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SARS-CoV-2USA-CA1/2020

CLIENT: The PYURE Company Inc

PROJECT: OH MYSPACE™ Bioaerosol Test

PRODUCT: OH AIR® MYSPACE™

CAP LIC NO: 886029801

CLIA LIC NO: O5D0955926

STATE ID: CLF 00324630

CHALLENGE VIRUS: SARS-CoV-2 USA_CA1/2020

INNOVATIVE BIOANALYSIS
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摘要：PYURE MYSPACE™裝置對霧化SARS-CoV-2的功效

背景：這項體外研究旨在確定MYSPACE™裝置的功效。該產品是由佛羅里達州博因頓海灘市的The PYURE Company（PYURE）生產的市售移動式消毒淨化設備。MYSPACE™設備設計為可獨立放置在房間中，並在運行時降低空氣中和物體表面上的病原體濃度，以對封閉空間及其內容物進行消毒淨化。對於此測試，使用了SARS-CoV-2-CA1 / 2020病原體。CDC估計，在美國，超過1,000萬人感染了SARS-CoV2，導致在2019年至2020年之間估計有24萬人死亡。冠狀病毒可以通過空氣傳播並通過接觸被污染的表面傳播。這些消毒淨化裝置必需要具有減少空氣中傳染性病原體的證明能力，從而減少人類感染和傳播的風險。PYURE提供了MYSPACE™獨立式單元來用於測試。在測試中，電源通過具有浪湧保護器和備用電池系統的電源調節120v穩壓電源插座供電。使用內部SOP進行檢測程式，以應對氣溶膠病毒病原體的挑戰和隨後的消毒淨化。所有內部SOP和流程都遵循GCLP準則和建議。

測試設備：

製造商：The PYURE Company

型號：MSPACE™

序列號：

20MYS01TC02008027740

MYSPACE™設備

設備從製造商預先包裝到達實驗室，並在抵達時檢查是否有損壞。製造商提供了兩個汞蒸氣紫外線光學元件，用於安裝在設備中。安裝了光學元件，設備已打開電源以檢查是否正常運行。開始測試之前，PYURE MYSPACE™裝置在密封的生物氣溶膠中進行了2個小時的空運行，以確認該設備正常運行。測試室是用於病毒測試的同一個BSL3測試室。

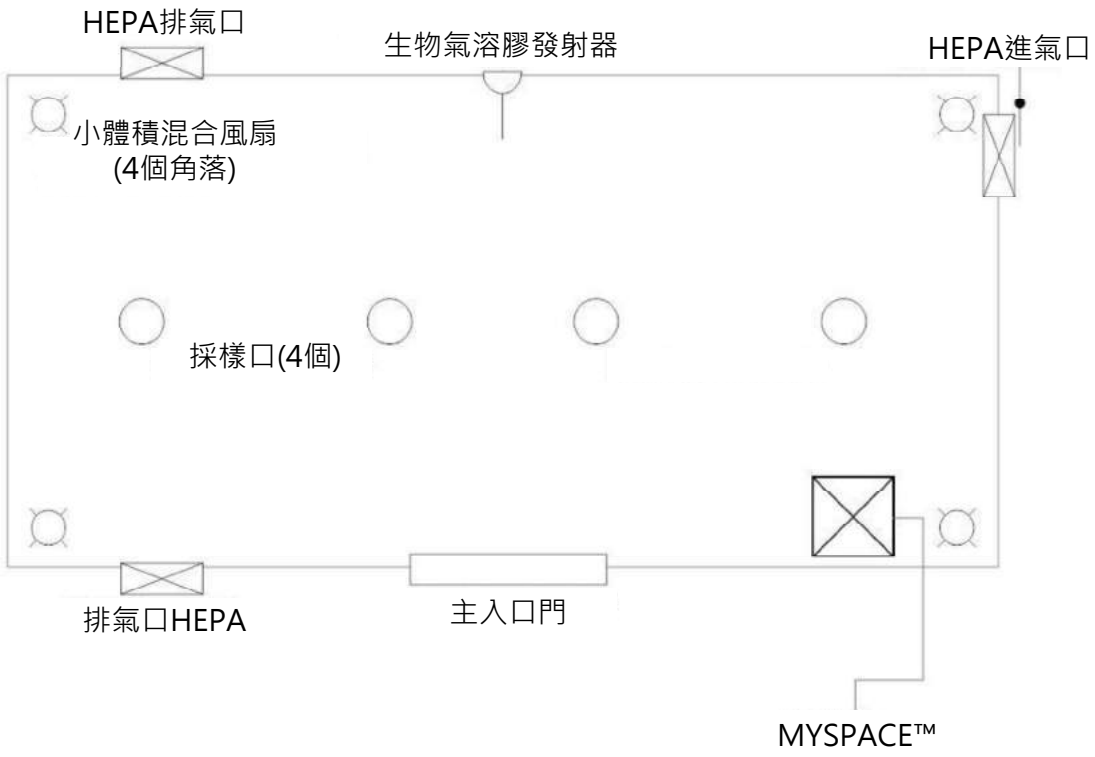
病毒測試室

測試室是一個大型的密封空氣體積測試室，由金屬牆和環氧樹脂地板組成，符合BSL3對氣溶膠病原體的密封控制要求。測試室設計為與外部環境完全密封，以防止測試介質潛在釋放到大氣中的可能。測試室配備了4個密封的檢測觀察窗和可進出且可鎖定的室門。試驗箱的總尺寸約為8x8x20英尺，排量為1280立方英尺。基於立方英尺的容積換算，該腔室具有36245.56升的空氣。

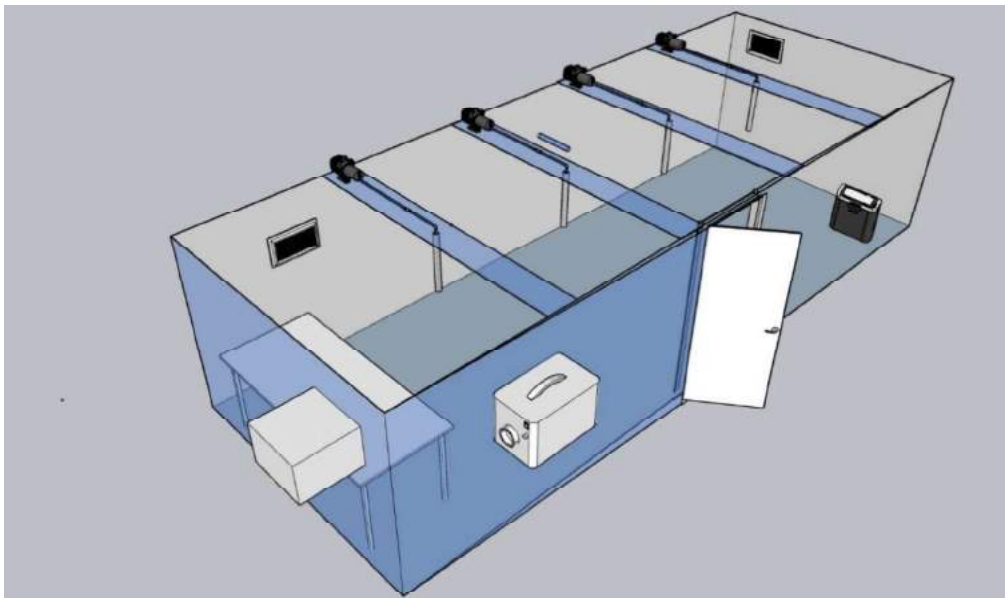
測試室具有經過HEPA過濾的進氣口和排氣口，在所有管道中均配有主動UV-C系統。使用校準的無線設備對室內的濕度和溫度進行監控。對於空氣樣品測試，該室裝有4個沿房間中心線並從天花板24英吋向下突出的探針。每個探管都連接到帶有Sensidine生產的批號18248的採樣盒的Gilian 10i可編程系統。在入口門對面的20英尺牆的中央有一個生物氣溶膠霧化端口。擴散端口從壁24英吋突出，並連接至可編程壓縮機霧化器系統。

