Evaluation of Improvements in an Innovative Disinfection System Using Gas Phase Nucleic Acid Digestion Technology to Support Laboratory Animal Management in NCGG

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Introduction

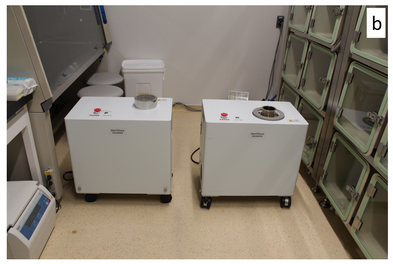
Sterilization of experimental animal facilities is performed not only on articles (breeding equipment, instruments, and laboratory equipment), but also on breeding rooms and laboratories to prevent the development of pathogenic microorganisms. Formaldehyde gas, hydrogen peroxide, and chlorine dioxide gas are used for non-heat-resistant sterilization.

Although any sterilization methods have their own characteristics, and have been reported that some methods caused corrosion and the accumulation of residues on breeding equipment (rubber products, gloves, etc.) and electronic equipment.

Our facility (NCGG) has kept many naturally-aged mice used in gerontology and geriatric research. Since the animals are kept for a long period of time, it is necessary to maintain a clean and appropriate rearing environment. Therefore, we evaluated a new system (steriXcure®) of combined gas equipment using the gaseous phase nucleic acid decomposition technology (Biovector®) with **methanol** as the raw material, **which allows decontamination with no corrosion and no residue.** According to the results, the system was excellent in terms of **occupational safety** and **workability** for workers.

Materials & Methods

The method was performed in two facilities of different sizes: the **disinfection room** (a) (DR, 10 m^3) and the **Infectious Diseases Laboratory** (b) (IL, 50 m^3).



Decontamination device:

① SteriXcure (SEAlive.Inc) Gas Generator

② Gas Decomposition device

③ Tablet operation: data log monitor

①

②

To evaluate the sterilization of each room, **chemical indicators (CI)** (F-Sign) and **biological indicators (BI)** (Bacillus atrophaeus (ATCC #9372) incubated for 7 days) were placed in four locations in the DR and eight locations in the IL. Gas decontamination was performed for 4.5 hours (hrs.) in the DR and 7 hrs. in the IL. Different equipment and electronic devices were decontaminated in both rooms. DNA and RNA degradation were evaluated using electrophoresis method and Qubit 4 Fluorometer (ThermoFischer) respectively.

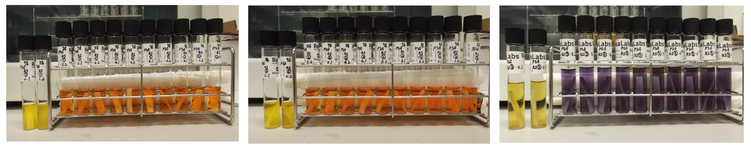
Results

<Biological indicators (BI)>

Fumigation method		Gas generation (2.5H)→	Gas exposure (4H)→	Gas decomposition (6H)→	Aeration (8.5H)	Positive (+) / Negative (-)		
IL	Culture Result Table		Number of culture days (7 days)					
1:*Bacillus atrophaeus* ATCC #9372 Mesa Strip (Glassine paper)	Sampling Location	Culture number	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Table surface	P3A_BI①1.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI①1.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Biosafety cabinet	P3A_BI①2.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI①2.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Chair surface	P3A_BI①3.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI①3.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Mouse cage	P3A_BI①4.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI①4.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Floor	P3A_BI①5.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI①5.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Control	P3A_BI①C.1	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		P3A_BI①C.2	(+)	(+)	(+)	(+)	(+)	(+)	(+)
2:*Bacillus pumilus* ATCC #27142 Mesa Strip (Glassine paper)	Sampling Location	Culture number							
	Table surface	P3A_BI②1.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI②1.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Biosafety cabinet	P3A_BI②2.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI②2.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Chair surface	P3A_BI②3.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI②3.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Mouse cage	P3A_BI②4.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI②4.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Floor	P3A_BI②5.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI②5.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Control	P3A_BI②C.1	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		P3A_BI②C.2	(+)	(+)	(+)	(+)	(+)	(+)	(+)
3:*Geobacillus stearothermophilus* ATCC #7953 Spore Strip (Glassine paper)	Sampling Location	Culture number							
	Table surface	P3A_BI③1.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI③1.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Biosafety cabinet	P3A_BI③2.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI③2.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Chair surface	P3A_BI③3.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI③3.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Mouse cage	P3A_BI③4.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI③4.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Floor	P3A_BI③5.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		P3A_BI③5.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Control	P3A_BI③C.1	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		P3A_BI③C.2	(+)	(+)	(+)	(+)	(+)	(+)	(+)

