27 יולי 2020

<u>הנדון : יעילות מכשיר פול-פיוריטי בהליך חיטוי המים</u>

א.ג.נ

בהתאם לתוצאות המעבדה המצ"ב, שנערכו בתאריך 16/7/2020, אכן המכשיר מוכיח יעילות של יותר מ 99% בניטרול וירוסים, כולל ממשפחת הקורונה המסוכנים לאדם.

לעניות דעתי קיימת חשיבות רבה לבריאות הציבור ולבטחונו בעת הזו, בשימוש במכשירי טיפול שנבדקו והוכחו כיעילים כנגד וירוסים ממשפחת הקורנה.

בברכה

דר' יעל לצטר

ראש המעבדה לטכנולוגיות סביבתיות

עזריאלי מכללה אקדמית להנדסה ירושלים

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Final Report

CONFIDENTIAL

Report No: Pool Purity-01

Work Plan 1.0 – Virus inactivation by exposure to Pool Purity AOP Treatment

Testing Facility:	Smart Assays Biotechnologies Ltd. 3 Pinhas Sapir St, POB 4182, Nes-Ziona, 7414003 Tel/Fax: 077-3001087
Sponsor Name:	Pool Purity Ltd.
Sponsor Address:	1st Beit Ha-Defus Street Jerusalem Tel: 052-4781920
Contact Person:	Jonathan Heller, PhD

Type of Test: **Virus inactivation assay**

Tested virus: 1. Human Cov 229E.

Tested inactivating materials: 1. Pool Purity AOP treatment for 10, 30 and 60 minutes.

Testing date:	14 July – 20 July 2020
Executed By:	Sagiv Weintraub, PhD
Reviewed by:	Merav Marom, PhD
Authorized by:	Raphael Mayer, PhD

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Assay Aim:

Pool Purity has developed an Advanced Oxidative Process (AOP) technology that efficiently kills a myriad of pathogens and degrades dissolved organic matter in water. The aim was to test this technology for its ability to kill HCoV 229E viruses. The human Cov 229E virus was applied on a slide and incubated for different time points in a device chamber in which oxidized air was flowed.

Overview:

The genetically diverse *Orthocoronavirinae* (CoV) family is divided into four genera (*alpha*, *beta*, *gamma*, and *deltacoronavirus*). Coronaviruses are large, enveloped, single-stranded, positive-sense RNA viruses, with a genome size of approximately 30 kb. Human coronaviruses (HCoV), with two known serogroups designated OC43 and 229E, are an important cause of upper respiratory tract illnesses, and are also implicated in diseases involving the digestive and the central nervous system. The recent SARS outbreak in 2002/2003 and the Middle East respiratory syndrome (MERS) in 2012 led to the discovery of a novel coronavirus (SARS-CoV). The new CoV, 2019 - nCoV (SARS-Cov-2), which belongs to *betacoronaviruses* based on sequence analysis can also infect the lower respiratory tract and cause pneumonia in human[1,2,3].

The efficiency of the Pool purity device (ASP) to kill human CoV 229E viruses was evaluated.

Human Coronavirus 229E was applied on slides and was exposed to Pool Purity APS treatment for three different time period (10, 30 and 60 min). Following period of time, the sample were collected and used to infect cells. The infectivity of the samples as function of incubation time on the test sample was determined. As a control a non-treated virus was used.

