Inactivation of Endotoxins at low temperature condition utilizing innovative gas phase sterilization system.

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Background

・We have developed innovative technology as gas phase sterilization system based on a catalytic reaction mechanism by use of methanol to generate mixed biogas (MBG), exhibiting remarkable performance of nucleic acid decomposition as well as sterilization.
(18th World Sterilization Congress at Bonn, Patent No. 5463378)
・Endotoxin is the continuing concern in the manufacturing and quality control of medical products.

Aim

There are limitations to the effective approaches available to inactivate bacterial endotoxin, especially **at low temperature**.

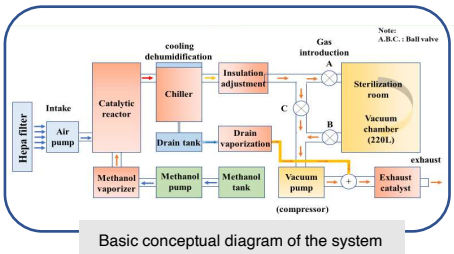
Evaluation challenge of additional function advantage for inactivation of endotoxins at low temperature

Conclusion

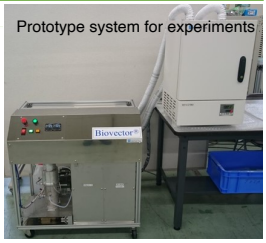
Newly developed innovative gas phase sterilization system revealed to exhibit remarkable effect of inactivation of endotoxin at low temperature condition (Patent PCT/JP2019/29834). It still require practical development aimed for medical devices.

Summary

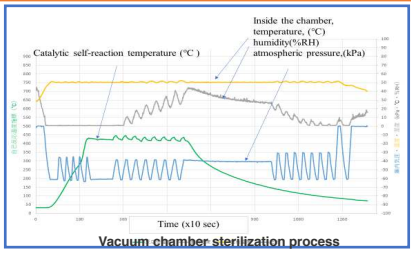
1) The mechanism of action of the system has been clarified. These two major effectors molecules work cooperatively to degradate nucleic acids in the synergistic greater than additive manner.
2) MBG generated by the system proved to have remarkable endotoxin inactivation effect, reaching at 3Log reduction even at low temperature condition under optimized condition.
3) The system exhibit equivalent endotoxin inactivation efficacy of gamma-rays.



Basic conceptual diagram of the system

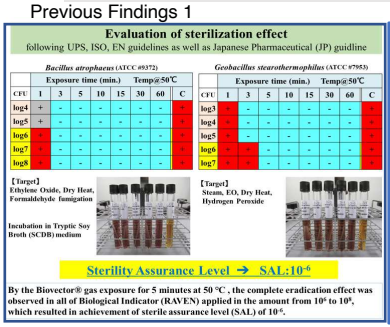


The outline of the core technology is to produce an optimal sterilization gas by controlling the oxidation-reduction reaction between copper oxide and methanol using copper as a catalyst.
This chemical reaction produces formaldehyde and water molecules. When formaldehyde is further oxidized, formic acid is produced. Formic acid is broken down into carbon dioxide and water when further oxidized. By controlling this chemical reaction, it becomes a sterilized gas containing methanol, formaldehyde, formic acid and moisture.
The catalyst's chemical reaction is regulated by the amount of methanol and air supplied.

