Title: Final Report for Reduced Incubation Time Study for *Geobacillus stearothermophilus*	
Document Type: Technical Report	**Report Number:** R-13-1001
	Page: 1 of 11
	Date: October 1, 2013

1. Summary

A Reduced Incubation Time (RIT) study was conducted by ClorDiSys Solutions, Inc (CSI) to validate a reduced incubation period for *Geobacillus stearothermophilus* Biological Indicator (BI) Spore Strips. The RIT study was conducted to determine the minimum incubation time at 55-65°C required to produce ≥ 97% correlation to seven day outgrowth of exposed biological indicators.

2. Introduction

The effectiveness of a sterilization or decontamination process is determined through the use of biological indicators placed within the area or items being exposed. Once a sterilization is complete, BI's are aseptically dropped into media and incubated for growth over a duration of several days. Color change or an increase in turbidity of the media indicates growth of the biological indicator brought about by an ineffective sterilization cycle. During the incubation period, the areas/items sterilized are still treated "as hot" (contaminated) until the end of the BI incubation period at which point the lack of growth means the cycle was successful and areas/items are free of contamination.

Currently, the required incubation period for *Geobacillus stearothermophilus* biological indicators is 7 days, during which time any area decontaminated must be treated as "hot". Large facilities can lose out on large profits during a 7-day production halt while waiting for BI results to come back. Pharmaceutical companies can run into a shortage of sterile equipment if stringent scheduling of sterilizations are not put in place to prevent a waiting period for BI results. In order to alleviate large profit losses, a reduced incubation time study can be performed to lower the current approved incubation period of 7 days.

3. References

"Guidance for Industry and FDA Staff Biological Indicators Premarket Notification [510(k)] Submissions", Documented Issued on: October 4, 2007. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071261.htm

Reduced Incubation Time Study (Per CDRH Guidelines) for Exposure to Chlorine Dioxide using *Geobacillus stearothermophilus.* NAMSA

4. Method

4.1. Material and Equipment

4.1.1. Qty: 100 from each of three lots: NAMSA biological indicators – *Geobacillus stearothermophilus* 10^6 (ATCC No. 7953) Spore strips packaged in Tyvek peel pouches Lot #'s S83104, S84901 and S84101

4.1.2. Qty: 300: NAMSA Tryptic Soy Broth (TSB) modified with Bromocresol Purple, tubes 16 x 100mm, 4.0 ± 0.2 mL fill volume

4.1.3. Incubator set to 57°C

4.1.4. ClorDisys Solutions, Inc. 17 ft^3 isolator for exposure of BI's

4.1.5. ClorDiSys Solutions, Inc. Minidox-M Chlorine Dioxide gas generator

5. Procedure

5.1. Exposure of biological indicators to partial cycle

Following NAMSA procedure "Reduced Incubation Time Study (Per CDRH Guidelines for Exposure to Chlorine Dioxide using *Geobacillus stearothermophilus"*, 100 biological indicators from the same lot were placed in the isolator for sterilization. A partial sterilization was performed using Chlorine Dioxide (CD) gas consisting of the following set points: 65% relative humidity for 30 minutes before exposure to 1 mg/L concentration of CD gas followed by 5 minutes of aeration. The length of exposure was altered to achieve fractional results in the range of 30-80% positive spore strips after 7 days of incubation.

Once the sterilization cycle was complete, the spore strips were aseptically transferred to individual tubes of TSB modified with Bromocresol purple. Tubes were then incubated for 7 days at 55-65°C; during which the number of positive spore strips were recorded at 24, 36, 48, 72, 96, 120, 144 and 168 hours of incubation. If 30-80% positive spore strips were not obtained after 7 days of incubation, the exposure time was adjusted the run was repeated using new BI's from the same lot.

Two additional lots of *Geobacillus stearothermophilus* spore strips were subjected to the steps above. After achieving valid runs with 3 different lots, the percentage of growth was calculated for each day of incubation by using the total number of positive spore strips on day 7 as the denominator and the number of positives on the current day as the numerator. The reduced incubation time is then determined by the greatest length of time between the three valid lots required to achieve ≥97% growth.

6. Results

The table below shows the successful runs for 3 lots of spore strips. See attachments for a summary of all runs performed.