Assay protocol:

Table 1: Cells & Virus were used for this project:

Cells / Virus	Vendor	Catalog #	Lot #
MRC-5 cells	ATCC	CCL-171	61926307
Human CoV 229E virus	ATCC	VR-740	70033323

Table 2: Reagents were used for this project:

Reagent	Vendor	Catalog #	Lot #
EMEM	ATCC	30-2003	80913222
FBS	Biological industries	04-121-1A	1414876
Pen/step	Biological industries	03-031-1C	1445472
Cell Titer glo kit	Promega	G7572	413394

Plate:

6 well plate, Greiner, Cat #657160, Lot #E190835K
U96-polypropylene plates, NUNC cat #267334, lot # 161413
96 well plate cell culture, Greiner, Cat #655-180, Lot #E18063RF

96 wells white Optiplates, Perkin Elmer, cat # 6005290, lot # 810-17081

Plate reader:
CLARIOstar (BMG, Serial number#430-0078)

Methods:
In this study, the virus killing effectivity of Pool Purity's Advanced Oxidative Process technology was investigated. Human CoV 229E viruses were exposed to Pool purity's ASP device and regular air flow for different incubation times (10, 30 and 60- minutes). Following incubation, the viruses were collected and serial diluted before being applied to the MRC-5 cells. The infected cells were incubated for 120 hours at 35°C in CO_2 incubator. At the end, cells' viability was determined using Cell titer glo reagent and viral infectivity and the corresponding tissue culture infective dose ($TCID_{50}$) was calculated.

The following samples were investigated:

- Not treated viruses at time 0 (in triplicates)
- Incubation time at ASP: 10, 30 and 60 min (in triplicates)
- Incubation time with air flow: 10, 30 and 60 min

Work was performed in an aseptic cell-culture hood as follows.

1. One day before the experiment MRC-5 cells were harvested, counted and plated in a 96-wells tissue culture plate at 15,000 cells/well in 100µl cell culture media (EMED, 10% FBS, 1% P/S).
2. On the experiment day, cover slips were placed in 6-well plates, one plate for each time point.
3. 100µl of human CoV 229E virus was applied onto each cover slips in three 6-wells plate. Each plate is for different incubation time in the Pool Purity's ASP device.
4. At the end of each incubation time, 900µl of medium was added to each well, and 1ml of viruses were collected to an eppendorf.
5. The collected viruses were further diluted 1:3 for six dilutions in cells medium.
6. The 96-wells plates were taken out from the incubator.
7. Medium was aspirated and MRC-5 cells were washed with PBS.
8. 100µl of each of the diluted viruses were added to the cells in tetra-plicates.
9. Plates were incubated for 120 hours at 35°C in CO_2 incubator.
10. At the end of the incubation, plates were equilibrated to room temperature and 100µl of CellTiter-Glo reagent was added to every well to induce cells lysis.
11. Following two minutes of incubation with orbital shaking, 200µl of cells lysis was transferred to white 96-wells Optiplates and luminescence was measured using ClarioSTAR BMG plate reader.

12. Data analysis: the results of each treatment was averaged and the results were analyzed using GraphPrism software.
13. Results were plotted as raw luminescence versus dilution factor using GraphPad Prism and fitted by a sigmoidal dose-response (variable slope) algorithm with nonlinear fit of log (inhibitor) vs. response (three parameters). Finally, $TCID_{50}$/ml was calculated.

Results:

To determine the effectivity of Pool Purity's APS device to kill Human CoV 229E viruses, we tested the ability of the viruses to infect MRC5 cells following treatment with the APS device for different incubation times. Cells' viability following infection is directly corelated to the virus virulence, that is, if the Pool Purity's APS device efficiently kills the viruses, the cells are more viable.

The Cell Titer Glo™ Assay is a homogeneous, add-mix-measure method to identify cytopathic effect induced by viral infection. The assay measures cellular ATP, a stable and tightly regulated surrogate of cell viability. Luminescent signals from the assay are proportional to viable cell number.

The Human CoV 229E viruses were exposed to oxidized air using the Pool Purity's APS device for three time points: 10, 30 and 60 minutes. Human CoV 229E viruses exposed to regular air flow, and without any exposure at time 0 were used as controls.

