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DECONTAMINATION OF ESCO CLASS II BIOSAFETY CABINET USING BIOQUELL EBDS HYDROGEN PEROXIDE VAPORIZER

by
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The Need for Decontamination

Biological safety cabinets should be decontaminated under the following conditions:
1. Accessing the contaminated plenum, to change the filters, blowers, etc.
2. Relocation of the cabinet from one room / building to another
3. When the type of micro-organisms is drastically changed
4. After a serious spill of a dangerous micro-organism
5. When there is product contamination contributed to the cabinet
6. Periodically, especially if BSL-3 or BSL-4 organisms are being manipulated inside the cabinet

Formalin / Paraformaldehyde Decontamination

Typically the decontamination is performed using formalin gas by either vaporizing 37% formalin solution or by depolymerization of solid paraformaldehyde, with concentration of > 8000 ppm.

Despite its widespread usage for decontamination, formalin presents the following health risks:
1. External contact can cause irritation to skin, eyes, and mucous membranes.
2. Inhalation in small concentrations can cause coughing, nausea, and diarrhoea.
3. Inhalation in large concentrations can cause convulsions, coma, and death.
4. Long term exposure can cause cancer.

Although the Permissible Exposure Level (PEL) for formalin is 0.75 ppm, many scientists believe that there is no safe level of carcinogen exposure to humans. Therefore, typically the room must be evacuated when the decontamination process is performed, which leads to lab down time.

The use of formalin decontamination also has other disadvantages:
1. The process is time-consuming.
2. The certifier needs to pulse the cabinet fan to circulate the formalin vapor. This can dislodge the tape holding the plastic sheet covering the exhaust filter.
3. Due to excessive residue extensive cleaning must be done after decontamination and before use.

The formalin decontamination process can require an extended period of time as outlined below:

#	Process	Time
1	Set-up & sealing the cabinet to make it air tight	1 hour
2	Formalin vaporization	½ hour
3	Formalin contact time to obtain target log of 4-6 kill	8 – 10 hours
4	Ammonia vaporization to neutralize formalin	½ hour
5	Ammonia contact time to neutralize formalin	2 hours
6	Exhausting the ammonia residue	1 hour
7	Tear-down & cleaning the (substantial) residue	1 hour
	TOTAL without ammonia neutralization	**10 ½ – 12 ½ hours**
	TOTAL with ammonia neutralization	**14 – 17 hours**

Due to the adverse health effect of formalin gas, its use has been banned in Germany, Austria, and Switzerland. Other European countries are expected to follow suit. Two primary candidates to replace formalin decontamination are chlorine dioxide gas and hydrogen peroxide vapor.

Chlorine Dioxide Decontamination

Chlorine dioxide decontamination is performed by injecting chlorine gas (Cl_2) into a cylinder filled with solid sodium chlorite ($NaClO_2$), which generates the greenish-yellow chloride dioxide gas (ClO_2).Chlorine dioxide

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decontamination is much faster than formalin. Being a true gas, it spreads quickly, without the need of pulsing the cabinet's blower. It can rapidly kill the micro-organisms with high efficacy with just 1 hour contact time. There is minimal residue to clean after the decontamination making the entire process much faster than formalin decontamination. The time required for the entire process of chlorine dioxide decontamination is as follows:

#	Process	Time
1	Set-up & sealing the cabinet to make it air tight	1 hour
2	Chlorine dioxide gassing	½ hour
3	Chlorine dioxide contact time	1 hour
4	Chlorine dioxide "scrubbing"	½ hour
5	Tear-down & cleaning the (minimal) residue	½ hour
	TOTAL	**3 ½ hours**

Chlorine dioxide has the Permissible Exposure Level (PEL) of 0.1 ppm, compared to 0.75 ppm for formalin. In both processes airtight cabinet sealing is required to protect personnel from the gas exposure.

D. Hydrogen Peroxide Decontamination

Hydrogen peroxide (H_2O_2) decontamination is performed by flash vaporization of an aqueous peroxide mixture, creating a vapor that is distributed throughout the inside the Biosafety cabinet.

Steris and Bioquell are two major vendors of hydrogen peroxide generators. There are significant differences in operating principles.

The Steris principle is to avoid condensation on surfaces to minimize corrosion and optimize vapor distribution. The relative humidity inside the cabinet must be lowered to 30% so that the remaining 70% relative humidity can be occupied by the hydrogen peroxide vapor.

The Bioquell principle is to seek micro-condensation to achieve the kill. The generator releases high-speed droplets inside the cabinet.

Hydrogen peroxide vapor is non-carcinogenic, but highly effective against micro-organisms. Hydrogen peroxide (H_2O_2) vapor breaks down under catalytic action to become air and water, making it environmentally friendly and it leaves no residues. The decontamination process is as fast as chlorine dioxide if the cabinet is ducted. However, if the cabinet is not ducted, the hydrogen peroxide must be aerated, which is time-consuming.