Table 1 – Determination of Reduced Incubation Time based on ≥97% Grow Out

Partial Cycle #1 - Lot# S83104

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator**	33	34	34	35	35	35	35	35
****Denominator**	35	35	35	35	35	35	35	35
Percent Growth	94.29	97.14	97.14	100.00	100.00	100.00	100.00	100.00

Partial Cycle #4 - Lot # S84901

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator**	55	56	56	57	57	57	57	57
****Denominator**	57	57	57	57	57	57	57	57
Percent Growth	96.49	98.25	98.25	100.00	100.00	100.00	100.00	100.00

Partial Cycle #5 - Lot # S84101

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator**	37	37	37	37	37	38	38	38
****Denominator**	38	38	38	38	38	38	38	38
Percent Growth	97.37	97.37	97.37	97.37	97.37	100.00	100.00	100.00

*The numerator is the number of positive BI's on the current day of incubation;
**The denominator is the total number of positive BI's after 7 days of incubation.

The greatest amount of time required to grow out ≥97% of the denominator biological indicators was 36 hours, indicating the new reduced incubation time.

7. Conclusion

The Reduced Incubation Time study performed by ClorDiSys Solutions, Inc. with *Geobacillus stearothermophilus* biological indicators exposed to chlorine dioxide gas resulted in a reduced overall incubation time. Three different lots of biological indicators were used, all of which resulted in 30-80% positive spore strips after 7 days of incubation. The longest amount of time to produce ≥97% positive spore strips for any of the three lots was 36 hours of incubation, therefore allowing the incubation time for *Geobacillus stearothermophilus* biological indicators to be reduced from 7 days to 36 hours. As a result of this reduced incubation time study, results can be obtained in a more timely fashion such that laboratories will now have less down time waiting for biological indicator results after a decontamination is performed.

8. Attachments

Attachment 1 – Reduced Incubation Time Study Results Geobacillus stearothermophilus, Lot# S83104

Attachment 2 – Reduced Incubation Time Study Results Geobacillus stearothermophilus, Lot# S84901

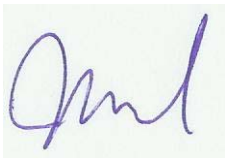
Attachment 3 – Reduced Incubation Time Study Results Geobacillus stearothermophilus, Lot# S84901 ***(invalid)***

Attachment 4 – Reduced Incubation Time Study Results Geobacillus stearothermophilus, Lot# S84901 ***(invalid)***

Attachment 5 – Reduced Incubation Time Study Results Geobacillus stearothermophilus, Lot# S84101

Attachment 6 and 7 –Biological Indicators Inside Isolator

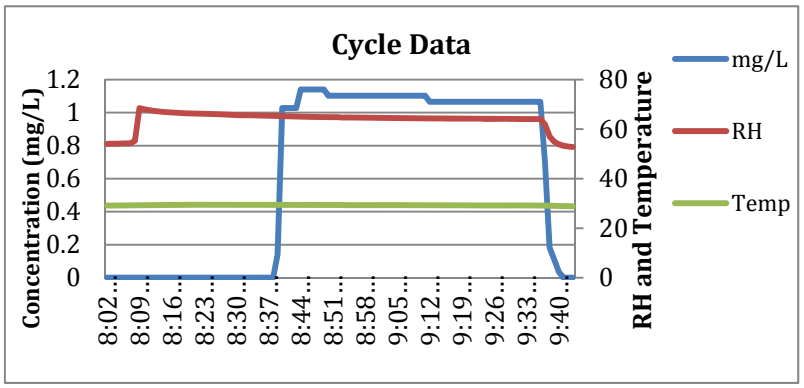
9. Approvals

Final Report Approvals			
Name	**Title**	**Signature**	**Date**
Mark A. Czarneski	Director of Technology		10/1/2013

Attachment 1 – Reduced Incubation Time Study Results Geobacillus stearothermophilus Lot# S83104

Cycle Parameters

RH set point:	*65%*
Condition time:	*30 min*
CD set point:	*1 mg/L*
Ppm-hrs:	*379 ppm-hrs*
Actual Aeration Time:	*5 minutes*



BI Organism:	*Geobacillus stearothermophilus*
Lot Number:	S83104
Weekday Incubated:	Wednesday
Date Incubated:	31 July 2013
Time Incubated:	10:30 AM