Cells' viability expressed as relative luminescence units (RLU) was plotted versus the Human CoV 229E dilution factor. As indicated in figure 1 and figure 2, following virus treatment for 10 minutes (figure 1) and 30 minutes (figure 2) with regular air flow, cells were slightly more viable compared to cells infected with untreated virus at T0. These findings suggest that the air flow per se partially kill the viruses. In addition, treatment of viruses for 10 and 30 minutes with oxidized air using the Pool Purity's APS device increased cells' viability compared to virus treated with air flow. No significant difference in cells' viability was observed between the 10 and 30 minutes.

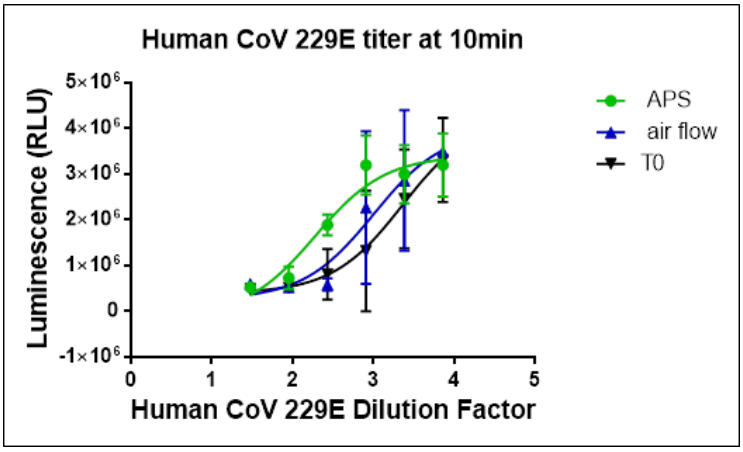

Figure 1: Human CoV 229E virus titer following different treatment for 10 minutes – Cells' viability indicated by luminescence was plotted vs. human CoV 229E dilution factor. In green, viruses exposed to 10 minutes of oxidized air (APS). In blue, viruses exposed to 10 minutes of regular air flow. In black viruses without any treatment (T0).
Error bars represent standard deviations from tetraplicates.

by human CoV 229E viruses without any treatment (T0).

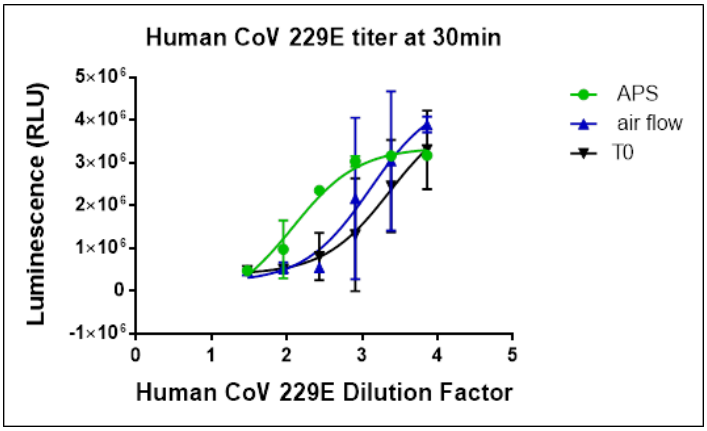

Figure 2: ***Human CoV 229E virus titer following different treatment for 30 minutes–*** Cells' viability indicated by luminescence was plotted vs. human CoV 229E dilution factor. In green, viruses exposed to 30 minutes of oxidized air (APS). In blue, viruses exposed to 30 minutes of regular air flow. In black viruses without any treatment (T0).
Error bars represent standard deviations from tetraplicates.

As indicated in figure 3, virus treatment for 60 minutes with regular air flow resulted in higher cells' viability compared to cells to cells infected with untreated virus at T0. This suggests that the air flow per se partially kills the viruses. Significantly, treatment of viruses for 60 minutes with oxidized air using the Pool Purity's APS device, led to more viable cells compared to virus treated with air flow. The increase of the exposure time from 10 and 30 minutes to 60 minutes increases significantly the cells viability following infection with viruses treated by APS device.