在測試之前，對測試室進行壓力測試以檢查是否洩漏，並使用有彩色吸煙裝置進行目視檢查。確認測試室的所有密封，並且所有使用的設備均進行了功能測試，以確認工作條件。對於已校準的設備，將檢查校準記錄以確認操作狀態。

測試室布局：



3D設計布局：



實驗設計

- 在進行初始對照測試之前，以及在每次試運行之後，均應按照內部程序對測試區域進行消毒和準備。
- 所有測試運行期間的溫度約為71F +/- 2F，相對濕度為49％。
- 在所有測試期間，在測試室的兩個部分中獲取相對濕度和溫度，以確認每側的偏差不超過3％。
- 製造商已於2020年9月3日對空氣採樣器進行了校準，並設置為5.02L / min的標準流量。校準記錄表明公差為0.20％。
- 將每個時間點的所有樣品收集量設置為5分鐘抽取。
- 在霧化之前打開小體積混合風扇，以確認測試室內的濃度均勻。
- 混合風扇保持在45度角並以45度角放置，以達到生物氣溶膠懸浮並降低自然粒子下降率。
- 以相同方式進行霧化以進行對照和病毒測試挑戰。
- 將樣品盒從收集系統中手動取出，並在每個時間點後存儲，並用新的盒帶替換。
- 在每個時間點取出盒帶後，將盒帶帶到相鄰的生物安全櫃中彙集存放。
- 每次生物氣溶膠收集六個總採樣時間。
- 使用相同的方法完成了1個對照，並完成了1個病毒測試。

生物氣溶膠的產生：

為了進行對照和病毒攻擊，將霧化器裝滿相同量的病毒原液（每毫升4.02 X 10^7 TCID50）並以1ml / min的流速霧化。霧化器由未經處理的當地大氣驅動。每次完成後稱量霧化器的剩餘病毒儲備量，以確認霧化後的病毒儲備量相同。

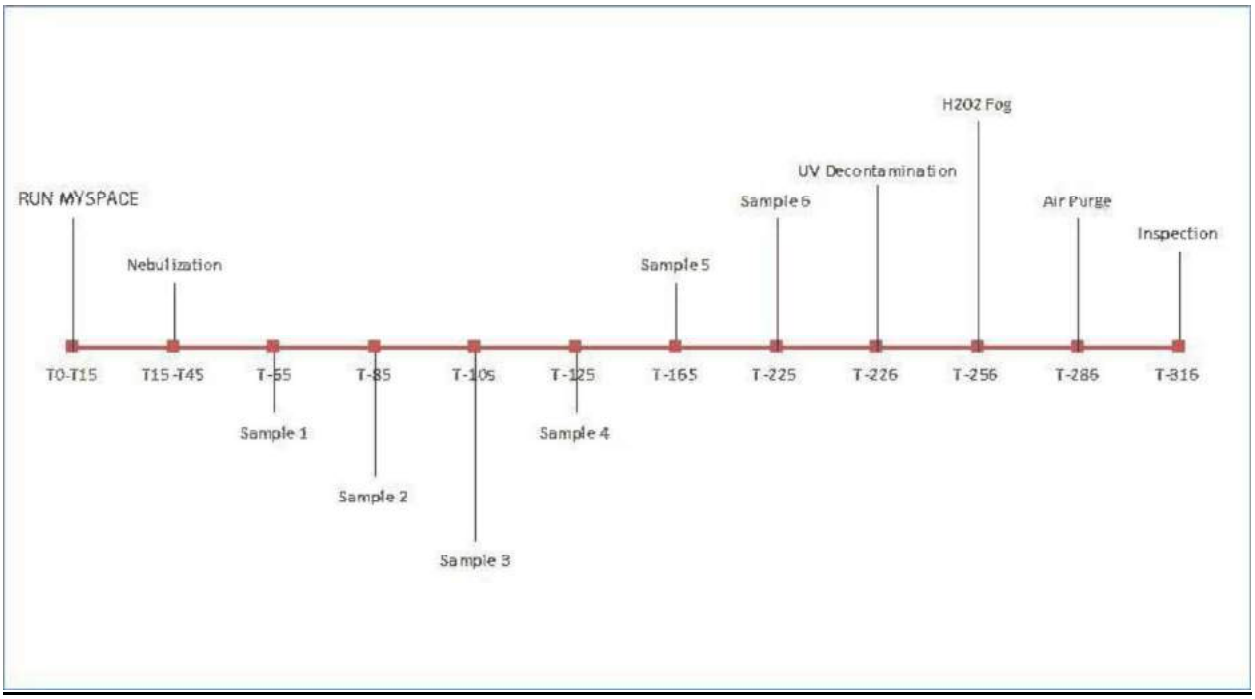
生物氣溶膠的採樣：

對於空氣採樣，使用了4種不同的Gillian 10i可編程真空設備。製造商已在2020年9月對空氣採樣器進行了校準，並在使用前檢查了證書。在與Gilian Gilibrator 2 SN-200700-12和高流量氣泡發生器SN-2009012-H一起使用之前，已確認空氣樣本量收集。空氣採樣器與可移動的密封盒配合使用，可在每個採樣時間點手動將其取出。盒式磁帶具有精密的內部過濾盤，可收集病毒樣品。

樣品接種：

表面接種包括使用校準的Eppendorf移液管，使用過濾的移液管吸頭，將精確的1 ml病毒培養基施加到每個試樣上。使用標準的無菌25mm x 75mm玻片。 施用後，用一次性刮鏟將培養基攤薄，然後乾燥10分鐘。 所施加的病毒的起始濃度為4.02X10^7 TCID50 / mL。經過幾次恢復測試，確定最有效的恢復活病毒的方法是在病毒培養基中漂洗2 mL，然後用拭子擦拭接種區域。達到的最大回收率為95.3%或3.83X10^7 TCID50 / mL。 該值用作所有表面測試的 " 0 分鐘" 起始濃度，以說明回收率。

採樣點時間軸：



病毒株背景：

以下試劑由疾病預防控制中心保管，並通過BEI資源、BIAID、NIH SARS相關冠狀病毒，分離株USA-CA1 / 2020，NR-52382獲得。之所以選擇這種病原體，是因為它來自A譜系的一部分，來自38歲的加州嚴重急性呼吸系統綜合症患者。這是一個非致命的案件，代表了美國大多數的案件。患者所屬年齡歸類為中等成年人，並非老年人或未成年人。

淨化後：

每次病毒攻擊測試結束時，將測試室內的紫外線系統啟動30分鐘。暴露於紫外線30分鐘後，將過氧化氫混合氣體霧化整個測試室，然後吹掃30分鐘。每天結束時用70%的酒精溶液清潔所有測試設備。將收集管線在漂白水混合物中浸泡30分鐘，然後用去離子水重複沖洗。霧化器和真空收集泵用過氧化氫混合物清洗淨化。

TCID50程序：

材料和設備：

- 認證的生物安全櫃
- 微量管和無菌的一次性抗氣溶膠吸頭20uL、200uL、1000uL。
- 倒置顯微鏡
- 稀釋管
- 帶蓋玻片的血細胞計
- 用於感染的細胞培養基
- 適用於細胞系的生長培養基
- 0.4%台盼藍溶液
- 浸有70%異丙醇的無絨濕巾
- 將CO_2培養箱設置為37°C或34°C或其他溫度。

