

Validation and Utility of a Bleach Chemical Effluent Decontamination System (CEDS) for Biocontainment Laboratories

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Celebrating 50 Years

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1969 - 2019

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Disclaimer

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Outline

- Laboratory testing of bleach efficacy
- Process for Chemical Effluent Decontamination System (CEDS) Validation
- Validation Results
- Utility of CEDS for Biocontainment Laboratories
- Discussion

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Laboratory Testing

- Initial proof of concept work performed using dialysis tubing in bleach solution
- Tested at various concentrations/contact times and with and without organics
- Relied on *B. thuringiensis* (*Bt*) spores for efficacy



Laboratory Testing Procedure

Prepare the bleach
solution and add 5%
FBS



Prepare spore
inoculum of Bt (in
Tween solution)



**Heat spore
suspension at 65C
for 30 min**



Perform serial
dilution of spores on
blood agar

Prepare dialysis
tubing by pre-
wetting in PBS



Load 2 ml of
spore suspension

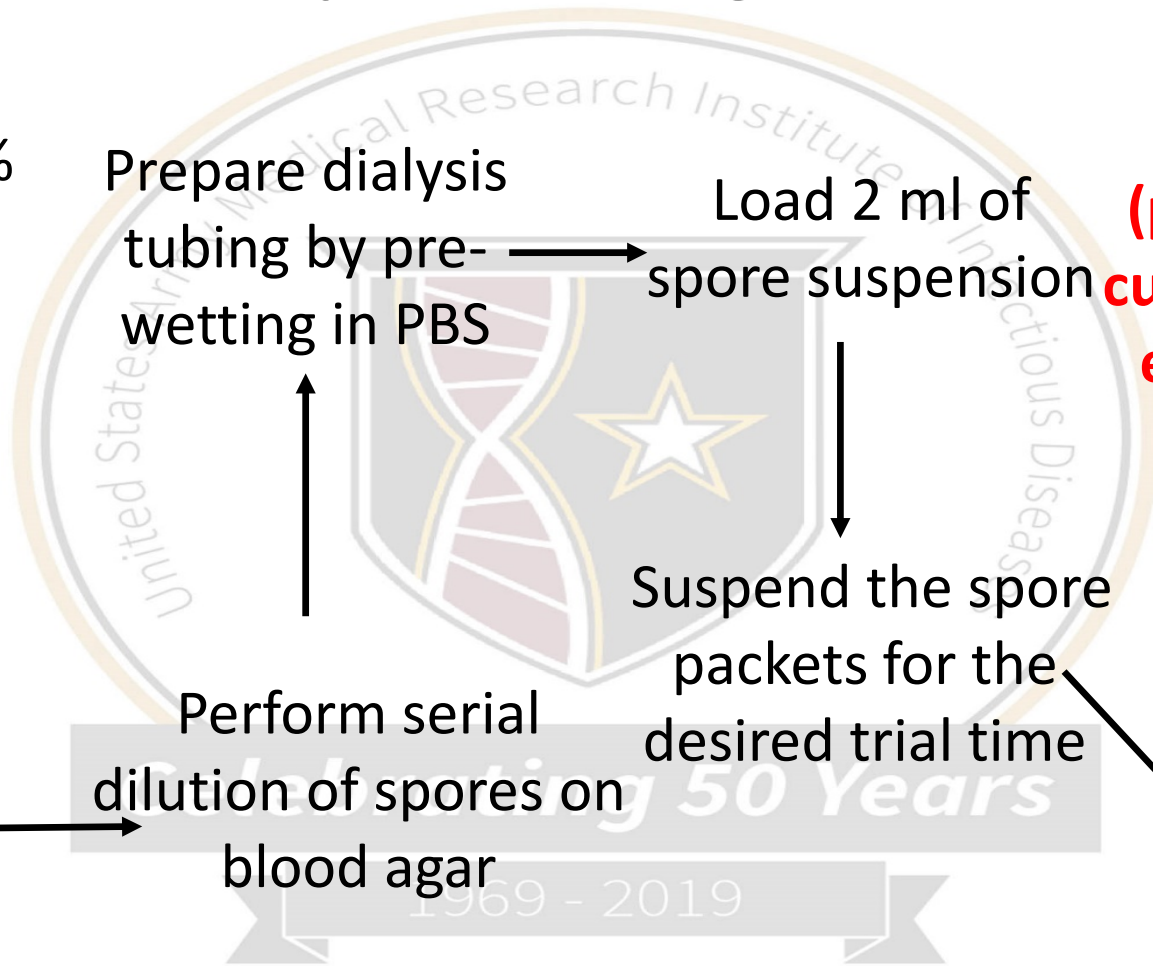


Suspend the spore
packets for the
desired trial time

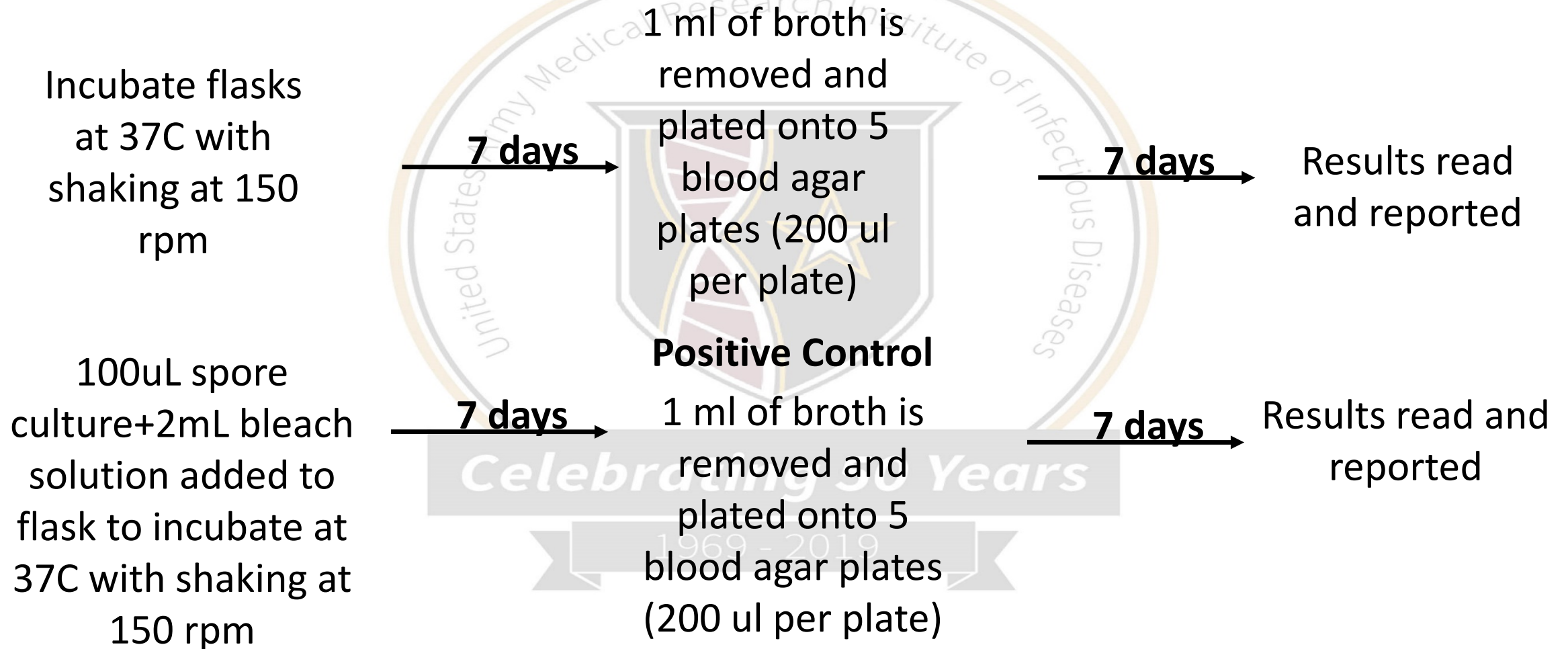
**With sterile scissors
(performed with 2 people)
cut the dialysis tubing at the
end with the alligator clip**



After desired contact
time, retrieve the
spore packet and
suspend over sterile
flask (1 liter flask with
500 ml Heart Infusion
Broth -HIB)



Laboratory Testing Procedure (continued)



Parameter tested ¹	total spore	time (h)	Interday variation	# total samples	pass	fail	failure rate ²
11,400 ppm	4x10 ⁵	2	no	3	3	0	0
11,400 ppm + 1% FBS	4x10 ⁵	2	no	3	3	0	0
5,700 ppm + 5% FBS	4.5-7.8 x10 ⁵	2	yes	9	9 ³	0	0
5,700 ppm + 5% FBS	3.6-4.5 x10 ⁵	1	yes	6	5	1	17%
5,700 ppm + 5% FBS	3.6-4.5 x10 ⁵	0.5	yes	6	3	3	50%
3,800 ppm + %5 FBS	3.6x10 ⁵	20	no	3	3 ⁴	0	0%
3,800 ppm + %5 FBS	3.6x10 ⁵	2	no	3	2	1	33%
3,800 ppm + %5 FBS	3.6x10 ⁵	1	no	3	0	3	100%
3,800 ppm + %5 FBS	3.6x10 ⁵	0.5	no	3	0	3	100%

