

VALIDATION STUDY FOR THE USE OF CHLORINE DIOXIDE GAS AS A DECONTAMINANT FOR BIOLOGICAL SAFETY CABINETS

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Validation Study for Biological Safety Cabinets (BSCs)

- ◎ BSCs used to simultaneously
 - protect biological material inside from external contamination
 - protect user and lab from biological material being manipulated
- ◎ HEPA filtration, blower(s), plenums
- ◎ Found in medical, veterinary, pharmaceutical, and biotech labs

BSC Decontamination

- Formaldehyde gas has been the standard method for >30 yrs
- National Sanitation Foundation holds the standard for BSC maintainance
- Until now, only formaldehyde considered “pre-validated”
- Study to confirm similar status to CD

NSF/ANSI 49 – 2002

Annex G

Recommended microbiological decontamination procedure

“Prior to decontamination with an alternative [note added: other than depolymerized paraformaldehyde] method (such as VHP), cycle parameters and validation of those parameters must be developed for each model and size of BSC.”

Alternative CD Methods – Fixed Quantity (Method 1)

- ⦿ A predetermined mass of CD created, dependent upon BSC volume
- ⦿ Similar concept to current use of paraformaldehyde
- ⦿ CD gas concentration not constant
- ⦿ While concentration monitored in study, would not be measured in typical field use.
- ⦿ Single exposure time for given CD/volume ratio

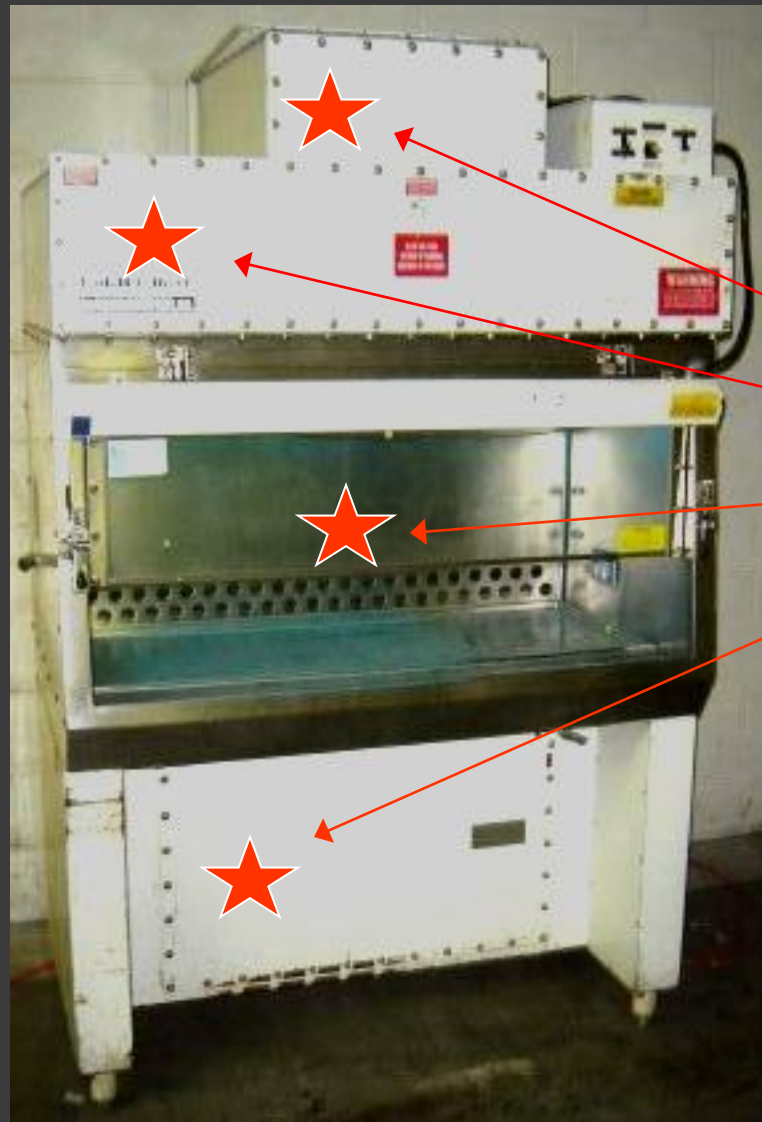
Alternative CD Methods – Fixed Concentration (Method 2)