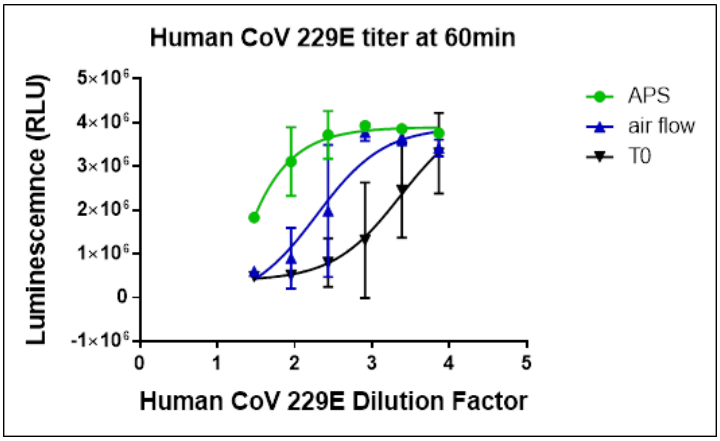

Figure 3: ***Human CoV 229E virus titer following different treatment for 60 minutes–*** Cells' viability indicated by luminescence was plotted vs. human CoV 229E dilution factor. In green, viruses exposed to 60 minutes of oxidized air (APS). In blue, viruses exposed to 60 minutes of regular air flow. In black viruses without any treatment (T0).
Error bars represent standard deviations from tetraplicates.

Using GraphPad Prism software the TCID50 values were calculated for all treatments. The $TCID_{50}$ value is the reciprocal of the dilution that produces a 50% decline in ATP levels compared to controls. $TCID_{50}$ was calculated by plotting raw luminescence versus the virus dilution factor and fitted by a sigmoidal dose-response (variable slope) algorithm. Table 3, summarizes the calculated $TCID_{50}$/ml. At the dilution of the $TCID_{50}$, half of the wells represent viable cells and the other half represent 100% cell death.

Table 3: The $TCID_{50}$/ml values for human CoV 229E viruses following different treatments.

	$TCID_{50}$/ml		
Treatment	**10 minutes**	**30 minutes**	**60 minutes**
T0	$2.3*10^4$	$2.3*10^4$	$2.3*10^4$
Regular air flow	$1.0*10^4$	$1.3*10^4$	$1.9*10^3$
Oxidized air (APS)	$1.9*10^3$	$1.2*10^3$	$3.3*10^1$

- The $TCID_{50}$ was calculated using GraphPad Prism software.

Conclusions and discussion:

The ability of the viruses to infect MRC5 cells following treatment with oxidized air or regular air for different time points was measured using cell titer glo reagent. This report summarizes the results for the virus $TCID_{50}$/ml following the different treatments.

In order to distinguish between the virus death due to oxidized air or due to air flow itself, control treatment with regular air flow at the same incubation times were tested in parallel.

The $TCID_{50}$/ml of the viruses treated by regular air flow for 10 and 30 minutes were similar and slightly lower than the $TCID_{50}$ /ml of non-treated viruses (T0). At 60 minutes, the $TCID_{50}$/ml of the viruses treated by regular air flow was lower than in 30 minutes by one magnitude (10-fold). The treatment of oxidized air by Pool Purity's APS device for 10- and 30-minutes led to a decrease in $TCID_{50}$/ml by one order of magnitude than the regular air flow treatment. Virus treatment with oxidized air by Pool Purity's APS device for 60 minutes significantly decreased the $TCID_{50}$/ml by two orders of magnitude.

References:

1. Yang P et al., Cellular & Molecular Immunology (2020) 17:555–557.
2. Brawn AJ et al., Antiviral Research 169 (2019) 104541.
3. Chen Y et al., Journal of medical virology (2020) 92:418–423.

Signatures:

The undersigned have reviewed the content of this report and have approved the report for final issuance.

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