程序：

1. 測試前一天，通過在DMEM中添加Vero E6細胞和7.5%胎牛血清，4mM谷氨酰胺和抗生素來接種96孔培養皿。
2. 在測試當天，用PBS稀釋病毒樣品。
3. 按原始病毒樣品的1:10進行一系列稀釋。第一支裝有2.0 mL PBS的試管，隨後一支有1.8 mL的試管
4. 渦旋病毒樣品，將20 uL病毒轉移到第一個試管中，渦旋，棄去針尖。
5. 使用新吸頭，串行稀釋後續吸頭，從而轉移200 uL。

向細胞添加病毒稀釋液

1. 通過繪製的網格劃出四邊形並標記每個網格與病毒樣品相對應的方式標記96孔培養皿的蓋子，並以進行稀釋。
2. 在每個板上包括4個不會被感染的陰性孔。
3. 通過真空抽吸從每個孔中除去除0.1 mL培養基外的所有培養基。
4. 從稀釋度最高的樣品開始，向每個一式四份的孔中添加0.1 mL病毒稀釋液。

5. 每次稀釋感染4孔，並反向操作。
6. 在37°C下使病毒吸收到細胞2小時。
7. 吸收後，除去病毒接種物。從稀釋度最高開始，然後倒退。
8. 向每個孔中添加0.5 mL感染培養基，注意不要用移液管接觸孔。
9. 將板置於37°C並使用倒置顯微鏡在1至4週內監控CPE。
10. 記錄正孔和負孔的數量。

控制：

在測試室內沒有PYURE MYSPACE™裝置的情況下進行了一項對照測試。在三個相同的挑戰試驗所用的每個相應採樣時間採集對照樣品（請參見上面的採樣時間線）。對照的病毒培養基和收集方法的霧化與病毒攻擊相同。當在挑戰試驗中操作PYURE MYSPACE™設備時，將對照測試用於比較基線以評估病毒減少，從而能夠進行淨減少計算。在對照測試過程中，在測試室的每個角落都操作了四個低風量風扇，以確保空氣均勻混合。緊急O_3警報器已安裝到房間併校準為1ppm。在對照測試期間，未觀察到O_3警報。在控制期間，監測並記錄溫度和相對濕度為+/- 5%。

病毒測試：

攻擊性病原體SARS-CoV-2-USA-CA1 / 2020用於測試PYURE MYSPACE™設備的功效。在挑戰測試期間，對挑戰室中的壓力進行監控，以確認沒有任何部分洩漏。生物氣霧劑功效挑戰是在三個不同的試驗中用活病原體完成的，以創建數據基準。對於每種病毒攻擊，PYURE MYSPACE™設備都放置在相同的位置，並以相同的方式操作。在霧化病毒病原體之前，將PYURE MYSPACE™設備打開並運行15分鐘，以模擬真實環境，並使設備達到標準操作條件。在整個對照測試和病毒病原體測試中，使用了四個低風量混合風扇。採樣時間如下，其中T等於分鐘，T-20、T-40 、T-60、T-80、T-120、T-180。使用4個自動風量採樣器進行採樣，每個採樣器同時運行。採樣器預設為在收集5分鐘後自動關閉。通過使用病毒培養基包被的過濾器的設備進行收集，以最大程度地捕獲病原體並保持穩定性。在每個採集時間點之後，將採集樣品提供給實驗室工作人員進行匯總。

病毒庫存：SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

通過Vero 6細胞中的感染性鑑定	單元舍入和分離	單元舍入和分離
使用Illumina®iSeq™100平台的完整基因組的下一代測序（NGS） 約940個核苷酸	與SARS-CoV 2≥98%的同一性，分離出USA-CA1 / 2020 GenBank：MN994467.1 與SARS-CoV 2≥98%的同一性FDAARGOS_983分離株USA-CA1 / 2020 GenBank：MT246667.1	與SARS-CoV 2有99.9%的同一性，分離出USA-CA1 / 2020 GenBank：MN994467.1 與SARS-CoV 2具有100%的同一性FDAARGOS_983株分離株USA-CA1 / 2020 GenBank：MT246667.1
TCID50通過細胞病變效應在Vero E6細胞中滴度	報告結果	在37°C和5%CO_2下的5天之內，每毫升2.8 X 10^5 TCID50
無菌（21天孵化） Harpos HTYE有氧肉湯 胰蛋白酶大豆肉湯，有氧 Sabourad湯，有氧 綿羊血瓊脂，有氧 羊血瓊脂，厭氧 硫代乙醇酸肉湯 具有10%FBS的DMEM	 沒有增長 沒有增長 沒有增長 沒有增長 沒有增長 沒有增長 沒有增長	 沒有增長 沒有增長 沒有增長 沒有增長 沒有增長 沒有增長 沒有增長
支原體污染 瓊脂和肉湯文化 通過PCR提取提取的測試物品核酸進行DNA檢測。	 未檢測到 未檢測到	 未檢測到 未檢測到

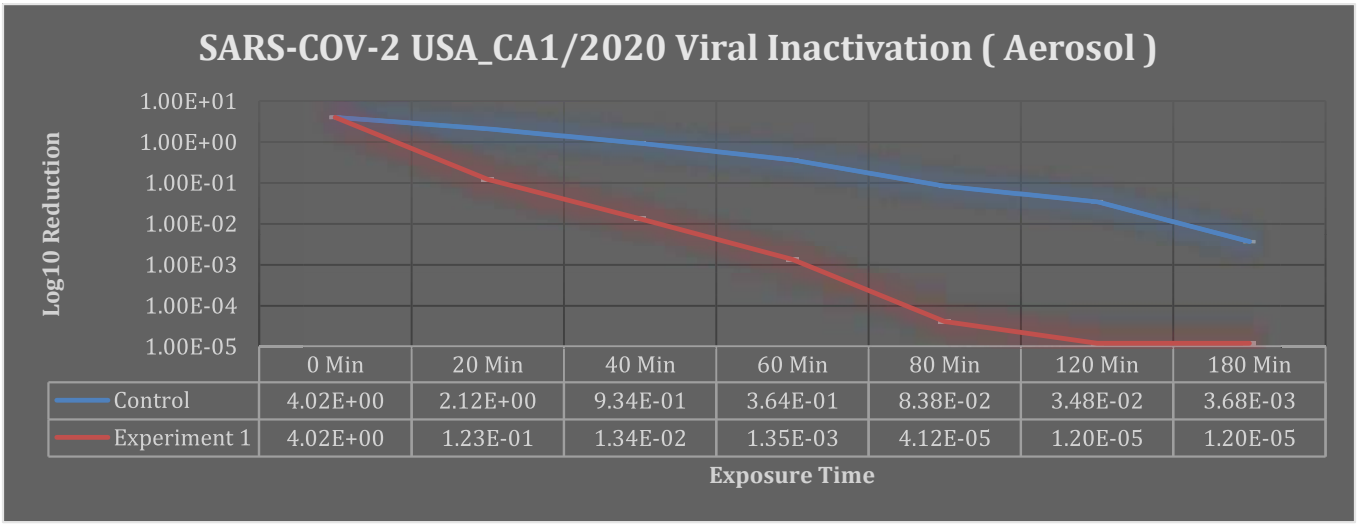
接種表面樣品

表面接種包括使用校準的Eppendorf移液管，使用過濾的移液管吸頭，將精確的1 ml病毒培養基施加到每個試樣上。使用標準的無菌25mm x 75mm玻片。 施用後，用一次性刮鏟將培養基攤薄，然後乾燥10分鐘。 所施加的病毒的起始濃度為4.02X10^7 TCID50 / mL。經過幾次恢復測試，確定最有效的恢復活病毒的方法是在病毒培養基中漂洗2 mL，然後用拭子擦拭接種區域。達到的最大回收率為95.3%或3.83X10^7 TCID50 / mL。 該值用作所有表面測試的“0分鐘”起始濃度，以說明回收率。