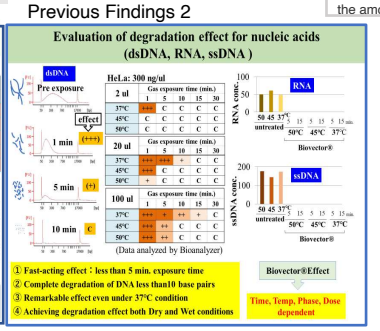


Vacuum chamber sterilization process

Previous Findings 1

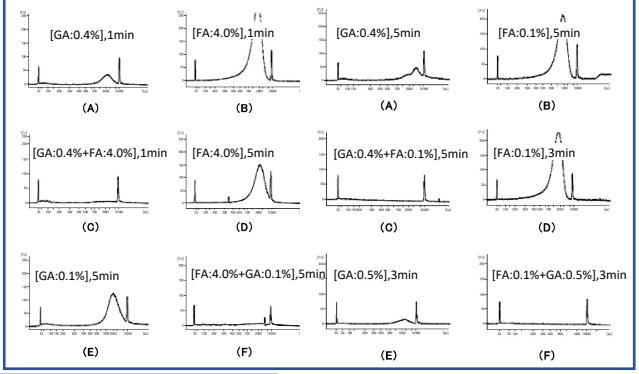


Previous Findings 2



Mechanism of action (MOA)

These two major effectors molecules work cooperatively to degradate nucleic acids in the synergistic greater than additive manner. GA:Formic Acid, FA: Folmaldehyde

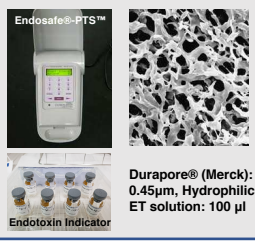


Molecular substance	Method	
Formaldehyde	InertSep mini AERO DNPH active capture➡HPLC (SPD-10, Shimazu)	2L/min, 10min. in 20L bag
Formic acid	HPLC (SPD-10, Shimazu)	2L/min, 10min. in 10ml water
Methanol	GC (GC-14B, Shimazu)	1L vaccume tube x5, 10min.

Composition (ppm)	MeOH (cc)			
	1.8	3.2	4.6	7.4
Methanol	11,000	22,000	33,000	55,000
Formaldehyde	200	180	160	120
Formic acid	25	58	91	157

Method

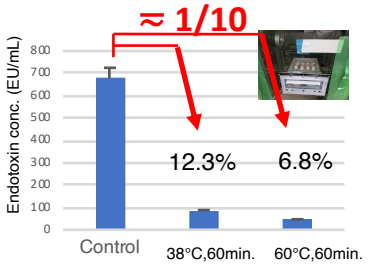
Endotoxin Indicator 2,000EU (Charles River) Limulus ES-IIplus CS Single Test Wako (0.01-1) Endosafe-®PTS™ Cartridge FDA (0.05-5.0) Measurement equipment: Endosafe®-PTS™



Durapore® (Merck): 0.45μm, Hydrophilic ET solution: 100 μl

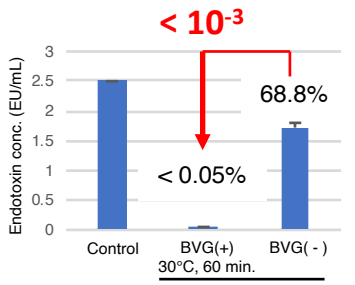
The United States Pharmacopeia suggests depyrogenation should reduce an Endotoxin Indicator by at least 1,000 fold (3-Log reduction) in endotoxic activity as measured by Limulus Ambocyte Lysate method.

Endotoxin inactivation effect by MBG
Sample:Endotoxin Indicator 2K bottle



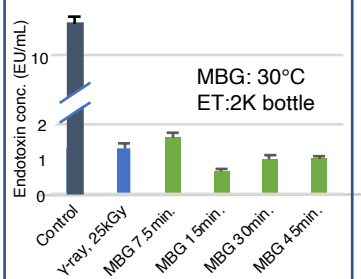
Endotoxin Indicator exhibited 1/10 reduction after MBG exposure even at low temperature. A thick layer sediment of dry endotoxin in the bottle might affect the efficacy.

Endotoxin inactivation effect by MBG
Sample preparation by Filter membrane



Preparation of thin layer of endotoxin by filter membrane exhibit remarkable effect of endotoxin inactivation, reaching 3 Log reduction !

Endotoxin inactivation effect:
MBG vs. γ-Ray



Comparative challenge test showed MBG has equivalent effect as γ-ray (25 kGy).