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NSF49 Certification of Gaseous Chlorine Dioxide for BSC Decontamination

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ICID International Centre for Infectious Diseases

ABSA CANADA

CREM Centre for Research on Environmental Microbiology
Designing a safer tomorrow

CRME Centre de recherche en microbiologie environnemental
Pour un futur plus sain

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Public Health Agency of Canada | Agence de santé publique du Canada

BSC Decontamination

- Formaldehyde gas has been the standard method for >30 yrs
- National Sanitation Foundation holds the standard for BSC maintenance
- Until now, only formaldehyde considered "pre-validated"
- Study to confirm similar status to chlorine dioxide (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)

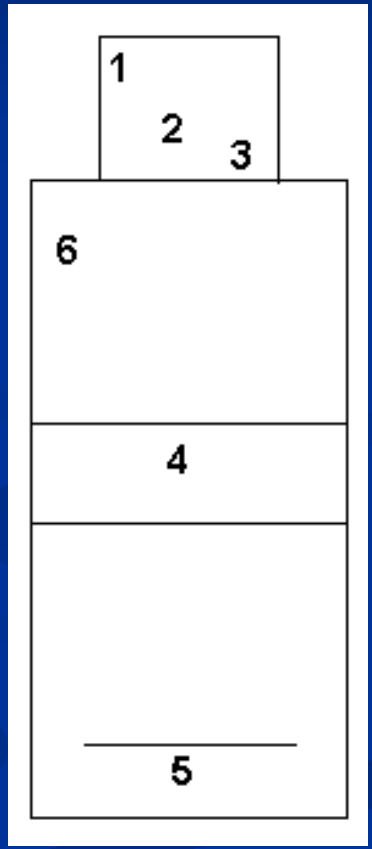
- A predetermined mass of CD created, dependent upon BSC volume
- Similar concept to current use of paraformaldehyde
- CD gas concentration not constant
- While concentration monitored in study, would not be measured in typical field use.
- Single exposure time for given CD/volume ratio

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
- Residual CD on BI's to be neutralized prior to culturing



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 kill
  - 50% probability "true" efficacy at least 6.3 log kill

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

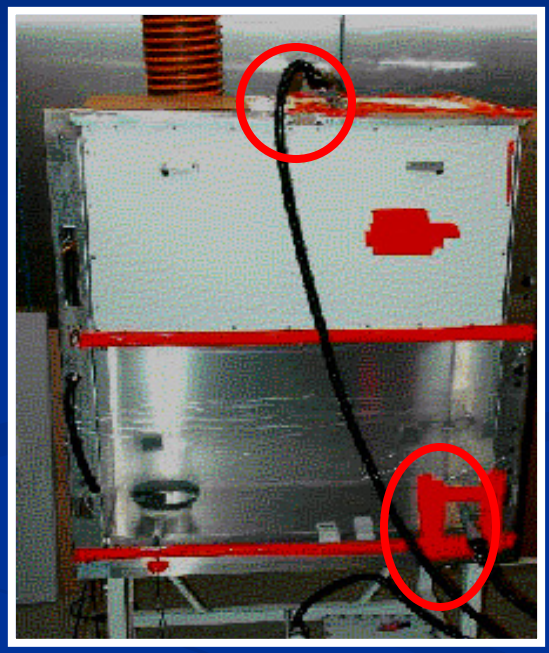
Validation Experiments

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/ft^3
  - Exposure time – 80 min

