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ESCHERICHIA COLI Surface Reduction Through Air Purification with PYURE MDU/Rx

CLIENT: The PYURE Company

PROJECT: MDU/Rx - ECOLI-1

REPORT NUMBER: PYURE2001

PRODUCT: MDU/Rx
CAP LIC NO: 886029801
CLIA LIC NO: O5D0955926
STATE ID: CLF 00324630

REPORT DATE: 01/28/2021

CHALLENGE ORGANISM: Escherichia Coli O157:H7
ATCC Number: 12600

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ABSTRACT: EFFICACY OF THE MDU/Rx AGAINST E. COLI O157:H7

Background: This in vitro study was designed to determine the efficacy of a PYURE MDU/Rx device against a known strain of bacteria. The MDU/Rx is a commercially available air purifier that is registered with the FDA as a class II medical device and manufactured by The PYURE Company. The MDU/Rx is designed to be placed free standing in a room and decrease the concentration of bacteria and certain viruses in the air while operational.

For this challenge, the Escherichia Coli O157:H7 **(E. COLI)** bacteria was used. According to public health studies each year in the United States, *E. coli* infections cause approximately 265,000 illnesses and about 100 deaths. Approximately 40 percent of these infections are caused by the strain *E. coli* O157:H7, a strain that is part of the shiga toxin-producing group of *E. coli* bacteria (STEC). The other 60 percent of *E. coli* cases are caused by non-0157:H7 shiga toxin-producing *E. coli* (STEC). There is a demand for disinfectant devices that have a proven ability to reduce the presence of bacteria in the air and on surfaces thereby reducing the risk of human infection and transmission.

Escherichia coli (E. coli) is a bacterium that normally is an important part of the healthy intestinal tracts of humans and animals. However, there are some kinds of *E. coli* that are harmful and can cause disease.
The most common type of *E. coli* infection that causes illness in people is called *E. coli* O157. *E. coli* O157 is naturally found in the intestinal tracts of many farm animals, including healthy cattle, sheep, and goats. Animals can carry *E. coli* O157 and shed the germs in their stool but still appear healthy and clean. The germs can quickly contaminate the animals' skin, fur, feathers, and the areas where they live and roam.

Most people become infected with *E. coli* O157 from contaminated food, such as undercooked ground beef or raw (unpasteurized) milk, but *E. coli* O157 can be passed directly to people from the stool of young calves and adult cattle. *E. coli* O157 *also* can be spread from person to person, particularly in places where frequent and close contact between people occurs, such as day-care facilities.
Animals can appear healthy and clean but can spread *E. coli* O157 to humans or other animals.

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EQUIPMENT PROVIDED:

MANUFACTURER: The PYURE Company, Boynton Beach Florida

MODEL: PYURE MDU/Rx (Class II medical device)

SERIAL#MDURXA0000069

PYURE MDU/Rx EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Prior to starting the challenge, the PYURE MDU/Rx device operated in several viral challenge studies in a BSL3 sealed bioaerosol chamber to confirm correct operations. The chamber for this challenge was the same BSL3 chamber used for the viral challenge testing. RKI air monitoring systems continuously sampled air for O3, H202, N20 production which could put staff members at risk. Air monitoring was in place as a safety mechanism for staff and no alarms for unsafe elevated ozone were activated during testing.

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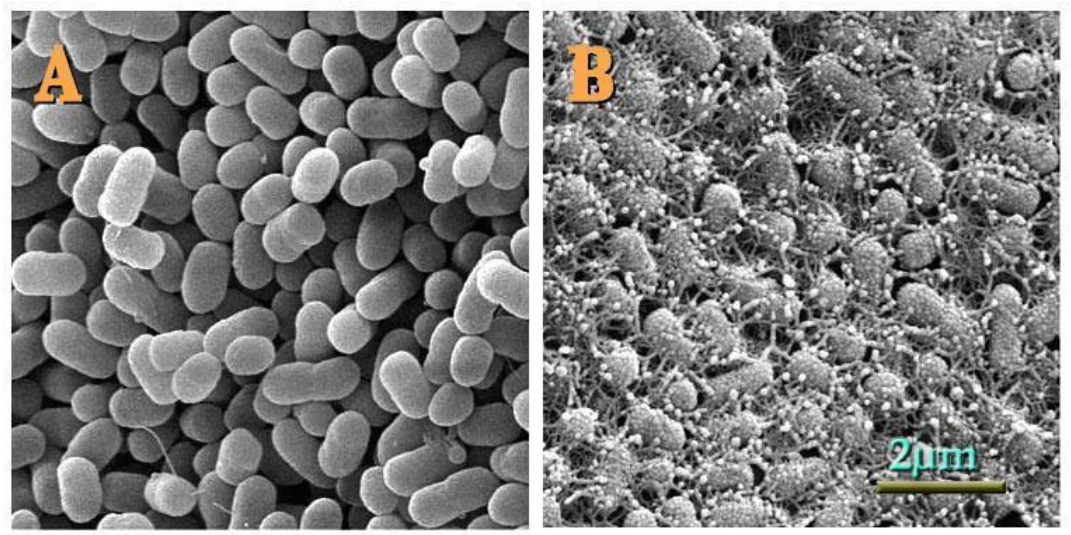
TESTING CHAMBER:

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 outside variables from entering the test chamber. 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 8'x8'x20'.

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. 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.

ORGANISM PREPARATION

Escherichia Coli was cultured by plating the thawed broth on Tryptic Soy Agar and allowed to incubate at 32°C with 5% CO2 for 24 hours. A single isolate colony was harvested and introduced to a tryptic soy broth and allowed to incubate at 32°C for an additional 24 hours. This process was replicated several times to reach higher concentrations of the organism and to be able to represent a potential for a greater log reduction. Upon completion of the incubation period, bacteria were harvested and rinsed 3 times in phosphate buffered saline. A 1 to 10 dilution was made by removing 1 mL of inoculated tryptic soy broth and adding it 9 mL of phosphate buffered saline. This solution was further diluted to a final concentration of 1:100.



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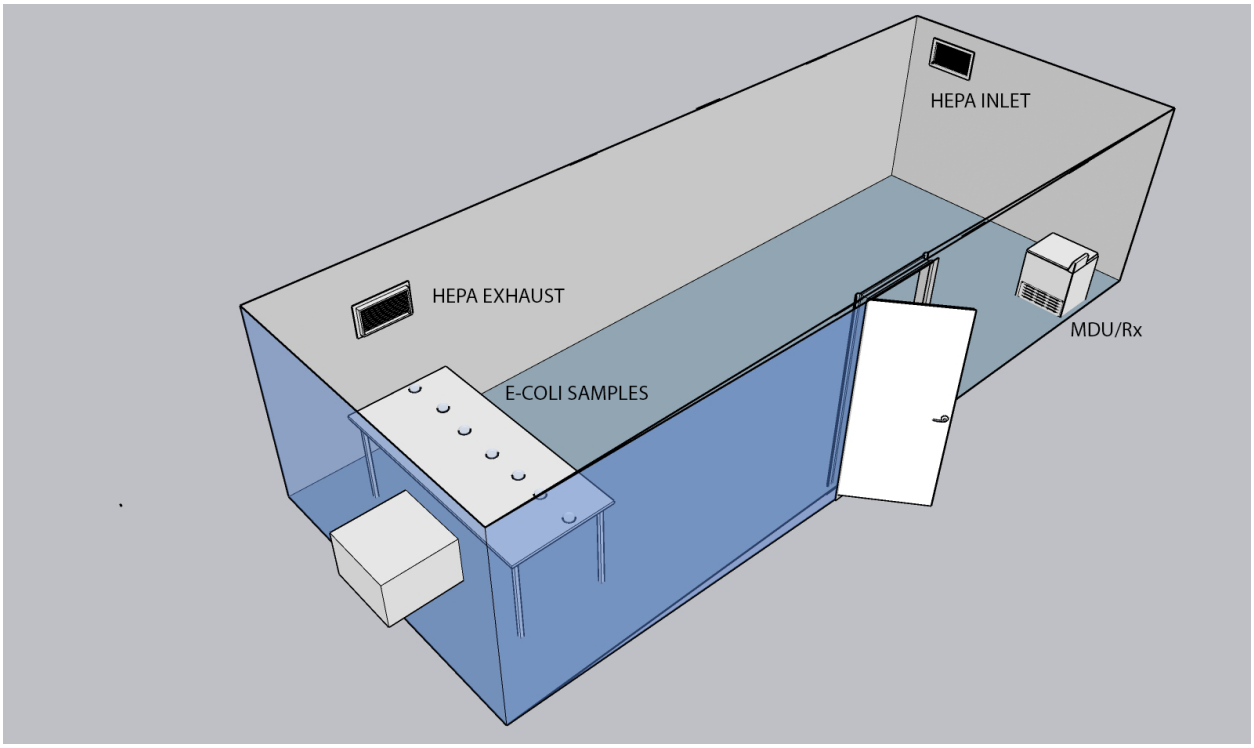
MATERIALS AND EQUIPMENT:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips – 20uL, 200 uL, 1000uL
- Microscope
- Tubes for dilution
- Hemocytometer with cover slip
- Tryptic Soy Broth
- Tryptic Soy Agar
- 10 uL Inoculation Loops
- CO2 Incubator set at 34°C