- ⦿ Concentration of CD maintained at set value
- ⦿ Requires real-time concentration monitoring
- ⦿ Requires ability to compensate for CD loss
- ⦿ Concentration independent of cabinet size

NSF Biological Indicator Criteria (for each trial)

- ◎ 6 pairs of BIs

- 3 pairs in downstream side of exhaust HEPA filter (center and opposite corners)
- 1 pair within positive pressure plenum
- 1 pair beneath cabinet workspace
- 1 pair in center of upstream (dirty) side of down flow HEPA



Test BI Locations

BI Validation Criteria

Site for single trial

- Success = If either of 2 samples is negative
- Failure = Both samples test positive
- Statistical analysis:
 - Assume spore strip has 2.3×10^6 spores (typical)
 - Assume of 2 strips, 1 tests positive and 1 negative
→
 - 95% probability “true” efficacy at least 5.8 log reduction
 - 50% probability “true” efficacy at least 6.3 log reduction

BI Validation Criteria (cont.)

◎ Single Trial

- Pass = all 6 sites pass
- Conditional Pass = 5 sites pass
- Fail = 2 or more sites fail

◎ Cabinet/Method Validation

- At least 3 trials
- Pass = 3 trials pass
- Pass = 3 trials have conditional or full passes, with failed sites not coinciding
- Trials may be repeated if cause for failure identified

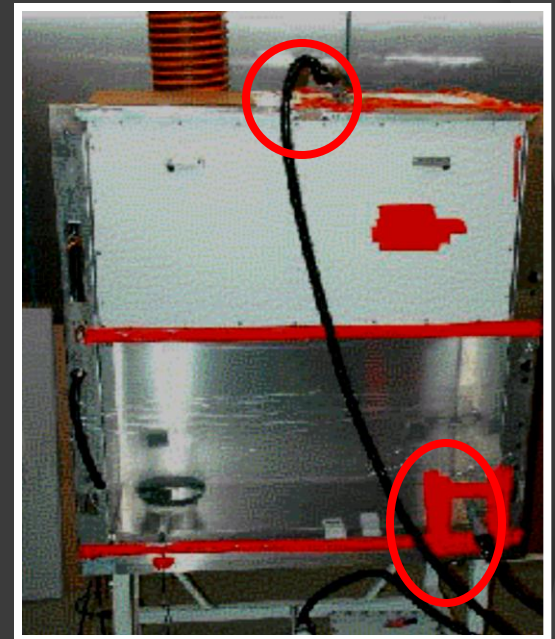
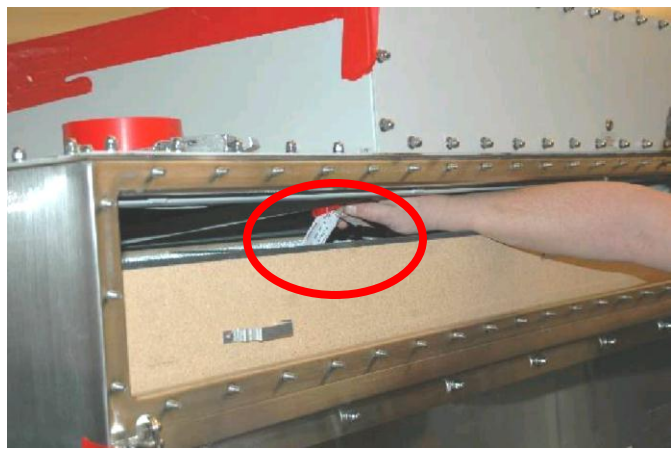
Validation Experiments

Method 1 (fixed mass of CD)

- ⦿ Experimenters – Micro-Clean
- ⦿ BSC Manufacturer – NuAire
- ⦿ Location – NuAire (Plymouth, MN)
- ⦿ Conditions:
 - 60 – 75% RH
 - CD amount - 0.1 g/ft³
 - Exposure time – 80 min