The time needed for the entire process is outlined below:

#	Process	Time
1	Set-up & sealing the cabinet to make it semi-airtight	½ hour
2	Conditioning and decontamination cycle	1 ½ hour
3	Ducting out H_2O_2 | H_2O_2 generator doing aeration	½ hour | 4 hours
4	Tear-down	½ hour
	TOTAL	**3 hours | 6 hours**

For hydrogen peroxide decontamination, the biosafety cabinets need to be equipped with two ports:
1. One port located in front opening or side wall, penetrating the work zone area
2. One port located on top of the exhaust filter.

The generator used defines the port function as described below:

	Steris	Bioquell
Hydrogen peroxide source	Injected into the cabinet	Generated inside the cabinet
Bottom front / side port	Hydrogen peroxide introduction	Hydrogen peroxide re-introduction
Top port	Hydrogen peroxide extraction	Hydrogen peroxide extraction

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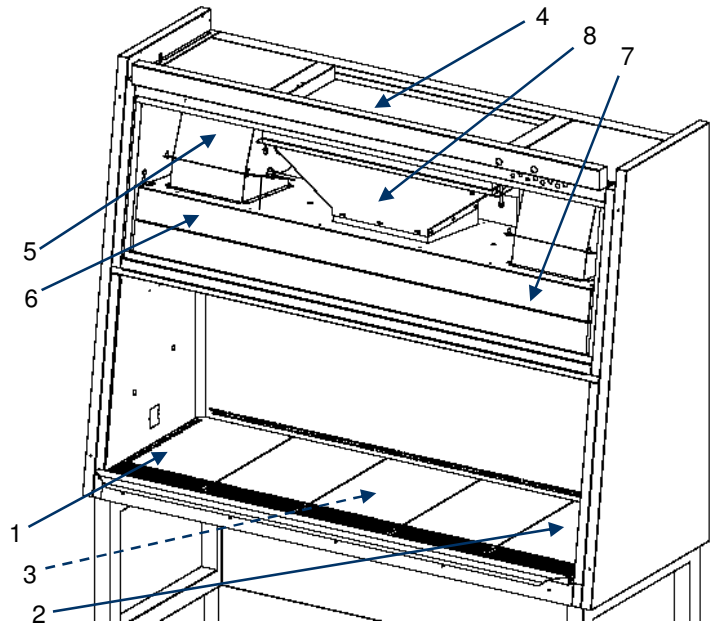
No	Aspect	Formalin Vapor	Chlorine Dioxide	Hydrogen Peroxide
1	Is it carcinogenic ?	Yes	No	No
2	Is it a genotoxin ?	Yes	No	No
3	Permissible Exposure Level (PEL)	0.75 ppm	0.1 ppm	1 ppm
4	Immediately Damaging to Life & Health (IDLH)	2 ppm	5 ppm	75 ppm
5	Sealing of the biosafety cabinet	Must be airtight	Must be airtight	Some small gaps are OK
6	Must people leave lab during the process ?	Yes, due to leakage danger	Yes, due to leakage danger	No, people can still work in lab
7	Is room humidity control required ?	Yes, above 60%	Yes, between 60 to 80 %	No
8	Residue	Substantial, needs extensive cleaning	Minimal, in the form of NaCl	No residue. Needs no cleaning at all.
9	Decontamination time per cabinet	11-17 hours	3-4 hours	3-10 hours
10	Equipment cost	USD $100	USD $1,500 + Cl gas canister	USD $18,000 to $52,000

Some people may argue that hydrogen peroxide vapor, being a non-true gas, may not reach and decontaminate all the corners inside the biosafety cabinet. They are also concerned that hydrogen peroxide being a strong oxidizer, may corrode various metals or components used in the cabinet. To determine if these concerns were valid the following experiment was conducted.

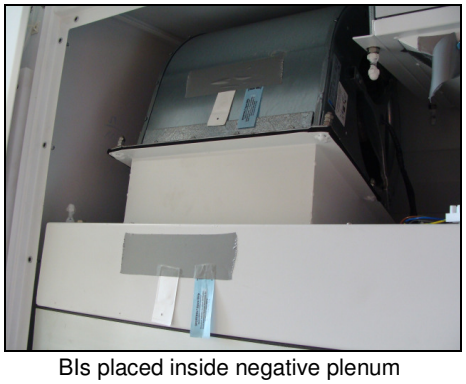
Validating Hydrogen Peroxide Decontamination Efficacy on ESCO Class II Biosafety Cabinet

To validate the hydrogen peroxide effectiveness on ESCO Biosafety cabinet, an experiment was conducted using one of the largest Esco cabinets, an Esco Infinity Class II Type A2 – 6ft cabinet (FC2-6ft). The test was performed at Camfil-Farr facilities in Brisbane, Australia.