Date of Run:	31 July 2013

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
Date:	1 Aug 2013	1 Aug 2013	2 Aug 2013	3 Aug 2013	4 Aug 2013	5 Aug 2013	6 Aug 2013	7 Aug 2013
Weekday:	Thursday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
Time:	10:30 AM	10:30 PM	10:30 AM	10:30 AM	10:30 AM	10:30 AM	10:30 AM	10:30 AM
Result: *# of Positive/100*	33/ 100	34/100	34/100	35/100	35/100	35/100	35/100	35/100

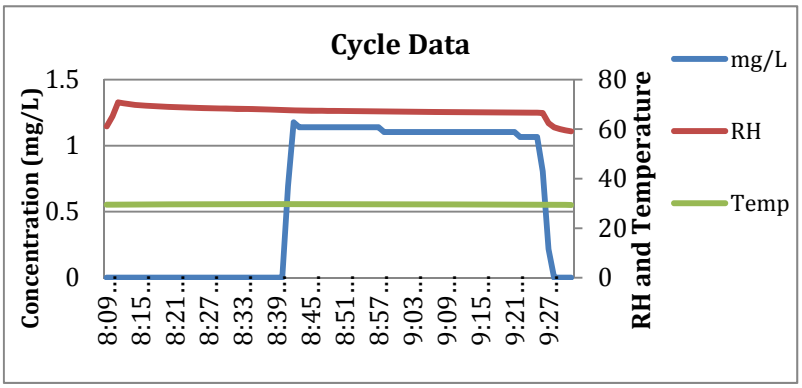
	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator:**	33	34	34	35	35	35	35	35
****Denominator:**	35	35	35	35	35	35	35	35
Percent Growth:	94	97	97	100	100	100	100	100

*The numerator is the number of positive BI's on the current day of incubation;
**The denominator is the total number of positive BI's after 7 days of incubation.

Attachment 2 – Reduced Incubation Time Study Results Geobacillus stearothermophilus Lot# S84901

Cycle Parameters

RH set point:	*65%*
Condition time:	*30 min*
CD set point:	*1 mg/L*
Ppm-hrs:	*305 ppm-hrs*
Actual Aeration Time:	*3 minutes*



BI Organism:	*Geobacillus stearothermophilus*
Lot Number:	S84901
Weekday Incubated:	Tuesday
Date Incubated:	20 Aug 2013
Time Incubated:	10:15 AM

Date of Run:	20 Aug 2013

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
Date:	21 Aug 2013	21 Aug 2013	22 Aug 2013	23 Aug 2013	24 Aug 2013	25 Aug 2013	26 Aug 2013	27 Aug 2013
Weekday:	Wednesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Time:	10:15 AM	10:15 PM	10:15 AM	10:15 AM	10:15 AM	10:15 AM	10:15 AM	10:15 AM
Result: *# of Positive/100*	55/ 100	56/100	56/100	57/100	57/100	57/100	57/100	57/100

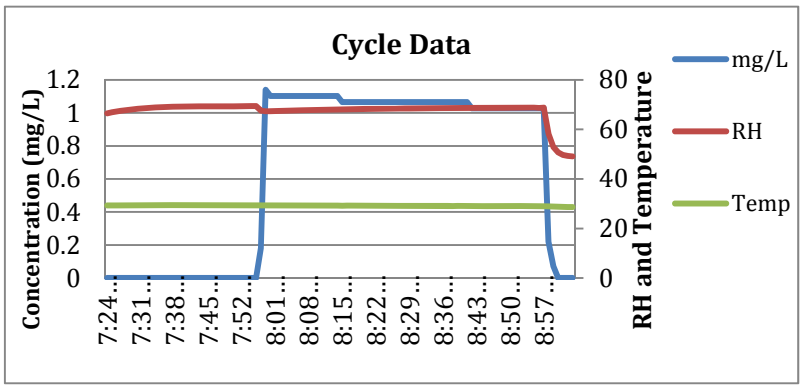
	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator:**	55	56	56	57	57	57	57	57
****Denominator:**	57	57	57	57	57	57	57	57
Percent Growth:	96	98	98	100	100	100	100	100

*The numerator is the number of positive BI's on the current day of incubation;
**The denominator is the total number of positive BI's after 7 days of incubation.