¹ppm is based upon % free chlorine per each bleach source

²failure rates reflective of past work at USAMRIID and published literature

³expt pending: 2 independent trials running, sterile after 30 h; so if sterility maintained N=9 samples, 3 distinct bottles of bleach

⁴passed through day 4 final results pending

EDS Validation Process

Chemical



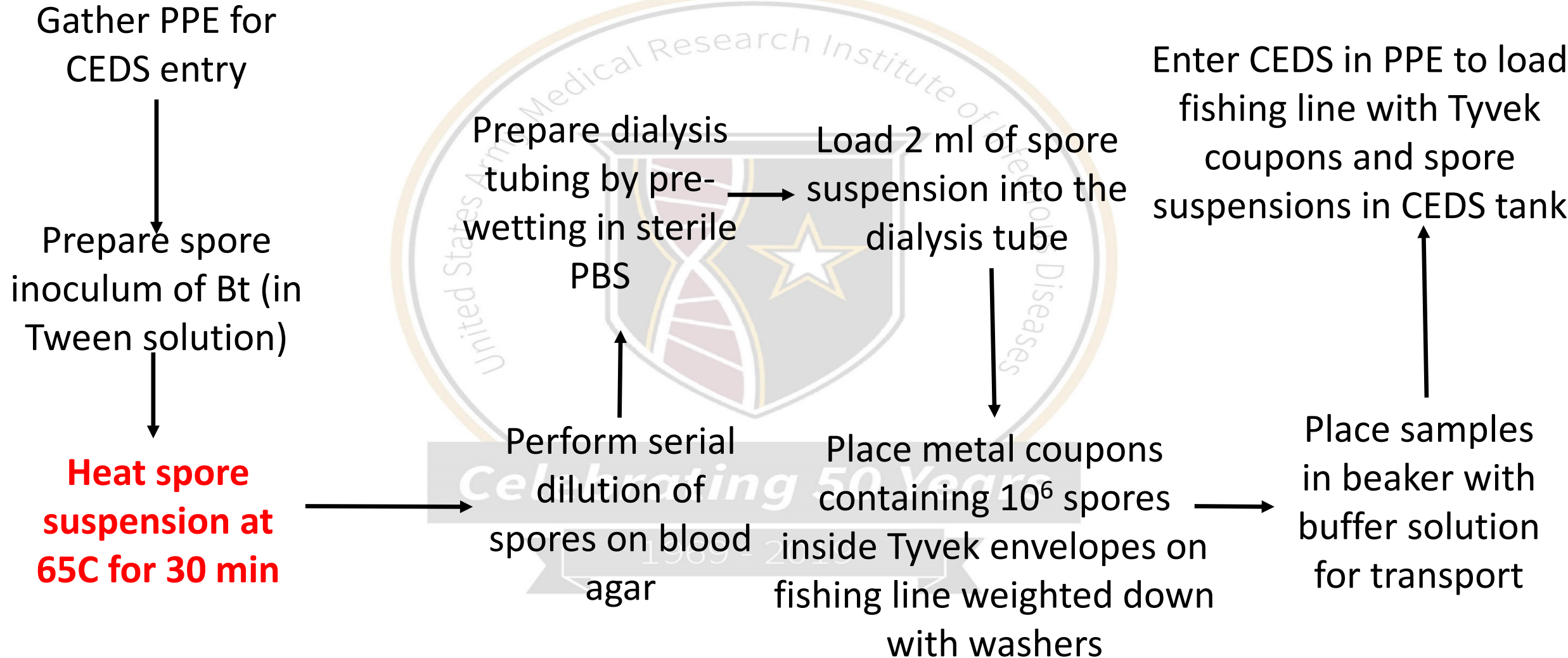
Thermal



Table 1. Free chlorine concentrations for consistency run testing/pre-validation

Sample	Free Chlorine (ppm) 0 min	Free Chlorine (ppm) 15 min	Free Chlorine (ppm) 120 min
Run A			
High	6600	6600	6400
Low	6800	6400	6400
Run B			
High	6600	6600	6400
Low	6600	6600	6600
Sample	Free Chlorine (ppm) 0 min	Free Chlorine (ppm) 15 min	Free Chlorine (ppm) 120 min
Run C			
High	6400	6400	6400
Low	6400	6400	6400
Run D			
High	6200	6400	6600
Low	6200	6400	6600
Run E			
High	6400	6400	6600
Low	6400	6400	6400
Run F			
High	6400	6200	6400
Low	6600	6400	6200
Run G			
High	6400	6800	6400
Low	6400	6600	6200
Run H			
High	6400	6600	6600
Low	6200	6400	6400
Run I			
High	6400	6400	6600
Low	6400	6600	6400
Run J			
High	6400	6400	6200
Low	6600	6600	6600