Method 1 Preparation

BI PLACEMENT

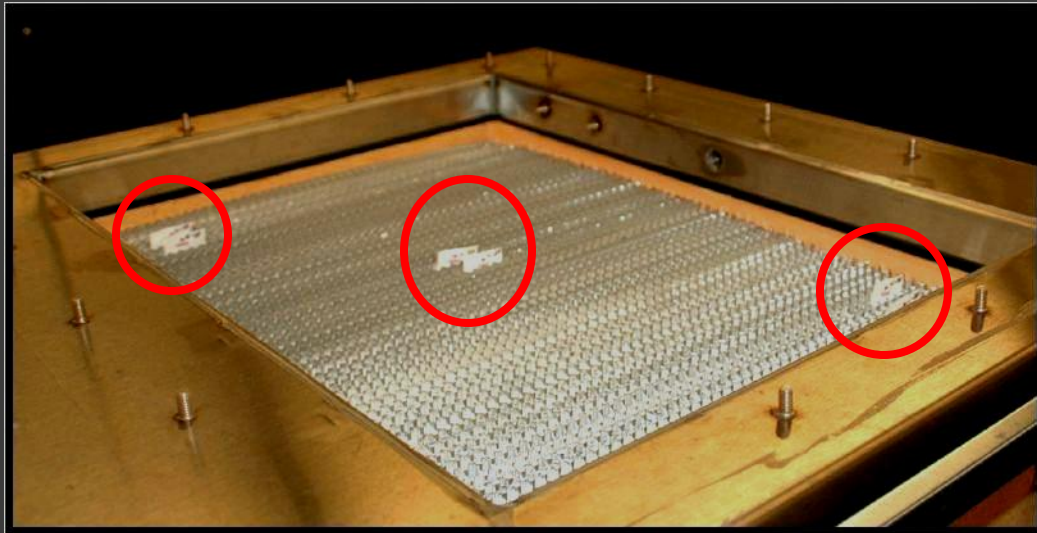


SEALED CABINET

LEAK TESTING

Method 1 - Preparation

BI PLACEMENT



BI RETRIEVAL



BI Analysis

REMOVAL OF BI FROM POUCH



TRANSFER TO THIOSULFATE



TRANSFER TO
GROWTH MEDIA

Results – Method 1

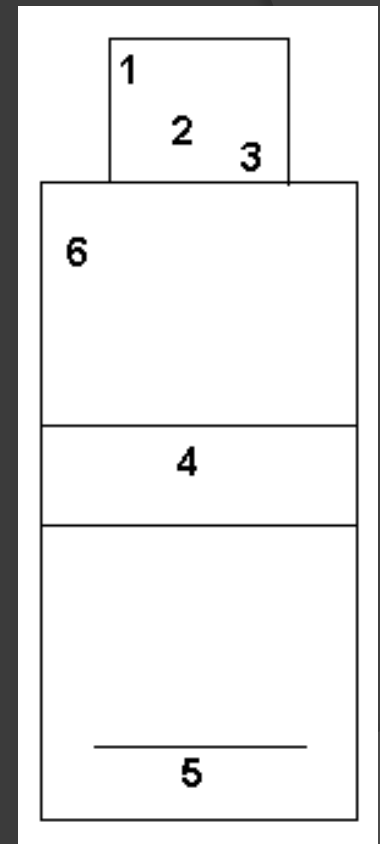
A2 -6' Console

A2 – 4' Bench Top

Trial #	1	3	5		2	4	6	13
Dose	3.1	3.9	3.1		3.5	3.1	3.5	3.9
Position								
1	2	1	1		1	1	0	0
2	2	1	1		2	1	0	0
3	1	0	2		2	0	0	0
4	1	1	1		1	0	0	0
5	1	2	0		0	0	0	0
6	1	1	0		0	0	1	0
	F	CP	CP		F	P	P	P