Method 1 Preparation

BI PLACEMENT

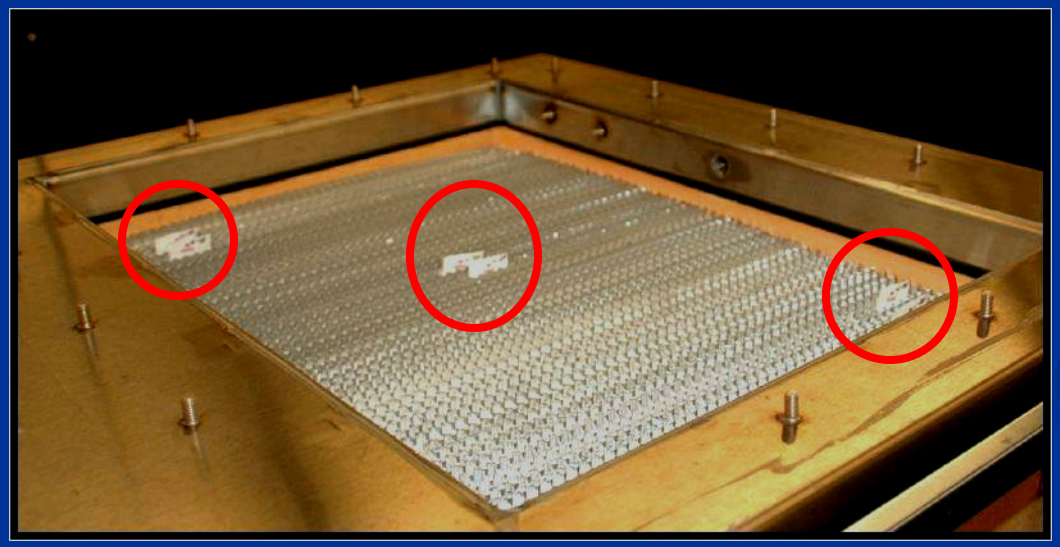


SEALED CABINET

LEAK TESTING

Method 1 - Preparation

BI PLACEMENT



BI RETRIEVAL

BI Analysis

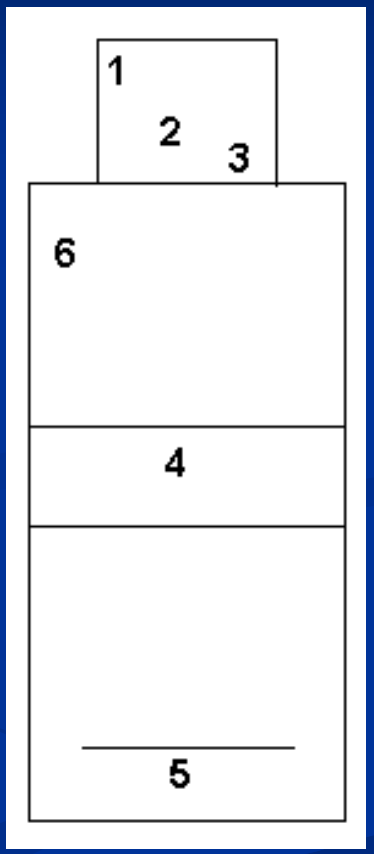
REMOVAL OF BI FROM POUCH

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 BIs out of 2

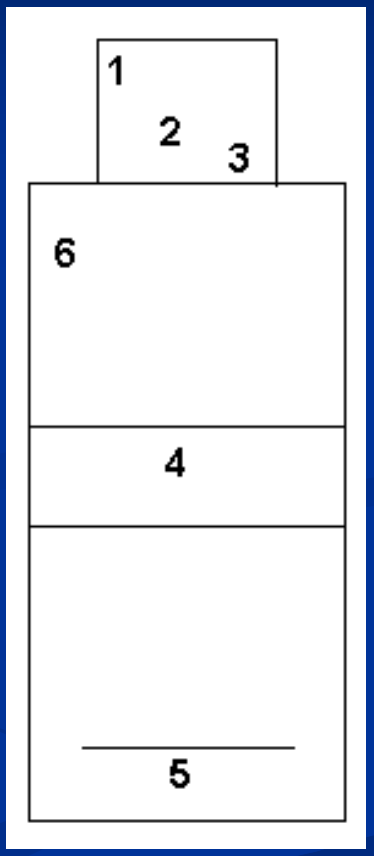
Dose – mg-hr/L

A2 Failure due to operator error. Alcohol is not sporicidal

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

B1 Failure due to operator error. Alcohol is not sporicidal

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

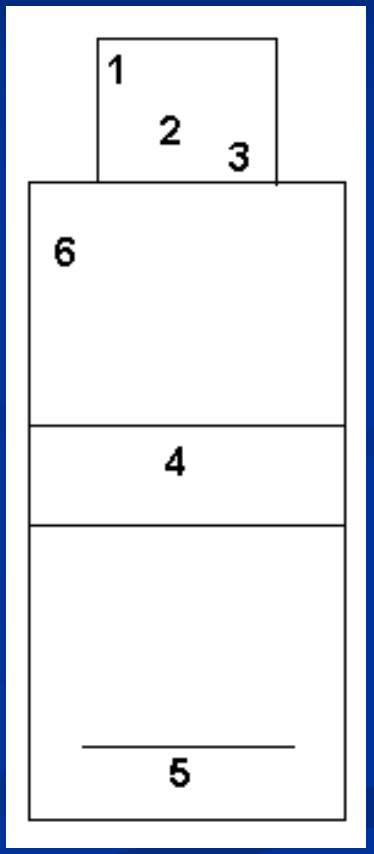
A2 & B1 Failure due to operator error. Alcohol is not sporicidal

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

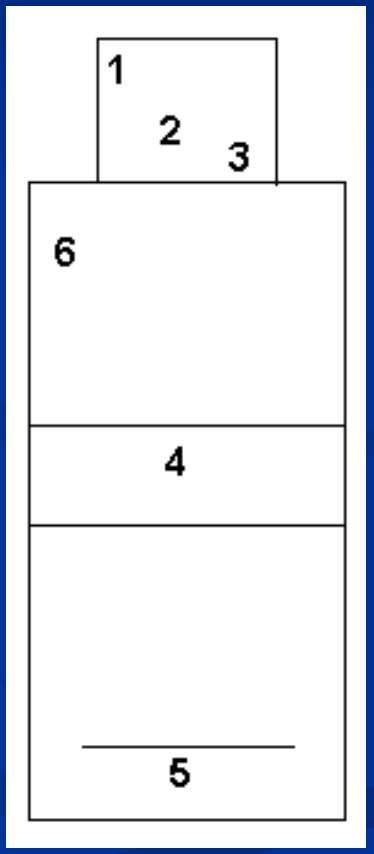
PLACING BIs

GAS MANIFOLD

Results – Method 2

4.8 mg/L for 40 min

	A1 -6' Console				A2 – 4' Console		
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		