A Bioquell EBDS was used as the hydrogen peroxide generator. To validate the decontamination efficacy, Biological Indicators (BI) containing $1.4x10^6$ *geobacillus stearothermophilus* spores, which is one of the most hard-to-kill spores, were placed at 8 locations as shown below:



No	BI Location
1	Work Tray: front left
2	Work Tray: back right
3	Drain pan (underneath work tray)
4	Above exhaust filter
5	Negative plenum: on left blower
6	Negative plenum: left side
7	Negative plenum: right side
8	Positive plenum: inside plenum



BIs placed inside negative plenum

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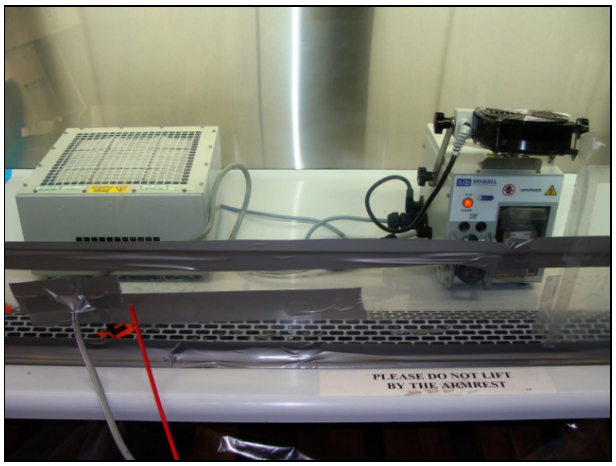
The Bioquell EBDS system consists of 3 units: the generator, the internal catalyst, and the external catalyst. The hydrogen peroxide generator and internal catalyst are placed on the work tray. Both are connected via cable to the control panel located outside the cabinet, placed on top of the external catalyst. A clear Perspex panel with two holes drilled on it was used to seal the cabinet front aperture while providing the holes for the cable and the pressure sensor tube. Future FC2 cabinets will have an extra ø 38 mm hole on the side wall for this cable and tube, so the sash window can be completely closed down to seal the front opening.

During the **decontamination (gassing) cycle**, the generator evaporates the liquid hydrogen peroxide inside the work zone, while the internal catalyst is turned off. The external catalyst extracts the air & vapor from the top of cabinet through the 3" hose to create negative pressure inside the cabinet, bypasses the carbon filter inside the catalyst, then injects it back into the work zone via a 1" hose connected to the inlet port on the right side wall. This loop re-circulates the hydrogen peroxide vapor while maintaining negative pressure of below -10 Pa inside the cabinet. To improve the glass sealing to achieve -10 Pa, three pieces of cardboard were lodged between the left & right sash track cover and sash window, also between the blue panel and sash window. Cabinet fan is kept off during this cycle.

During the **aeration (neutralization) cycle**, the internal catalyst is turned on, to absorb the hydrogen peroxide vapor. This process is expedited by passing the re-circulating air & vapor through the carbon filter inside the external catalyst, that was bypassed during the decontamination cycle. Cabinet fan is kept off during this cycle.



Internal catalyst and HPV generator placed on tray. Control cable and pressure tube exits thru perspex.

Vapor flows through 3" hose from top of cabinet to external catalyst, then via 1" hose back into cabinet.

Based on the cabinet volume, below is the table indicating the suggested hydrogen peroxide solution to decontaminate Esco Class II biosafety cabinets, referring to the standard H_2O_2 bottle supplied by Bioquell:

Width (ft)	Actual Volume (ml)	"Set" Volume (ml)	Gassing Time	Aeration Time
3	50	65	45 min	2 hours 30 min
4	65	100	60 min	3 hours 20 min
5	100	155	80 min	4 hours 10 min
6	100	155	80 min	4 hours 10 min

Note: There is no bottle with volume of between 65 to 100 ml. For the 5ft cabinet, please use the 100 ml bottle.

Based on the table above, 100 ml solution was used. According to Bioquell manual, if we use 100 ml solution, then we should set the control panel to 100 ml, then the machine will be set to 60 minutes gassing cycle and 3 hours and 20 minute aeration cycle. However, based on practical experience verbally taught by Bioquell, it's advisable to increase the setting on the control panel to one step higher. Therefore, is 100 ml is actually used, we need to "set" the machine to 155 ml. The table above shows the corresponding gassing and aeration time if we "set" the machine to one step higher. To investigate the need to increase this setting one, three decontamination runs were performed at 155 ml setting following Bioquell's verbal suggestion, and one run was performed at 100 ml setting, following the manual

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Below is the table describing the time span and the required ambient condition:

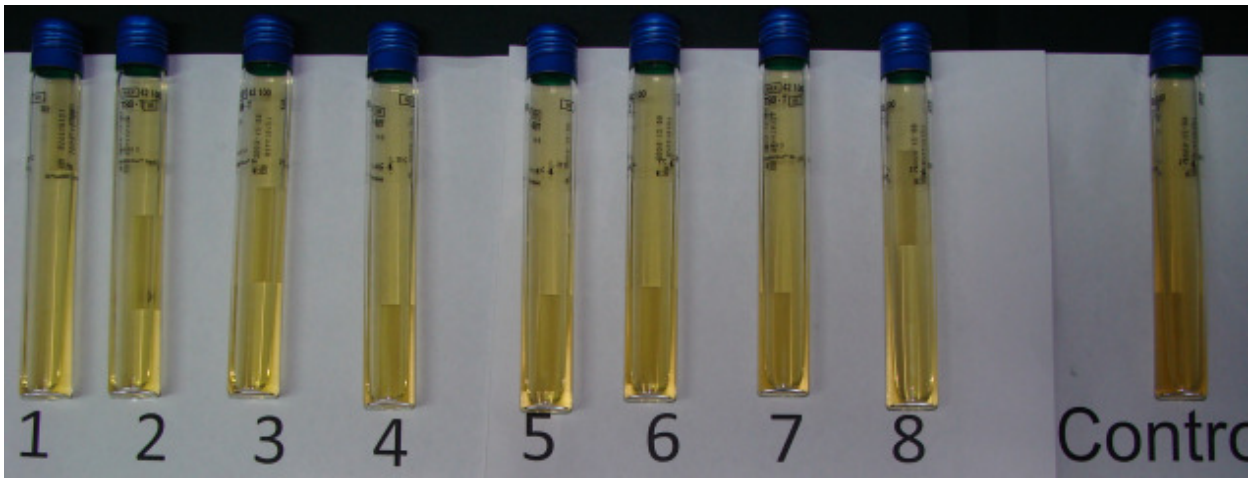
No	Process	Time
1	Set up	30 min
2	Decontamination	80 min
3	Aeration	4 hrs 10 min
4	Tear down	30 min
	TOTAL	**6 hrs 30 min**

Ambient Condition	Requirement
Temperature	15-35℃ (60-95℉)
Relative humidity	85% maximum

Note: If the ambient condition is outside this requirement, the decontamination will not be effective.

After the aeration process was finished, the tape that seals the cable hole on the Perspex is opened and a Dräger gas detection probe was inserted to measure the hydrogen peroxide concentration. Once the concentration is below 1ppm, the Perspex and sash window are opened, and the BIs are removed, and the cabinet blowers are turned on, to prove that it's not detrimentally affected by the hydrogen peroxide.

After the BIs are removed from the cabinet, they are individually immersed inside vials containing liquid soy broth, and incubated at 37℃ for 48 hours. After 48 hours, the results of the 1st Run at 155 ml are shown below:



The soy broth vials containing sterilized BIs remain clear, whereas spore growth in other vials is visible as clouds of microorganisms suspended in the liquid. The results of the four runs are summarized as below:

No	Spore Strip Location	155 ml Setting			100 ml Setting
		1st Run	2nd Run	3rd Run	1st Run
1	Work Tray: front left	No growth	No growth	No growth	No growth
2	Work Tray: back right	No growth	No growth	No growth	No growth
3	Drain pan (underneath work tray)	No growth	No growth	No growth	No growth
4	Above exhaust filter	No growth	No growth	No growth	**Some growth**
5	Negative plenum: on left blower	No growth	No growth	No growth	**Some growth**
6	Negative plenum: left side	No growth	No growth	No growth	No growth
7	Negative plenum: right side	No growth	No growth	No growth	No growth
8	Positive plenum: inside plenum	No growth	No growth	No growth	**Some growth**
X	Control	Full growth	Full growth	Full growth	Full growth

The cabinet is fully operational after each decontamination cycle, indicating that there is no adverse effect on the hydrogen peroxide vapor to the materials used on the cabinet. Additionally, there was no residue or vapor leftover after the decontamination process. The cabinet remained clean and dry after the process is finished, so there is absolutely no cleaning job needs to be performed by the certifier.

Conclusions

The Bioquell EBDS hydrogen peroxide generator is an effective decontamination instrument for ESCO Biosafety cabinets, if the setting is increased by one notch, following the verbal recommendation from Bioquell. Esco

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Biosafety cabinets are compatible with hydrogen peroxide vapor. The overall decontamination process of one of ESCO's largest biosafety cabinets using Bioquell EBDS takes about 6½ hours, which is much faster than formalin decontamination. There is also no residue to clean. In summary this translates to a safer and efficient process for the user, field certifiers, and the cabinet itself, and much shorter process time compared to formalin decontamination.

Acknowledgement

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