Biological Challenge Procedure



Biological Challenge Procedure Continued

After 2 hours, retrieve
the fishing line with
Tyvek coupons and
spore suspensions

Tyvek coupons to
microbiology core lab

Results read and
reported

Spore suspensions to
lab

**With sterile scissors (performed with 2
people) cut the dialysis tubing at the
end with the alligator clip**

Sample and positive controls
are incubated and analyzed in
same manner and laboratory
testing procedures

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Sample	Free Chlorine (ppm)
Run #1	
High	6400
Low	6600
Run #2	
High	6400
Low	6400
Run #3	
High	6800
Low	6600
Run #4	
High	6400
Low	6400
Run #5	

Sample	Free Chlorine (ppm)
Run #6	
High	6600
Low	6400

Biological Challenge Results: Tyvek Coupons

Sample	Broth Culture (72 hours)	Agar culture (72 hours)
Run #1-positive ctl	Positive	Positive
High	Negative	Negative
Middle	Negative	Negative
Low	Negative	Negative
Run #2-positive ctl	Positive	Positive
High	Negative*	Negative*
Middle	Negative	Negative
Low	Negative	Negative
Run #3-positive ctl	Positive	Positive
High	Negative	Negative
Middle	Negative	Negative
Low	Negative	Negative
Run #4-positive ctl	Positive	Positive
High	Negative	Negative
Middle	Negative	Negative
Low	Negative	Negative
Run #5-positive ctl	Positive	Positive
High	Negative	Negative
Middle	Negative	Negative
Low	No recovery	No recovery
Run #6-positive ctl	Positive	Positive
High	Positive**	Positive**
Middle	Negative	Negative
Low	Positive**	Positive**

**Indicates growth was seen in broth and agar but colonies were gram negative bacteria consistent with environmental contamination*

***Indicated growth was seen in broth and agar due to insufficient contact between bleach and spore coupons. It is likely that there was inadequate space for bleach solution to penetrate the Tyvek envelope preventing inactivation. This explanation is corroborated by the negative results from the USAMRIID-prepared spore packets from the same runs.*

Biological Challenge Results: Spore Suspensions

Sample	Broth Culture (7 day)	Optical Density (OD ₆₂₀)	Agar culture (7 day)
Run #1-positive ctl	Positive	0.78	Positive
High	Negative	0	Negative
Middle	Negative*	0.36*	Negative*
Low	Negative	0	Negative
Run #2-positive ctl	Positive	0.85	Positive
High	Negative	0	Negative
Middle	Negative	0	Negative
Low	Negative	0	Negative
Run #3-positive ctl	Positive	0.78	Positive
High	Negative	0	Negative
Middle	Negative	0	Negative
Low	Negative	0	Negative
Run #4-positive ctl	Positive	0.96	Positive
High	Negative	0	Negative
Middle	Negative	0	Negative
Low	Negative	0	Negative
Run #5-positive ctl	Positive	0.82	Positive
High	Negative	0	Negative
Middle	Negative	0	Negative
Low	Negative	0	Negative
Run #6-positive ctl	Positive	0.94	Positive
High	Negative	0	Negative
Middle	Negative	0	Negative
Low	Negative	0	Negative

*Culture was negative for *B. thuringiensis*, but was a pure culture of *B. atrophaeus*

Additional Considerations for CEDS application in Biocontainment Labs



- Discharge costs to neutralize or disposal of chemically treated effluent
- Additional PPE considerations during operations
- Difficulty of monitoring chemical concentrations
- Efficacy in presence of high organics
- CEDS units do not function properly unless system design permits complete drainage at end of each cycle
- Chemical procurement
- Transport of chemicals between storage and CEDS
- Extensive laboratory testing needed for validation

Types of Chemical Effluent Decontamination Systems

	Bleach	Chlorine Dioxide (CD)	Peroxyacetic Acid (PAA)
Affected by organics	Yes (trihalomethane generation)	No	No
Delivery	Concentrated liquid	Solid, liquid (through acid and sodium chlorite) or gas	Concentrated liquid
Neutralization required	Yes (both pH and dechlorination)	Yes (dechlorination only)	Yes (both PAA and pH)
Efficacy	6000ppm for 2h	1000ppm for 3h	1500ppm for 1h (est. by EPA)
Cost	\$5/gal in bulk for 12.5 % bleach	\$5/gal for sodium chlorite	

Discussion

- We have validated a bleach based CEDS using a minimum concentration of 6200ppm free chlorine and a two hour contact time
- Bleach procurement and disposal considerations make it very difficult to operate a bleach based CEDS over an extended period
- As a general rule, the lowest initial cost solution may not be the right one
- Extensive laboratory work is required both upfront and during any CEDS validation process
- Bleach based CEDS units only have limited utility in biocontainment without significant financial resources

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