測試結果：霧化病毒

每個時間點的採集都是通過Gillian 10i空氣採樣器完成的。在指定的時間點，由技術人員在容器內收集樣品，並將其存儲在密封的冷藏容器中，直到完成測試和回收為止。 技術人員穿著全面的隔離工作服，並且在任何時候都沒有直接暴露於病原體中。下圖代表與霧化病毒有關的實驗和對照數據。

當針對SARS-CoV-2-CA1 / 2020病毒進行測試時，PYURE MYSPACE™裝置在操作期間顯示出侵襲性降低，從而導致該病毒的嚴重破壞。與對照組相比，霧化病毒的減少率在20分鐘採樣點為94.81%，而在80分鐘採樣點則穩定地進一步降低到99.95%以上。在120分鐘的時間點，由於定量限制，不再檢測到病毒。



氣溶膠測試數據表 - 對照試驗組

Time Point	TCID50/mL	Percent Reduction
0 MINUTES	4.02×10^7	N/A
20 MINUTES	2.37×10^7	41.04
40 MINUTES	9.34×10^6	76.77
60 MINUTES	3.64×10^6	90.95
80 MINUTES	8.38×10^5	97.92
120 MINUTES	3.48×10^5	99.13
180 MINUTES	3.68×10^4	99.91

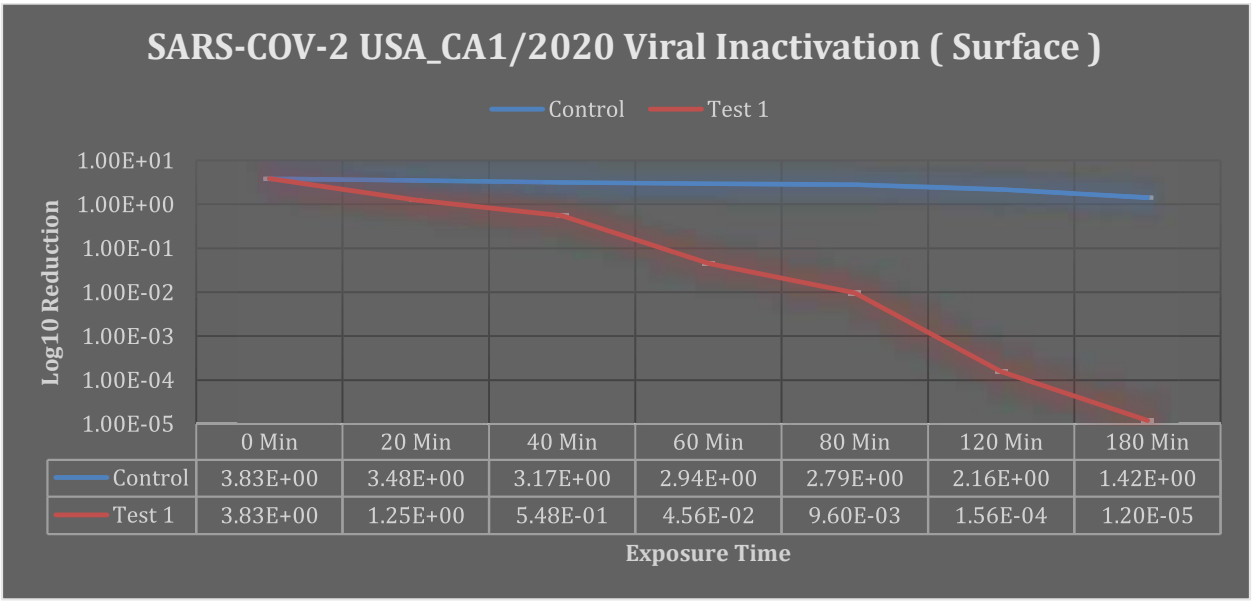
氣溶膠測試數據表 - 測試試驗組

TIME POINT	TCID50/mL	Percent Reduction
0 MINUTES	4.02×10^7	N/A
20 MINUTES	1.23×10^6	96.94
40 MINUTES	1.34×10^5	99.66
60 MINUTES	1.35×10^4	99.966
80 MINUTES	4.10×10^2	99.999
120 MINUTES	$< 1.2 \times 10^2$	>99.999
180 MINUTES	$< 1.2 \times 10^2$	>99.999

測試結果：表面接種

以與對照測試相同的方式進行，在測試試驗中觀察到以下失活率，用於直接表面接種。通過拭子和沖洗試樣在每個時間點進行收集。在指定的時間點，由技術人員在容器內收集樣品，並將其存儲在病毒培養基中，直到完成測試和回收為止。 技術人員穿著全面的隔離工作服，並且在任何時候都沒有直接暴露於病原體中。下圖表示表面接種實驗和對照的數據。

在60分鐘的採樣點，接種的表面樣本的平均減少率為98.44%，並且在120分鐘的採樣點處穩定減少，甚至超過99.993%。在180分鐘的時間點，由於定量限制，不再檢測到該病毒。



表面測試數據表 - 對照試驗組

Time Point	TCID50/mL	Percent Reduction
0 MINUTES	3.83×10^7	4.72
20 MINUTES	3.48×10^7	9.14
40 MINUTES	3.17×10^7	17.23
60 MINUTES	2.94×10^7	23.23
80 MINUTES	2.79×10^7	27.15
120 MINUTES	2.16×10^7	43.60
180 MINUTES	1.42×10^7	62.92

表面測試數據表 - 三組測試實驗組平均數據表

Time Point	TCID50/mL	Percent Reduction
0 Min	3.83×10^7	4.72
20 Min	1.25×10^7	67.36
40 Min	5.48×10^6	85.69
60 Min	4.56×10^5	98.81
80 Min	9.60×10^4	99.75
120 Min	1.56×10^3	>99.995
180 Min	$< 1.20 \times 10^2$	>99.999

結論：

PYURE　MYSPACE™裝置的性能符合製造商的規格，並證明在以氣霧劑形式暴露120分鐘和在表面測試中暴露180分鐘後，活性病毒逐漸減少。在這些時間點之後，未檢測到活的SARS-CoV-2病毒（水平低於120 TCID50 / ml的定量限）。

在考慮使用生物安全3級病原體時需要採取的特殊預防措施的情況下，已竭盡全力模擬室內的真實環境。考慮到活性SARS-CoV-2病毒的起始濃度，氣霧化的體積和接種的體積，可以假設在現實生活中不太可能攜帶這種數量的病原體進入環境。

當霧化病原體並收集所述病原體時，存在無法完全解釋的變量，即病原體的位置、收集量、收集點、滴落率、表面飽和度、收集時的病毒破壞、霧化時的病毒破壞等。盡一切努力通過試驗的設計和執行來解決這些限制。這些努力反映在對照測試中有意義的病毒回收中。

考慮到這些變量，在最初的60分鐘內，MYSPACE™設備實現了大量滅菌。 空氣和表面上的減少非常明顯，並且與製造商聲稱的MYSPACE™設備產生的有機氧化劑擴散到經過處理的空間中並破壞了空氣和表面上的病毒有關。 總體而言，MYSPACE™設備在破壞空氣和表面的SARS-CoV-2USA_CA1 / 2020中顯示出顯著的功效。