DESIGN LAYOUT:

The PYURE MDU/Rx device was placed in the room as indicated in the diagram below. The stainless-steel table on which the eight inoculated dishes were placed was located at the far end of the chamber, approximately 18 feet from the PYURE MDU/Rx device.



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CONTROL SUMMARY:

Eight sterile dishes inoculated with E. Coli were provided by lab staff and labeled with time point designation and organism. Dishes were placed on a stainless-steel table inside the room and the door sealed to prevent outside environmental contaminants. Swabs were taken at eight pre-defined time points: 0 minutes, 10 minutes, 20 minutes, 40 minutes, 60 minutes, 120 minutes, 180 minutes, and 360 minutes. All swabs were sealed after collection and provided to lab staff for analysis after study completion. The door to the chamber remained closed the entirety of the test.

EXPERIMENT SUMMARY:

The challenge study was performed in the same manner as the control study, with one difference: a PYURE MDU/Rx device was placed in the chamber and operated during the entire period of the trial. The following procedures were followed during the challenge trial:

- Prior to the control test and prior to the challenge trial, the test chamber was decontaminated and prepped per internal procedures.
- Temperature during all test runs was approximately 73F +/- 2F with a relative humidity of 48%.
- 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.
- Swabs were taken at eight predefined time points: 0 minutes, 10 minutes, 20 minutes, 40 minutes, 60 minutes, 120 minutes, 180 minutes, and 360 minutes. The test chamber was sealed throughout the trial and was not breached.
- 8 round petri dishes provided by the lab and inoculated with 1mL of E. Coli with a concentration of 29228 CFU/mL were placed on the stainless-steel table.
- All sample dishes were labeled with their bacterium and the time point they were to be used with. A sample swab was taken from each dish, as well as a swab collected for residual bacteria at each predetermined timepoint.
- Four small fans were operated in the corners of the chamber to ensure air movement and simulate air exchange. The PYURE MDU/Rx device was started 15 minutes prior to the T=0.
- All petri dishes were brought into the Chamber sealed and unsealed at T=0. Each swab sample was sealed at the time of swabbing.
- Upon testing completion, the sealed samples were provided to lab staff for analysis.

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RESULTS:

CONTROL RESULTS			EXPERIMENT RESULTS		
Timepoint	**CFU/mL**	**% Reduction**	**Timepoint**	**CFU/mL**	**% Reduction**
0 Min	**29228**	**N/A**	**0 Min**	**29228**	**N/A**
10 Min	**27649**	**5.40**	**10 Min**	**23256**	**20.43**
20 Min	**26345**	**9.86**	**20 Min**	**18523**	**36.63**
40 Min	**25163**	**13.91**	**40 Min**	**12535**	**57.11**
60 Min	**23936**	**18.11**	**60 Min**	**8634**	**70.46**
120 Min	**19623**	**32.86**	**120 Min**	**523**	**98.21**
180 Min	**15635**	**46.51**	**180 Min**	**No Growth**	**100**
360 Min	**11652**	**60.13**	**360 Min**	**No Growth**	**100**

CONCLUSION:

The overall reduction of the experiment trial relative to the control test (natural degradation) confirms the increased degradation of E. Coli on a non-porous glass surface when exposed to the PYURE MDU/Rx device. The utilization of the PYURE MDU/Rx device in the test environment significantly decreased the concentration of E. Coli vs. control (> 4 log), such that there was no growth after 180 minutes.

DISCLAIMER:

The Innovative Bioanalysis, LLC. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emission claims pertaining to ozone, hydrogen peroxide gas, reactive oxygen species, volatile organic compounds, or byproduct of any device. Innovative Bioanalysis makes no claims to the overall efficacy of tested products. The experiment results are solely applicable to the device used in the trial. 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, bacteria strain, collection method, inoculation, 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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