AND DISCUSSION

The spiking virus used for in the execution of this study is listed in Table 4.

Table 4: Virus Titers

Virus Lot	Titer (Log PFU/mL)	Used for
292088/5	5.5	Spike Recovery Assay, UVC Inactivation

3.1 Spike Recovery Assay

The results of the spike recovery assays are found in the Certificates of Analysis that follow this report. Summary data are shown in Table 5.

Table 5: Data Summary for Spike Recovery Study

Sample Number	Step	Run #	Log Total Virus		Viral Clearance Factor	95% Confidence Limit
			Untreated Sample	Treated Sample		
STUQL21AA0059-1:a	Spike Recovery – Wet – TGEV	1	4.53	4.55	-0.01	0.07
STUQL21AA0059-1:b	Spike Recovery – Dry – TGEV	1	3.76	3.94	-0.18	0.09

3.2 Removal/Inactivation study

Results for each assay are included in the Certificates of Analysis that follow this report. A data summary is shown in Table 6.

Table 6: Data Summary for Inactivation Study

Sample Number	Step	Run #	Log Total Virus		Viral Clearance Factor	95% Confidence Limit
			Before processing	After processing		
STUQL21AA0059-1:c	Inactivation – Height 1 – TGEV (Position A)	1	3.91	1.95	1.96	0.26
STUQL21AA0059-1:c	Inactivation – Height 1 – TGEV (Position B)	1	3.91	3.72	0.20	0.07
STUQL21AA0059-1:c	Inactivation – Height 1 – TGEV (Position C)	1	3.91	2.95	0.96	0.16
STUQL21AA0059-1:c	Inactivation – Height 1 – TGEV (Position D)	1	3.91	2.43	1.48	0.14
STUQL21AA0059-1:d	Inactivation – Height 2 – TGEV (Position A)	1	3.82	<1.53	≥2.29	0.09
STUQL21AA0059-1:d	Inactivation – Height 2 – TGEV (Position B)	1	3.82	2.23	1.60	0.39
STUQL21AA0059-1:d	Inactivation – Height 2 – TGEV (Position C)	1	3.82	3.16	0.66	0.12
STUQL21AA0059-1:d	Inactivation – Height 2 – TGEV (Position D)	1	3.82	<1.53	≥2.29	0.09

Note: Log total virus titers after processing displayed as a "<" value represent that no detectable, infectious virus was observed in the results of the plaque assay. The value represented is the limit of quantitation for the assay.

4. CONCLUSION

This study was performed for R&D purposes only. Sample generation was performed per the study protocol and the results of the assays are considered an accurate representation of the virus present in each sample.

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5. DOCUMENTATION

Notebook 334942
335572
331624
329312
331625
331626

Reviewed and electronically signed for Study Quality Assurance Release by
Caleb Faboye, Quality Assurance
for Eurofins Lancaster Laboratories, Inc., on 05-Mar-2021 16:45:20 UTC-05:00

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Certificate of Analysis

LEDpac - A008556891D9

Study Activity Code: STUQL21AA0059-1:a

Material Item Reference: N/A

Submitted On:	N/A
Sample Description:	N/A
Protocol ID:	QL-21-A-01411
Date Analyzed:	2/3/2021
Method Reference:	21-045466-262813.01
Effective Date:	1/23/2021
Notebook Reference:	329312
Test Name:	Spike Recovery - Wet (TGEV)

Sample	Titer (Log PFU/mL)	95% Confidence Limit	Sample volume (mL)	Virus Titer (Log PFU)	Clearance (Log PFU)	95% Confidence Limit
Untreated Sample	3.83	0.04	5.00	4.53		
Treated Sample	3.85	0.06	5.00	4.55	-0.01	0.07
Positive Control	5.05	0.04				
Negative Control	<0.40	0.00				

Assay was performed for R&D purposes only.

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for Eurofins Lancaster Laboratories, Inc., on 05-Mar-2021 16:45:20 UTC-05:00

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Certificate of Analysis

LEDpac - A008556891D9

Study Activity Code: STUQL21AA0059-1:b

Material Item Reference: N/A

Submitted On:	N/A
Sample Description:	N/A
Protocol ID:	QL-21-A-01411
Date Analyzed:	2/3/2021
Method Reference:	21-045466-262813.01
Effective Date:	1/23/2021
Notebook Reference:	331624
Test Name:	Spike Recovery - Dry (TGEV)

Sample	Titer (Log PFU/mL)	95% Confidence Limit	Sample volume (mL)	Virus Titer (Log PFU)	Clearance (Log PFU)	95% Confidence Limit
Untreated Sample	3.06	0.06	5.00	3.76		
Treated Sample	3.24	0.06	5.00	3.94	-0.18	0.09
Positive Control	4.91	0.02				
Negative Control	<0.40	0.00				

Assay was performed for R&D purposes only.

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for Eurofins Lancaster Laboratories, Inc., on 05-Mar-2021 16:45:20 UTC-05:00

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Certificate of Analysis

LEDpac - A008556891D9

Study Activity Code: STUQL21AA0059-1:c

Material Item Reference: N/A

Submitted On: N/A
Sample Description: N/A
Protocol ID: QL-21-A-01411
Date Analyzed: 2/10/2021
Method Reference: 21-045466-262813.01
Effective Date: 1/23/2021
Notebook Reference: 331625
Test Name: Inactivation - Height 1 (TGEV)

Sample	Titer (Log PFU/mL)	95% Confidence Limit	Sample volume (mL)	Virus Titer (Log PFU)	Clearance (Log PFU)	95% Confidence Limit
Untreated at 0.25 Inches	3.21	0.07	5.00	3.91		
Position A at 0.25 Inches	1.26	0.25	5.00	1.95	1.96	0.26
Position B at 0.25 Inches	3.02	0.03	5.00	3.72	0.20	0.07
Position C at 0.25 Inches	2.26	0.15	5.00	2.95	0.96	0.16
Position D at 0.25 Inches	1.73	0.12	5.00	2.43	1.48	0.14
Positive Control	4.85	0.04				
Negative Control	<0.40	0.00				

Assay was performed for R&D purposes only.

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for Eurofins Lancaster Laboratories, Inc., on 05-Mar-2021 16:45:20 UTC-05:00

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Certificate of Analysis

LEDpac - A008556891D9

Study Activity Code: STUQL21AA0059-1:d

Material Item Reference: N/A

Submitted On: N/A
Sample Description: N/A
Protocol ID: QL-21-A-01411
Date Analyzed: 2/10/2021
Method Reference: 21-045466-262813.01
Effective Date: 1/23/2021
Notebook Reference: 331626
Test Name: Inactivation - Height 2 (TGEV)

Sample	Titer (Log PFU/mL)	95% Confidence Limit	Sample volume (mL)	Virus Titer (Log PFU)	Clearance (Log PFU)	95% Confidence Limit
Untreated at 1 Inch	3.12	0.09	5.00	3.82		
Position A at 1 Inch	<0.83	0.00	5.00	<1.53	>=2.29	0.09
Position B at 1 Inch	1.53	0.38	5.00	2.23	1.60	0.39
Position C at 1 Inch	2.46	0.09	5.00	3.16	0.66	0.12
Position D at 1 Inch	<0.83	0.00	5.00	<1.53	>=2.29	0.09
Positive Control	4.82	0.04				
Negative Control	<0.40	0.00				

Assay was performed for R&D purposes only.

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for Eurofins Lancaster Laboratories, Inc., on 05-Mar-2021 16:45:20 UTC-05:00