Attachment 3 – Reduced Incubation Time Study Results Geobacillus stearothermophilus Lot# S84901

Cycle Parameters

RH set point:	*65%*
Condition time:	*30 min*
CD set point:	*1 mg/L*
Ppm-hrs:	*381 ppm-hrs*
Actual Aeration Time:	*3 minutes*



BI Organism:	*Geobacillus stearothermophilus*
Lot Number:	S84901
Weekday Incubated:	Monday
Date Incubated:	5 Aug 2013
Time Incubated:	10:00 AM

Date of Run:	5 Aug 2013

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
Date:	6 Aug 2013	6 Aug 2013	7 Aug 2013	8 Aug 2013	9 Aug 2013	10 Aug 2013	11 Aug 2013	12 Aug 2013
Weekday:	Tuesday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday
Time:	10:00 AM	10:00 PM	10:00 AM	10:00 AM	10:00 AM	10:00 AM	10:00 AM	10:00 AM
Result: *# of Positive/100*	6/ 100	6/100	6/100	6/100	6/100	6/100	6/100	6/100

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator:**	6	6	6	6	6	6	6	6
****Denominator:**	6	6	6	6	6	6	6	6
Percent Growth:	100	100	100	100	100	100	100	100

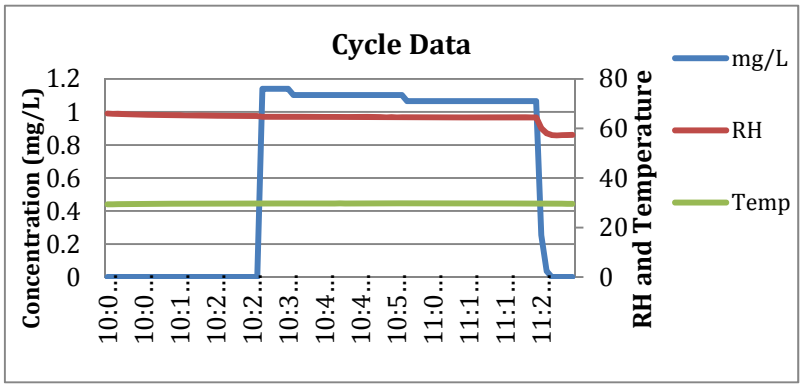
*The numerator is the number of positive BI's on the current day of incubation;
**The denominator is the total number of positive BI's after 7 days of incubation.

This test was invalid because the percent of positive biological indicators after 7 days of incubation was not within the range of 30-80% positive.

Attachment 4 – Reduced Incubation Time Study Results Geobacillus stearothermophilus Lot# S84901

Cycle Parameters

RH set point:	*65%*
Condition time:	*30 min*
CD set point:	*1 mg/L*
Ppm-hrs:	*355 ppm-hrs*
Actual Aeration Time:	*3 minutes*



BI Organism:	*Geobacillus stearothermophilus*
Lot Number:	S84901
Weekday Incubated:	Tuesday
Date Incubated:	13 Aug 2013
Time Incubated:	5:00 PM

Date of Run:	13 Aug 2013

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
Date:	14 Aug 2013	15 Aug 2014	15 Aug 2013	16 Aug 2013	17 Aug 2013	18 Aug 2013	19 Aug 2013	20 Aug 2013
Weekday:	Wednesday	Thursday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Time:	5:00 PM	5:00 AM	5:00 PM	5:00 PM	5:00 PM	5:00 PM	5:00 PM	5:00 PM
Result: *# of Positive/100*	8/ 100	8/ 100	8/ 100	8/ 100	8/ 100	8/ 100	8/ 100	8/ 100

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator:**	8	8	8	8	8	8	8	8
****Denominator:**	8	8	8	8	8	8	8	8
Percent Growth:	100	100	100	100	100	100	100	100

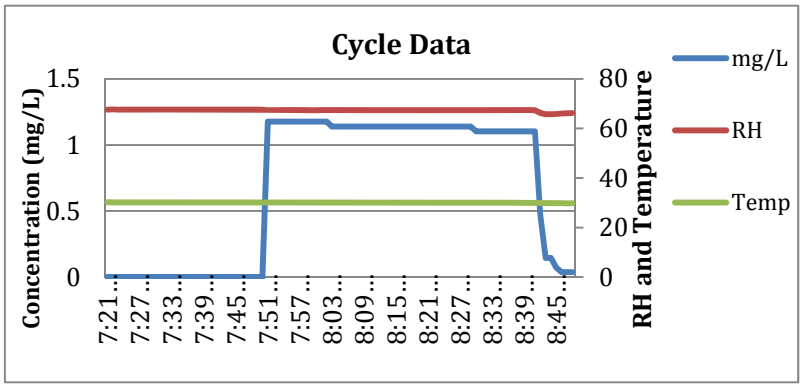
*The numerator is the number of positive BI's on the current day of incubation;
**The denominator is the total number of positive BI's after 7 days of incubation.