FAIL

PASS*



0, 1, 2 = # positive
Blis out of 2

Dose – mg-hr/L

Results – Method 1

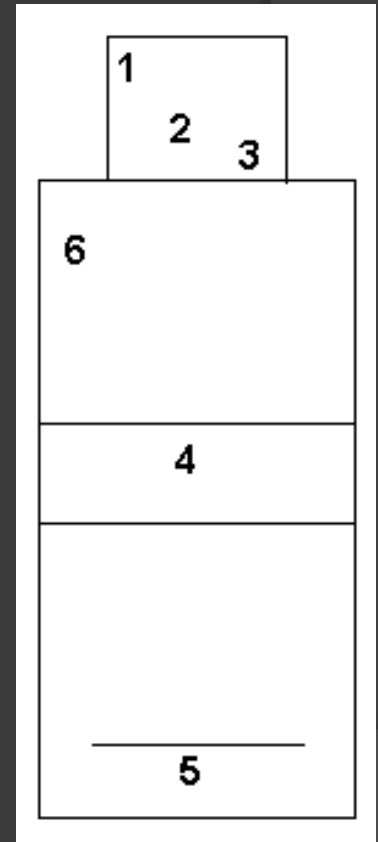
B1 -4' Console

B2 – 6' Console

Trial #	7	9	11		8	10	12
Dose	4.4	4.7	5.0		2.0	2.5	2.6
Position							
1	2	0	0		0	0	0
2	0	1	0		0	0	0
3	2	2	0		0	0	0
4	0	1	0		0	0	0
5	0	0	0		0	0	0
6	0	1	0		0	0	0
	F	CP	P		P	P	P

FAIL

PASS



0, 1, 2 = # positive
BIs out of 2

Method 1 – Issues

- ⦿ Likely BI handling problem through Run #7
 - Used forceps to transfer BIs from thiosulfate
 - Performed positive controls first
 - “Sterilized” forceps in alcohol between BIs
- ⦿ Repeated one run on A2-4’ to replace “bad” run – successful
- ⦿ Possible penetration issue for exhaust HEPA on B1 cabinet

Results – Method 1

Repeat Study

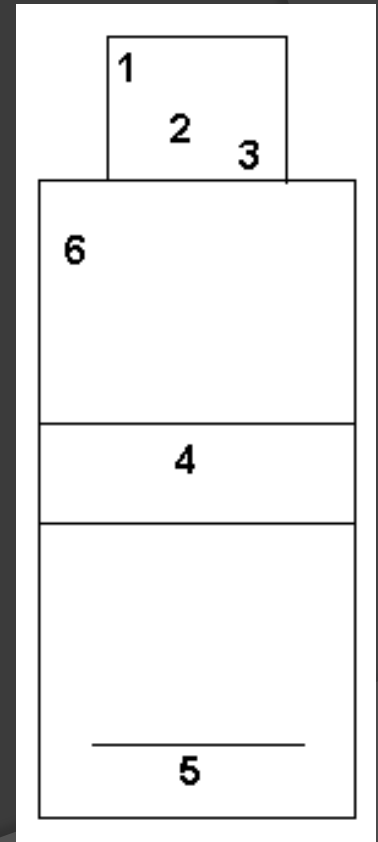
A2 -6' Console

B1 – 6' Console

Trial #	15	17	19		14	16	18	20
Dose	3.0	3.7	3.7		2.6	3.7	3.3	3.3
Position								
1	0	0	0		0	0	0	0
2	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	0	0		0	0	0	0
6	0	0	0		0	0	0	0
	P	P	P		P	P	P	P