免責聲明：

創新的生物分析實驗室（“Innovative Bioanalysis”）實驗室未經美國認證或許可
美國環境保護署（EPA）並沒有就任何臭氧、過氧化氫氣體、活性氧、揮發性有機化合物或任何MYSPACE™裝置的副產品提出任何設備排放聲明。創新的生物分析對任何MYSPACE™的整體功效不作任何聲明，實驗結果僅是適用於試用版的設備，序列號：20MYS01TC02008027740。結果僅代表本報告中描述的實驗設計。
鑑於即使在相同的測試環境，病毒株、收集方法、接種、霧化、病毒培養基、細胞類型和培養程序相同的情況下，實驗結果也可能發生變化，創新性生物分析法對實驗結果的可重複性沒有任何要求。創新的生物分析不對第三方提出任何要求，也不對由於使用或依賴第三方的實驗結果而導致的任何後果承擔任何責任。

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SARS-CoV-2USA-CA1/2020

CLIENT: The PYURE Company Inc

PROJECT: OH MYSPACE™ Bioaerosol Test

PRODUCT: OH AIR® MYSPACE™

CAP LIC NO: 886029801

CLIA LIC NO: O5D0955926

STATE ID: CLF 00324630

CHALLENGE VIRUS: SARS-CoV-2 USA_CA1/2020

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ABSTRACT: EFFICACY OF THE PYURE MYSPACE™ DEVICE AGAINST AEROSOLIZED SARS-CoV-2

Background: This in vitro study was designed to determine the efficacy of the MYSPACE™ unit. The product is a commercially available mobile disinfection device manufactured by The PYURE Company (PYURE) out of Boynton Beach, Florida. The MYSPACE™ unit is designed to be placed free standing in a room and decrease the concentration of pathogens in the air and on surfaces when it is operating, to sanitize enclosed spaces and their contents. For this challenge, the SARS-CoV-2USA_CA1/2020 pathogen was used. Coronavirus can be spread through the air and by touching contaminated surfaces. There is a demand for disinfectant devices that have a proven ability to reduce infectious pathogens in the air thereby reducing the risk of human infection and transmission. PYURE supplied a pre-packaged MYSPACE™ free standing unit for testing purposes. For the testing, power was supplied through a power regulated 120v outlet with surge protector and backup battery system. Test procedures were followed using internal SOPs for aerosolized viral pathogen challenges and subsequent decontamination. All internal SOPs and processes follow GCLP guidelines and recommendations.

EQUIPMENT PROVIDED:

MANUFACTURER: The PYURE Company

MODEL: MYSPACE™

SERIAL #: 20MYS01TC02008027740

MYSPACE™ EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The device was powered on to check for normal operations. Prior to starting the challenge, the PYURE MYSPACE™ unit was operated for over 2 hours of dry runs in a sealed bioaerosol chamber to confirm correct operation of the unit. The chamber was the same BSL3 chamber used for the viral challenge testing.

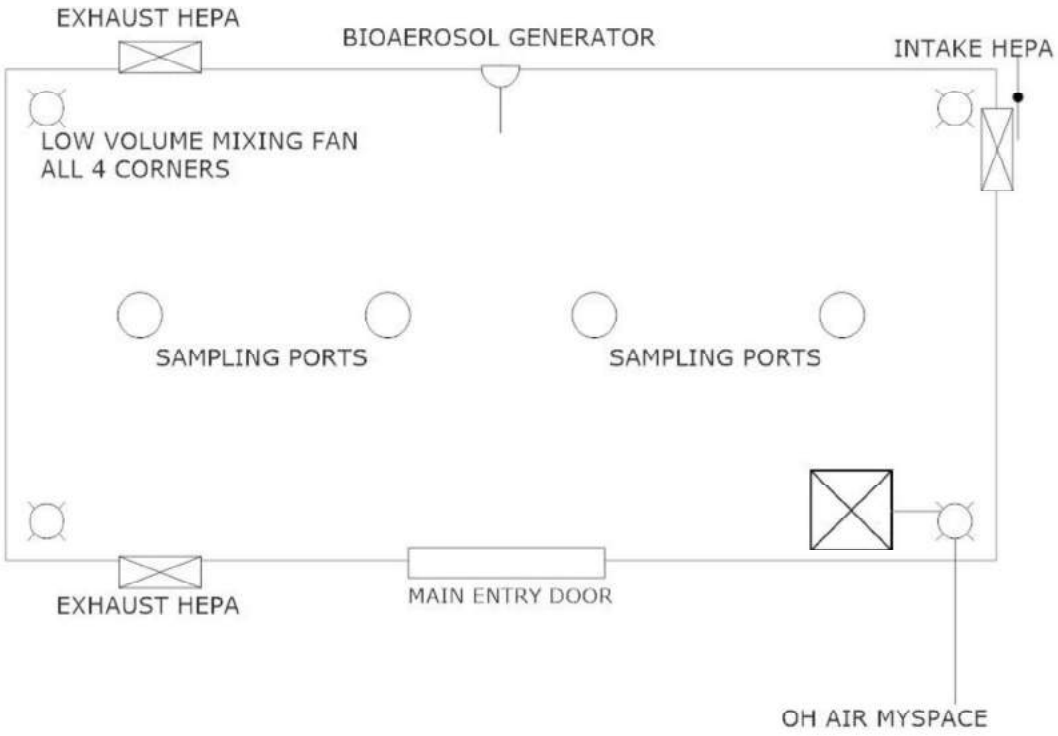
VIRAL CHALLENGE TESTING CHAMBER:

The testing chamber was a large, sealed air volume testing chamber consisting of metal walls and epoxy floor which complied with BSL3 standards. The chamber was designed to be completely sealed from the outside environment to prevent any potential release of testing media into the atmosphere. The testing chamber was equipped with 4 sealed viewing windows and a lockable chamber door for entry and exit. The overall dimensions of the test chamber were approximately 8'x8'x20' with a displacement volume of 1280 cubic feet. Based on cubic foot volume the chamber had 36,245.56 liters of air.

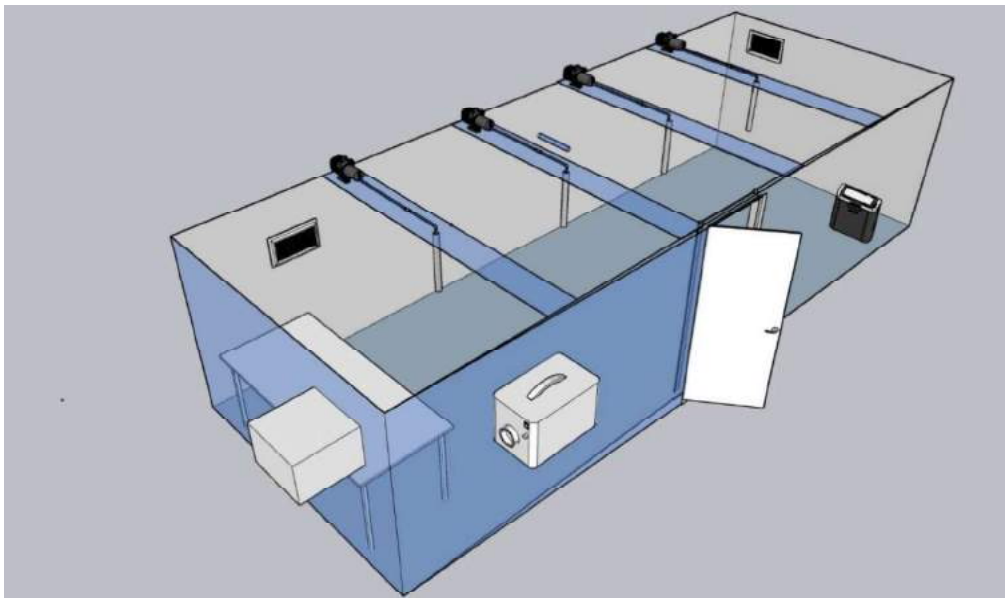
The testing chamber had HEPA filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. For air sample testing, the chamber was equipped with 4 probes that were along the centerline of the room and protruded down from the ceiling 24". Each probe tube was connected to a Gilian 10i programmable system with sampling cassettes from lot # 18248 made by Sensidine. A single bioaerosol nebulizing port was in the center of the 20' wall opposite of the entry doors. The dissemination port protruded from the wall 24" and was connected to a programmable compressor nebulizer system.