Fumigation method		Gas generation (2.5H)→	Gas exposure (4H)→	Gas decomposition (6H)→	Aeration (8.5H)	Positive (+) / Negative (-)		
DR	Culture Result Table		Number of culture days (7 days)					
1: *Bacillus atrophaeus* ATCC #9372 Mesa Strip (Glassine paper)	Sampling Location	Culture number	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Entrance door	SL_BI①1.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI①1.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Right wall	SL_BI①2.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI①2.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Exit door	SL_BI①3.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI①3.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Left wall	SL_BI①4.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI①4.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Control	SL_BI①C.1	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		SL_BI①C.2	(+)	(+)	(+)	(+)	(+)	(+)	(+)
2: *Bacillus pumilus* ATCC #27142 Mesa Strip (Glassine paper)	Sampling Location	Culture number							
	Entrance door	SL_BI②1.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI②1.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Right wall	SL_BI②2.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI②2.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Exit door	SL_BI②3.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI②3.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Left wall	SL_BI②4.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI②4.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Control	SL_BI②C.1	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		SL_BI②C.2	(+)	(+)	(+)	(+)	(+)	(+)	(+)
3: *Geobacillus stearothermophilus* ATCC #7953 Spore Strip (Glassine paper)	Sampling Location	Culture number							
	Entrance door	SL_BI③1.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI③1.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Right wall	SL_BI③2.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI③2.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Exit door	SL_BI③3.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI③3.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Left wall	SL_BI③4.1	(–)	(–)	(–)	(–)	(–)	(–)	(–)
		SL_BI③4.2	(–)	(–)	(–)	(–)	(–)	(–)	(–)
	Control	SL_BI③C.1	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		SL_BI③C.2	(+)	(+)	(+)	(+)	(+)	(+)	(+)

7 days culture tubes (Control: First two tubes from the left)



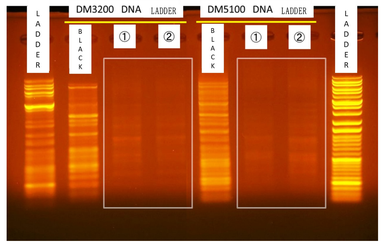
B. atrophaeus *B. pumilus* *G. stearothermophilus*

To evaluate the sterilization *Bacillus atrophaeus*, *Bacillus pumilus* and *Geobacillus stearothermophilus* were incubated for 7 days. All BI culture were negative from day 1 to day 7.

<Chemical indicators (CI)>

To evaluate the sterilization of each room, chemical indicators (CI) (F-Sign) were placed in four locations in the DR (circle) and eight locations in the IL. All the test resulted negative.

<DNA/RNA Degradation>



DNA/RNA degradation were confirmed in both rooms.

CI Test Positive Control

CI Test Negative Control

<Equipment costs and workability>

Costs: The running cost of the decontamination system is as low as 10 dollars per operation using only methanol.

Learning and manageability: Learning for a new operator lasted an average of 30-60 min. The controls were easy to operate and intuitive. The treatment lasts 4 to 10 hours depending on the surface of the room. The equipment could be moved easily and without too much effort from the operator. Our highest capacity breeding room (13,200 gal) was disinfected in about 7 hours. Recent tests showed that it can be used in breeding rooms up to 150,000 gal (In preparation).

The main disadvantage is preparation, which takes between 10-20 minutes. Disinfectant replacements should be performed 2 to 3 times per week if used daily.

Summary & Future plans

These results confirmed the adequate decontamination of both rooms. Furthermore, **no residues or corrosion** of instrumentation, electronic devices or experimental equipment were observed. Gas phase nucleic acid digestion method is effective for **complete DNA degradation**, and therefore for disinfection of COVID-19 (SARS-CoV-2) research laboratories and is expected to become a next generation sterilization solution. **Biovector®** system was introduced in Nature index (28 Oct. 2021).

Sealive, Inc.
https://www.sealive.co.jp/sterix