PASS

PASS



0, 1, 2 = # positive
BIs out of 2

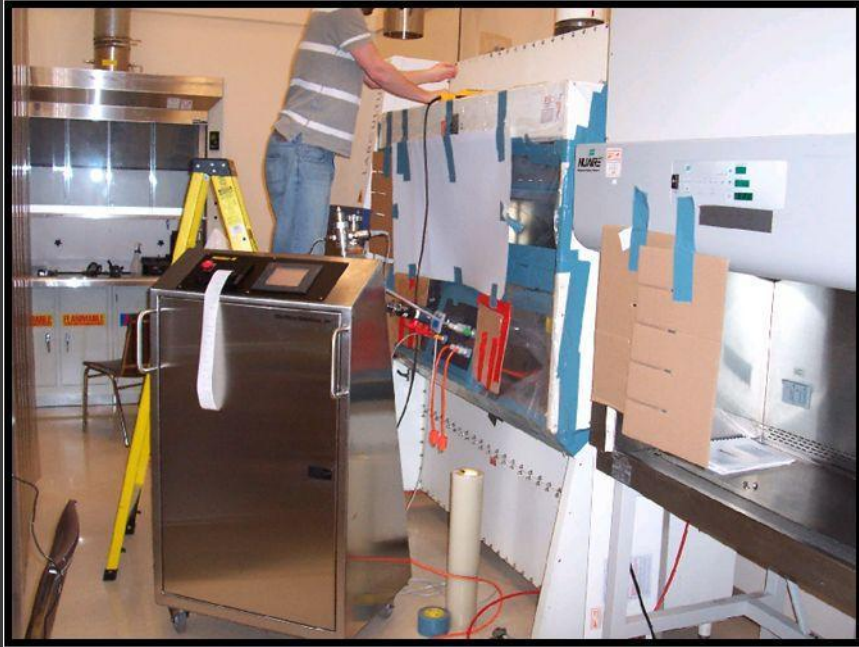
Dose – mg-hr/L

Validation Experiments

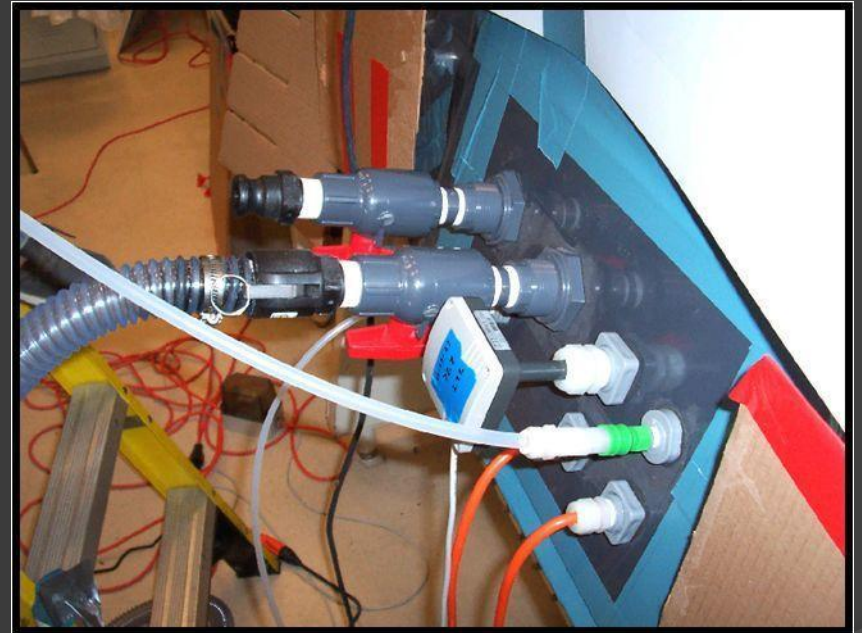
Method 2 (fixed concentration of CD)

- ⦿ Experimenters – ClorDiSys Solutions
- ⦿ BSC Manufacturer – Baker
- ⦿ Location – Micro-Clean (PA)
- ⦿ Conditions:
 - 70% RH
 - Two concentrations (24+ trials)
 - 4.8 mg/L for 40 min
 - 2.8 gm/L for 55 min