Prior to testing, the chamber was pressure tested for leaks and visual inspections were made using a colored smoking device. All seals for the chamber were confirmed and all equipment used had a function test to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

CHAMBER LAYOUT OVERHEAD:



DESIGN LAYOUT 3D:



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EXPERIMENTAL SUMMARY:

- Prior to the initial control test and following the trial run the testing area was decontaminated and prepped per internal procedures.
- Temperature during all test runs was approximately 71F +/- 2F with a relative humidity of 49%.
- Relative humidity and temperature were taken in two sections of the chamber during all tests to confirm there was no more than a 3% deviation from each side.
- Air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance.
- All sample collection volumes were set to 5-minute draws per time point.
- Low volume mixing fans were turned on prior to nebulization to confirm homogenous concentrations in the test chamber.
- Mixing fans remained on and positioned at a 45-degree angle to encourage bioaerosol suspension and reduce natural particle descent rates.
- Nebulization for control and viral test challenges were performed in the same manner.
- Sample cassettes were manually removed from the collection system and stored after each time point and replaced with new cassettes.
- Upon cassette removal at each time point, cassette sets were taken to an adjacent bio safety cabinet and pooled.
- Six total sample times were collected per bioaerosol challenge.
- 1 control was completed, and 1 viral challenge were completed using the same methodology.

BIOAEROSOL GENERATION:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock (4.02 x 10^7 TCID50 per ml) and nebulized at a flow rate of 1ml/min. Nebulizer was driven by untreated local atmospheric air. The nebulizer's remaining viral stock volume was weighed after completion to confirm the same amount of viral stock that was nebulized was consistent with the control.

BIOAEROSOL SAMPLING:

For air sampling, 4 different Gillian 10i programmable vacuum devices were used. Air samplers were calibrated by the manufacturer in September 2020 and certificates were inspected prior to use. Air sample volume collections were confirmed prior to use with a Gilian Gilibrator 2 SN- 200700-12 and a high flow bubble generator SN-2009012-H. Air samplers were operated in conjunction with removable sealed cassettes, which were manually removed after each sampling time point. Cassettes had a delicate internal filtration disc to collect viral samples.

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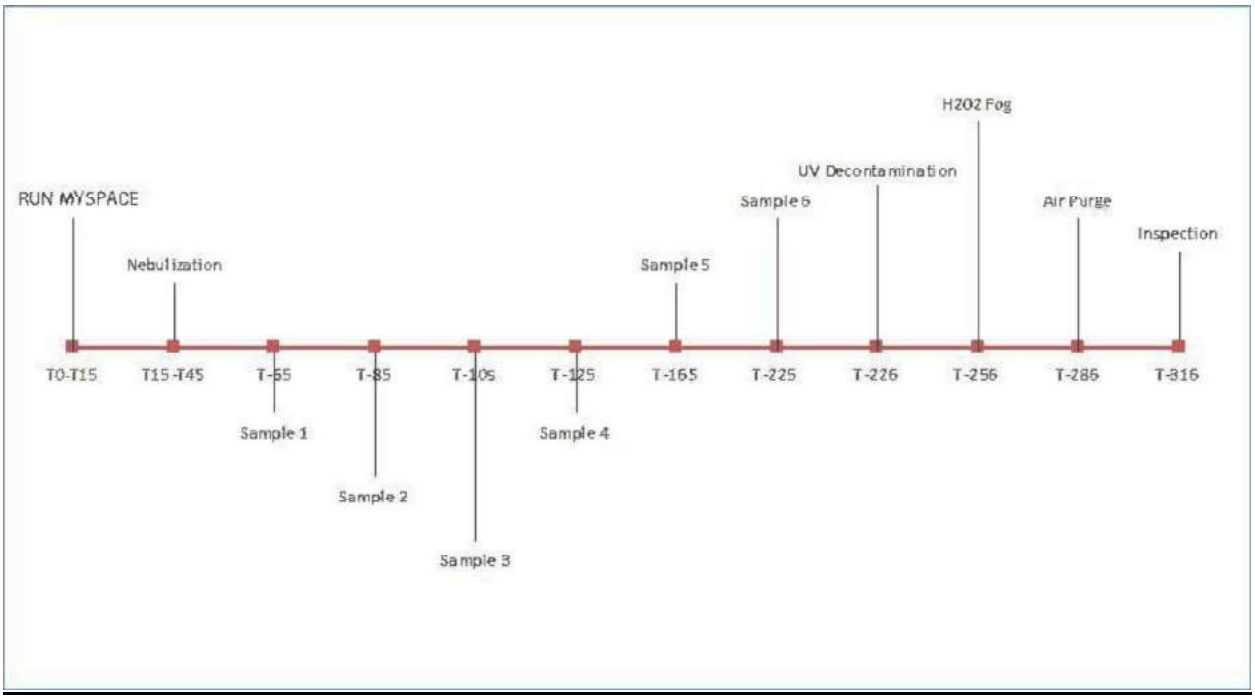
SAMPLE INOCULATION:

Surface inoculation consisted of applying exactly 1 ml of viral media to each coupon with a calibrated Eppendorf pipette utilizing filtered pipette tips. Coupons were standard sterile 25mm x 75mm slides. Once applied, the media was spread thin using a disposable spatula and allowed to dry for 10 minutes. The starting concentration of virus that was applied was 4.02X10^7 TCID50/mL. After several tests for recovery, it was determined that the most efficient method of recovering viable virus would be a 2 mL rinse in viral media followed by a swab of the inoculated area. The maximum recovery achieved was 95.3% or 3.83X10^7 TCID50/mL. This value was used as the "0 Minute" starting concentration for all surface testing to account for the recovery.

SAMPLING POINT TIMELINE:



VIRUS STRAIN BACKGROUND:

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through the BEI Resources, BIAID, NIH SARS-Related Coronavirus 2, Isolate USA_CA1/2020, NR-52382. This was the chosen pathogen strain because it was taken from a 38-year-old subject with severe acute respiratory syndrome in California as was part of the A lineage. This was a non-fatal case which represents most of the cases in the United States. The age group the patient belonged to was not elderly or juvenile, which was determined to be a good medium average.

POST DECONTAMINATION:

At the conclusion of the viral challenge test the UV system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV exposure the chamber was fogged with a Hydrogen Peroxide gas mixture followed by a 30-minute air purge. All test equipment was cleaned at the end of each day with a 70% alcohol solution. Collection lines were soaked in a bleach bath mixture for 30 minutes then rinsed repeatedly with DI water. Nebulizer and Vacuum collection pumps were decontaminated with Hydrogen Peroxide mixtures.