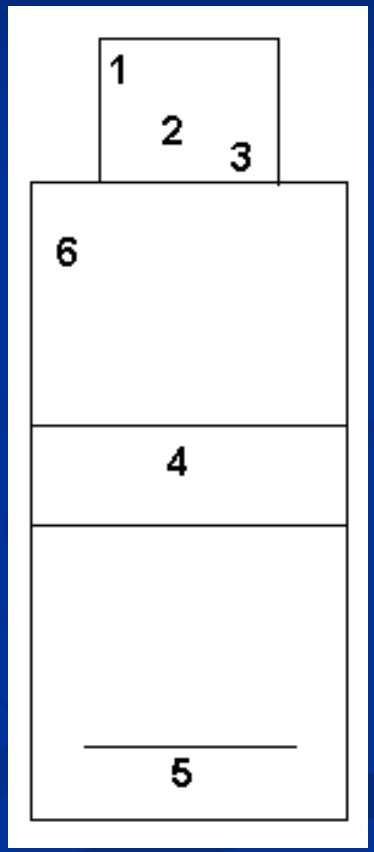
0, 1, 2 = # positive BIs out of 2

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 BIs out of 2

B2 Failure due to NO recirculation. External circulation added for B2 cabinets

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

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 accepted and available
  - Protocols use values of CD concentration and exposure time greater than used in validation

Annex G NSF / ANSI 49-2008

- Humidity 60-75%
- Method 1 Fixed Mass
  - 0.13 g / cu ft
- Method 2 Fixed Concentration
  - 3mg/L for 60 min
  - 5mg/L for 45 min
  - B Cabinets must have external circulation

Generators

Cloridox - Manual Single/Half
Method 1

Cloridox - Manual Double Pumper
Method 1

Mini Chlorine Dioxide System (MCS)
Method 1

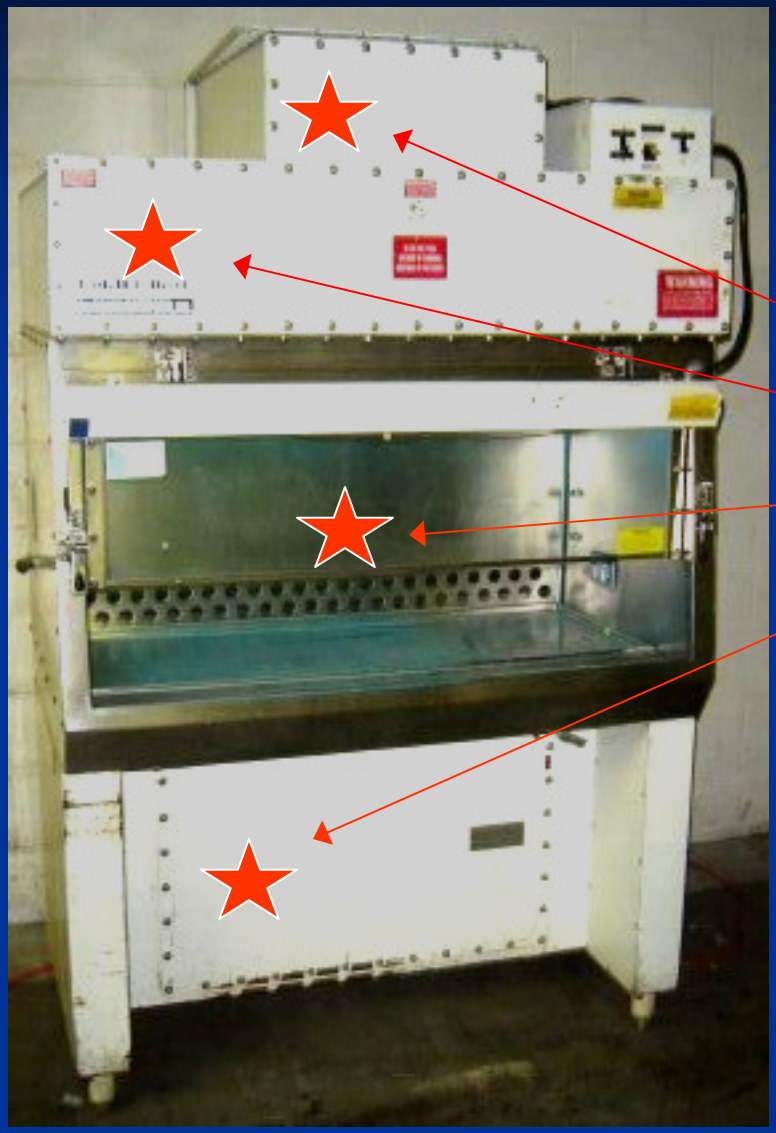
Minidox-M
Method 2

Chem-CD
Method 1

Cloridox-GMP
Method 2

Acknowledgements

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



Test BI Locations

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