Method 2 - Preparation



PLACING BIs



GAS MANIFOLD

Results – Method 2

A1 -6' Console

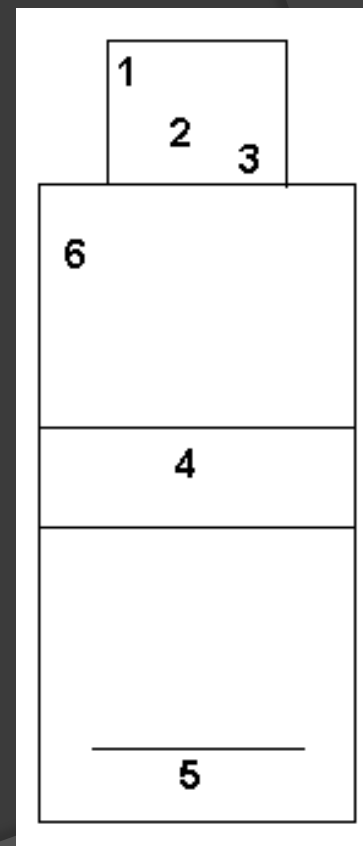
A2 – 4' Console

4.8 mg/L for 40 min

Trial #	1	6	7		2	5	15
Dose	3.2	3.2	3.2		3.2	3.2	3.2
Position							
1	0	0	0		0	0	0
2	1	0	0		0	0	0
3	0	0	0		0	0	0
4	0	0	0		0	0	0
5	0	0	0		0	0	0
6	0	0	0		0	0	0
	P	P	P		P	P	P

PASS

PASS



0, 1, 2 = # positive
Bls out of 2

Dose – mg-hr/L

Results – Method 2

4.8 mg/L for 40 min

B1 -6' Console

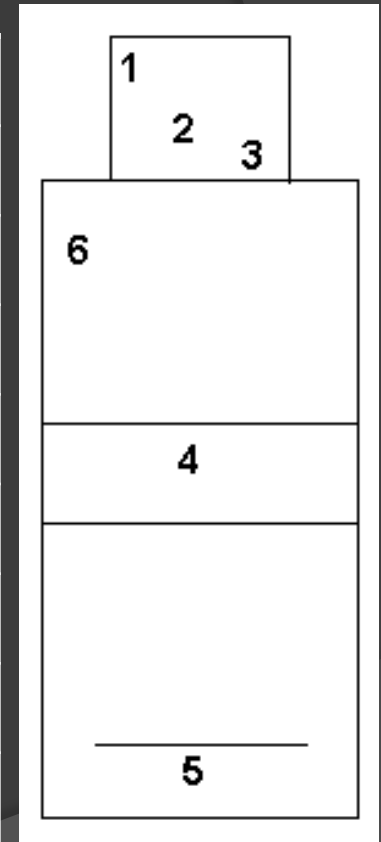
B2 – 4' Console

Trial #	3	4	10	11		12	13	14	16
Dose	3.2	3.2	3.2	3.2		3.2	3.2	3.2	3.2
Position									
1	0	0	0	0		2	0	1	0
2	1	0	1	0		1	0	0	0
3	0	0	0	0		2	0	0	0
4	0	0	0	0		1	0	0	1
5	0	0	0	0		0	0	0	0
6	0	ND	0	0		2	0	0	0
	P	P	P	P		F	P	P	P

PASS

PASS

0, 1, 2 = # positive
Blis out of 2



Results – Method 2

2.8 mg/L for 55 min

	A1				A2				B1				B2		
Trial #	8	9	27		23	24	29		17	18	19		18	20	21
Dose	2.8	2.8	2.8		2.8	2.8	2.8		2.8	2.8	2.8		2.8	2.8	2.8
Position															
1	0	0	0		0	0	0		0	0	0		0	0	0
2	0	0	0		0	0	0		0	0	0		0	0	0
3	0	0	0		0	1	0		0	0	0		0	0	0
4	0	0	0		0	0	0		0	0	0		0	0	0
5	0	0	0		0	0	1		1	0	0		0	0	0
6	0	0	0		0	0	0		0	0	0		0	0	0
	P	P	P		P	P	P		P	P	P		P	P	P
PASS					PASS				PASS				PASS		

Results

- ◎ Method 1 successfully validated on A2 console, A2 bench top, B1 and B2 cabinets
 - External circulation loop for A and B cabinets (from above exhaust HEPA to work space)
- ◎ Method 2 successfully validated on A1, A2, B1 and B2 cabinets
 - External circulation loop for B cabinets only

NSF Considerations

- ◎ NSF Task Force accepted results of validation study
- ◎ A revision to Annex G of NSF/ANSI 49 which includes chlorine dioxide gas as an alternative to formaldehyde and protocols for both methods has been drafted
 - Protocols use values of CD concentration and exposure time greater than used in validation

Acknowledgements

- Micro-Clean, Inc.
- Nu-Aire, Inc.