TCID50 PROCEDURE:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips – 20uL, 200uL, 1000uL.
- Inverted Microscope
- Tubes for dilution
- Hemocytometer with cover slip
- Cell Media for infection
- Growth Media appropriate for cell line
- 0.4 % Trypan Blue Solution
- Lint Free Wipes saturated with 70% isopropyl alcohol.
- CO_2 Incubator set at 37°C or 34°C or other temperature indicated.

Procedure:

1. One day prior to infection, prepare 96 well dishes by seeding each well with Vero E6 cells in DMEM plus 7.5 % fetal bovine serum, 4mM Glutamine, and antibiotics.
2. On the day of infection, make dilutions of virus sample in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. First tube with 2.0 mL PBS and subsequent tubes with 1.8mL
4. Vortex Viral samples, transfer 20 uL of virus to first tube, vortex, discard tip.
5. With new tip, serial dilute subsequent tips transferring 200 uL.

Additions of virus dilutions to cells

1. Label lid of 96 well dish by drawing grid lines to delineate quadruplicates and number each grid to correspond to the virus sample and label the rows of the plate for the dilution which will be plated.
2. Include 4 Negative wells on each plate which will not be infected.
3. Remove all but 0.1 mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1 mL of virus dilution to each of the quadruplicate wells for that dilution

5. Infect 4 wells per dilution, working backward.
6. Allow the virus to absorb to cells at 37°C for 2 hours.
7. After absorption, remove virus inoculum. Start with the most dilute and work backwards.
8. Add 0.5 mL infection medium to each well being careful to not touch the wells with the pipette.
9. Place plates at 37°C and monitor CPE using the inverted microscope over a period of 1 to 4 weeks.
10. Record the number of positive and negative wells.

CONTROL:

One Control test was conducted without the PYURE MYSPACE™ unit in the testing chamber. Control samples were taken at each of the corresponding sample times used for the challenge trial (see sampling timeline above). Nebulization of viral media and collection methods were the same for the control as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the PYURE MYSPACE™ device was operated in the challenge trials, to enable net reduction calculations to be made. During the control test, four low volume fans were operated in each corner of the testing chamber to ensure homogenous mixing of the air. An emergency O3 alarm was outfitted to the room and calibrated to 1ppm. During the control test no O3 alarms were observed. During the control temperature and relative humidity were monitored. Prior to running the viral challenges temperature and humidity were confirmed to be in relative range to the control +/- 5%.

VIRAL CHALLENGE:

The challenge pathogen, SARS-CoV-2USA_CA1/2020, was used for testing the efficacy of the PYURE MYSPACE™ device. During the challenge tests the pressure in the challenge chamber was monitored to confirm no portion of the chamber was leaking. The bioaerosol efficacy challenge was completed in single distinct trial with the live pathogen to create a baseline of data. Prior to nebulizing the viral pathogen, the PYURE MYSPACE™ device was turned on and allowed to run for 15 minutes to simulate a real-world environment and allow the device to reach standard operating conditions. Four low volume mixing fans were used throughout the entire control test and viral pathogen test. Sample times were as follows with T equal to minutes, T-0, T-20, T-40, T-60, T-80, T-120, T-180. Sampling occurred using 4 automatic air volume samplers that operated simultaneously for each collection. Samplers were pre-set to automatically shut off after 5 minutes of collection. Collections were made via the equipment utilizing viral media coated filters for maximum pathogen trapping and stability. Collection samples were provided to lab staff for pooling after each collection time point.

VIRAL STOCK: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next Generation Sequencing (NGS) of complete genome using Illumina® iSeq™ 100 Platform (Approx. 940 Nucleotides)	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 ≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1
Titer by TCID50 in Vero E6 Cells by Cytopathic effect	Report Results	2.8 X 10^5 TCID50 per mL in 5 days at 37°C and 5% CO2
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth
Mycoplasma Contamination Agar and Broth Culture DNA Detection by PCR of extracted Test Article nucleic acid.	 None Detected None Detected	 None Detected None Detected

Inoculation of Surface Samples

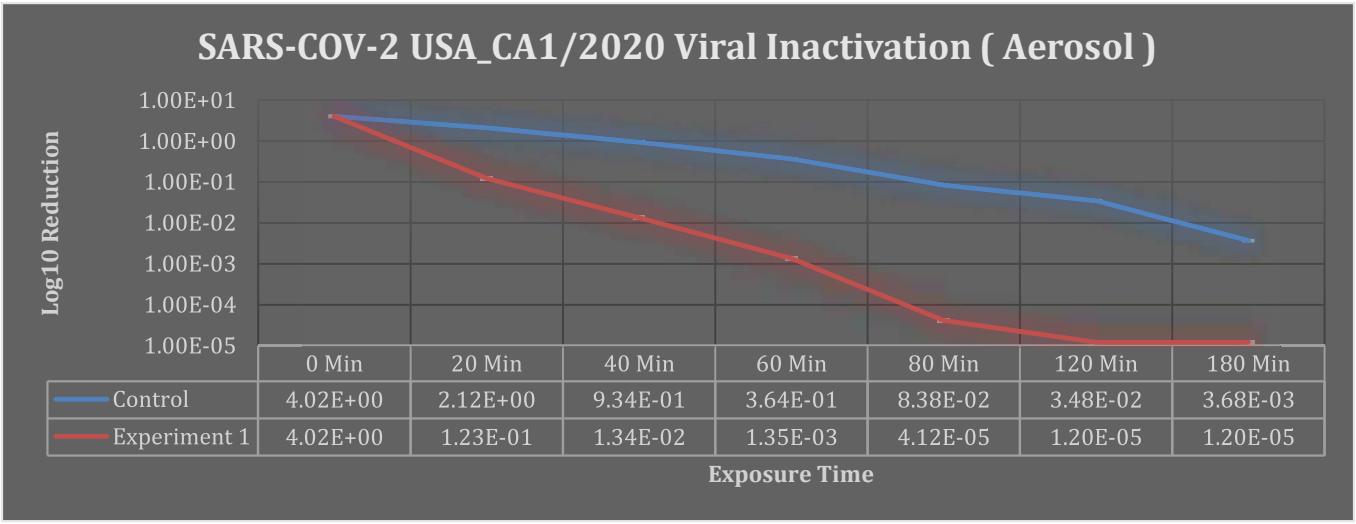
Surface inoculation consisted of applying exactly 1 ml of viral media to each coupon with a calibrated Eppendorf pipette utilizing filtered pipette tips. Coupons were standard sterile 25mm x 75mm slides. Once applied, the media was spread thin using a disposable spatula and allowed to dry for 10 minutes. The starting concentration of virus that was applied was 4.02 X 10^7 TCID50/mL. After several tests for recovery, it was determined that the most efficient method of recovering viable virus would be a 2 mL rinse in viral media followed by a swab of the inoculated area. The maximum recovery achieved was 95.3% or 3.83 X 10^7

TCID50/mL. This value was used as the "0 Minute" starting concentration for all surface testing to account for the recovery.

Test Results: Aerosolized Virus

Collection at each time point was done via the Gillian 10i air samplers. Samples were collected by a technician at specified time points inside the container and stored in a sealed refrigerated container until testing and recovery were complete. Technicians were wearing full hazmat coveralls and had no direct exposure to the pathogen at any time. The graph below represents the data for the experiment and the control, as it pertains to aerosolized virus.

When tested against SARS-CoV-2-CA1/2020 virus, the PYURE MYSPACE™ unit showed a progressive reduction during the time it was operated resulting in significant destruction of the virus. The reduction of aerosolized virus versus controls was 94.81% at the 20-minute sampling point and a steady, further reduction to greater than 99.95% at the 80-minute sampling point. At the 120-minute time point the virus was no longer detectable due to the limit of quantification.



