

Synoptis of BSC decontamination (6log sporicidal)

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Objective

- Objective is to validate decontamination of biosafety cabinets by hydrogen peroxide (H₂O₂) with biological indicator for sterilization efficiency.
- Biosafety cabinets should be decontaminated under the following conditions :
 - ✓ Accessing the contaminated plenum (to change the filters, blowers etc.),
 - ✓ Relocation of the cabinet from one room / building to another,
 - ✓ When the type of micro-organisms is drastically changed,
 - ✓ After a serious spill of dangerous micro-organism,
 - ✓ When there is product contamination contributed to the cabinet,
 - ✓ Periodically, especially if BSL-3 organisms are being manipulated inside the cabinet.

Material & Equipment

Item	Specification	remarks
Diffuser	Phileas 20D	DEVEA/ Flow rate : 450ml/h
Disinfectant	O2SAFE	Dosage : 6,54% (H ₂ O ₂)
Biological Indicator	Apex Biological Indicator for Gaseous Hydrogen Peroxide <i>Geobacillus Stearothermophilus 12980</i>	2,3*10 ⁶ CFU per stainless steel carrier
Culture media	Tryptic soy Broth Cat : 77-04-TSB-BP16	
BSC	Class II Type A2, 4ft Size : 138*156*79,2 cm	Estimated Volume 1,7m ³ ; Real V. 1,4m ³

BI locations

6 BI have been positioned in the BSC (locations previously determined to be the most difficult to sterilize) before the diffusion :

N° BI	BI locations
1 & 2	On top of the exhaust filter (corner & center)
3 & 4	Under surface of supply filter (corner & center)
5	on the left side
6	on the floor in a corner of the working plate

Program of generator

N°	Mode	Set value
1	P Performance cycle	06 (6 cycles)
2	R Delay	0:07 (7 minutes)
3	D Diffusion time	02:30 (2min 30s)

Decontamination of BSC

Phasis of protocol :

1. Place generator on the working place
2. Seal the BSC with plastic sheet
3. Close the BSC'sash and put it into night smart mode (low speed mode)
4. Conduct the diffusion and aeration (cycles)
5. After 2h, remove indicators and deliver them to the laboratory for sterility testing

Estimated volume of disinfectant :

$$2,5 \text{ (min)} * 7,5 \text{ (ml/min)} * 6 \text{ (cycles)} = 112,5 \text{ ml}$$

Estimated H₂O₂ concentration (in case of Vol =1,7m³) : d :m/V

$$112,5 \text{ (ml)} * 6,54 \text{ (%) } * 1,1 \text{ (g/ml;gravity)} / 1700 \text{ (m}^3\text{)} = 4,76 \text{ mg/L}$$

Decontamination cycle :

N°	Cycle	Time (hh:mm)	Remarks
1	Dehumidification (Drying)		
2	H ₂ O ₂ diffusion	00:57	H ₂ O ₂ concentration : <4,76mg/L
3	Aération (circulation)	01:03	
4	Ventilation	00:00	
TOTAL		02:00	

Results

Day	+ control	- control	N°1	N°2	N°3	N°4	N°5	N°6
Day 0	+		-	-	-	-	-	-
Day 2	+		-	-	-	-	-	-
Day 5	+		-	-	-	-	-	-

Incubate at 55-60°C

If the spores survive the sterilization cycle, the culture medium will turn yellow (positive) . If the spores have been killed, the culture medium will remain the purple color (Negative)

Conclusions

The Phileas 20D hydrogen peroxide generator is an effective decontamination instrument for Biosafety cabinets.

Biosafety cabinets are compatible with hydrogen peroxide fogging.