	0 Min	20 Min	40 Min	60 Min	80 Min	120 Min	180 Min
Control	4.02E+00	2.12E+00	9.34E-01	3.64E-01	8.38E-02	3.48E-02	3.68E-03
Experiment 1	4.02E+00	1.23E-01	1.34E-02	1.35E-03	4.12E-05	1.20E-05	1.20E-05

Data Table for Aerosol Testing - Control Trial

Time Point	TCID50/mL	Percent Reduction
0 MINUTES	4.02 X 10^7	N/A
20 MINUTES	2.37 X 10^7	41.04
40 MINUTES	9.34 X 10^6	76.77
60 MINUTES	3.64 X 10^6	90.95
80 MINUTES	8.38 X 10^5	97.92
120 MINUTES	3.48 X 10^5	99.13
180 MINUTES	3.68 X 10^4	99.91

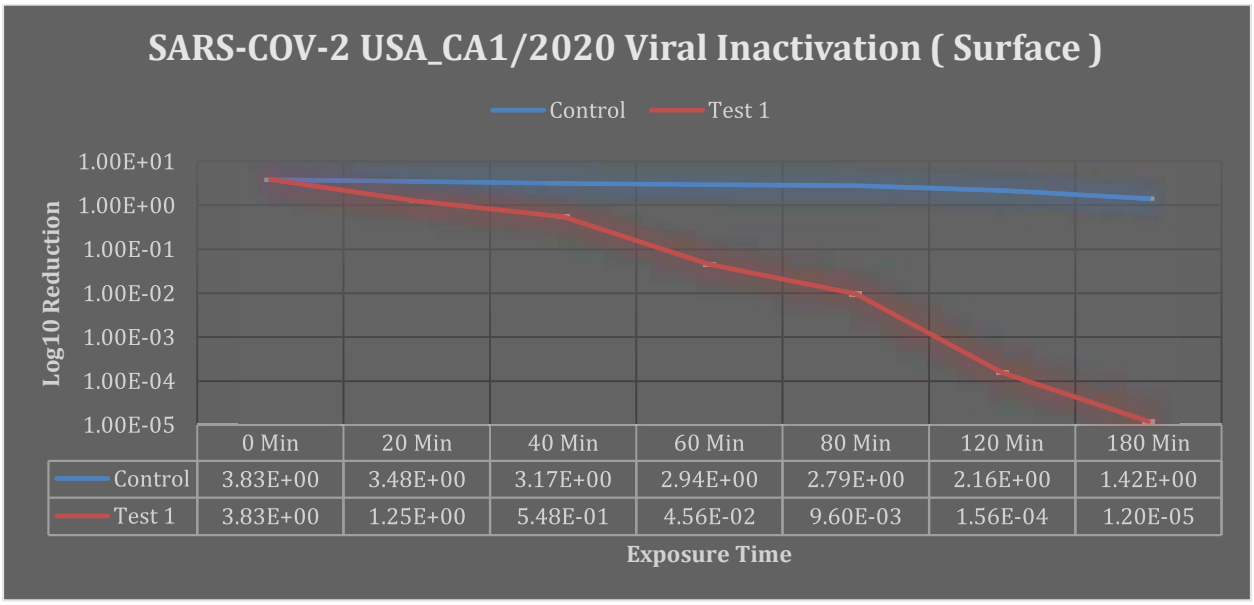
Data Table for Aerosol Testing. Challenge Trial

TIME POINT	TCID50/mL	Percent Reduction
0 MINUTES	4.02 X 10^7	N/A
20 MINUTES	1.23 X 10^6	96.94
40 MINUTES	1.34 X 10^5	99.66
60 MINUTES	1.35 X 10^4	99.966
80 MINUTES	4.10 X 10^2	99.999
120 MINUTES	< 1.2 X 10^2	>99.999
180 MINUTES	< 1.2 X 10^2	>99.999

Test Results: Surface Inoculation

Performed in the same manner as the control testing, the following deactivation rates were observed for direct surface inoculation in the challenge trial. Collection at each time point was done by swab and rinse of the coupon. Samples were collected by a technician at specified time points inside the container and stored in viral media until testing and recovery were complete. Technicians were wearing full hazmat coveralls and had no direct exposure to the pathogen at any time. The graph below represents the data for the surface inoculation experiment and the control.

The reduction of inoculated surface samples versus controls was 98.44% at the 60-minute sampling point and steady, further reduction to greater than 99.993% at the 120-minute sampling point. At the 180-minute time point, the virus was no longer detectable due to the limit of quantification.



Data Table for Surface Testing - Control Trial

Time Point	TCID50/mL	Percent Reduction
0 MINUTES	3.83 X 10^7	4.72
20 MINUTES	3.48 X 10^7	9.14
40 MINUTES	3.17 X 10^7	17.23
60 MINUTES	2.94 X 10^7	23.23
80 MINUTES	2.79 X 10^7	27.15
120 MINUTES	2.16 X 10^7	43.60
180 MINUTES	1.42 X 10^7	62.92

Data Table for Surface Testing – Averages of Three Challenge Trials

Time Point	TCID50/mL	Percent Reduction
0 Min	3.83 X 10^7	4.72
20 Min	1.25 X 10^7	67.36
40 Min	5.48 X 10^6	85.69
60 Min	4.56 X 10^5	98.81
80 Min	9.60 X 10^4	99.75
120 Min	1.56 X 10^3	>99.995
180 Min	< 1.20 X 10^2	>99.999

CONCLUSIONS:

The PYURE MYSPACE™ device performed to manufacturer specifications and demonstrated a progressive and dramatic reduction of active virus after 120 minutes of exposure in aerosol form and 180 minutes of exposure in surface testing. The live SARS-CoV-2 virus was not detectable after these timepoints, (levels were below the 120 TCID50 / ml limit of quantification).

Every effort was made to simulate a real-life environment in the chamber while taking into consideration the special precautions needed when working with a Biosafety Level 3 Pathogen. Taking into consideration the starting concentration of active SARS-CoV-2 virus, the volume aerosolized, and the volume inoculated, one could assume that the likelihood of entering an environment with this quantity of pathogen in a real-life circumstance to be unlikely.

When aerosolizing pathogens and collecting said pathogens, there are variables that cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction on collection, viral destruction on nebulization and possibly others. Every effort was made to address these constraints with the design and execution of the trials. And these efforts are reflected in the meaningful recovery of virus in the control test.

Taking these variables into account, there was a large amount of sterilization achieved by the MYSPACE™ device in the first 60 minutes. The reduction in air and on surfaces was significant and consistent with the manufacturer's claims that the organic oxidants produced by the MYSPACE™ device diffuse into a treated space and destroy the virus in air and on surfaces. Overall, the MYSPACE™ device showed substantial efficacy in the destruction of SARS-CoV-2USA_CA1/2020 in air and on surfaces.

DISCLAIMER:

The Innovative Bioanalysis, LLC. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone, hydrogen peroxide gas, reactive oxygen species, volatile organic compounds, or byproduct of any MYSPACE™ device. Innovative Bioanalysis makes no claims to the overall efficacy of any MYSPACE™. The experiment results are solely applicable to the device used in the trial, serial number: 20MYS01TC02008027740. The results are only representative of the experiment design described in this report. Innovative Bioanalysis makes no claims as to the reproducibility of the experiment results given the possible variation of experiment results even with an identical test environment, viral strain, collection method, inoculation, nebulization, viral media, cell type, and culture procedure. Innovative Bioanalysis makes no claims to third parties and takes no responsibility for any consequences arising out of the use of, or reliance on, the experiment results by third parties.

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2/24/2021

Dr. Dana Yee M.D
Clinical Pathologist and Medical Director

Date

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