This test was invalid because the percent of positive biological indicators after 7 days of incubation was not within the range of 30-80% positive.

Attachment 5 – Reduced Incubation Time Study Results Geobacillus stearothermophilus Lot# S84101

Cycle Parameters

RH set point:	*65%*
Condition time:	*30 min*
CD set point:	*1 mg/L*
Ppm-hrs:	*356 ppm-hrs*
Actual Aeration Time:	*7 minutes*



BI Organism:	*Geobacillus stearothermophilus*
Lot Number:	S84101
Weekday Incubated:	Thursday
Date Incubated:	12 Sep 2013
Time Incubated:	9:30 AM

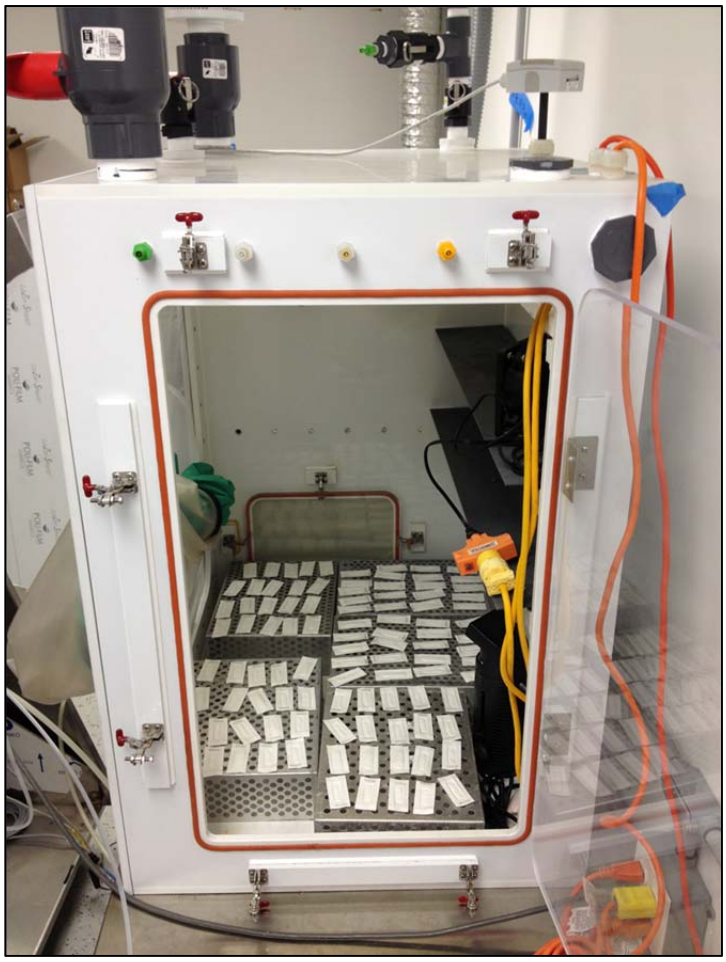
Date of Run:	12 Sep 2013

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
Date:	13 Sep 2013	13 Sep 2013	14 Sep 2013	15 Sep 2013	16 Sep 2013	17 Sep 2013	18 Sep 2013	19 Sep 2013
Weekday:	Thursday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
Time:	9:30 AM	9:30 PM	9:30 AM	9:30 AM	9:30 AM	9:30 AM	9:30 AM	9:30 AM
Result: *# of Positive/100*	37/ 100	37/100	37/100	37/100	37/100	38/100	38/100	38/100

	1 Day	1.5 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	(24 hours)	*(36 hours)*	*(48 hours)*	*(72 hours)*	*(96 hours)*	*(120 hours)*	*(144 hours)*	*(168 hours)*
***Numerator:**	37	37	37	37	37	38	38	38
****Denominator:**	38	38	38	38	38	38	38	38
Percent Growth:	97	97	97	97	97	100	100	100

*The numerator is the number of positive BI's on the current day of incubation;
**The denominator is the total number of positive BI's after 7 days of incubation.

Attachment 6 and 7 –Biological Indicators Inside Isolator



The above images portray the orientation in which the 100 biological indicators were placed inside the isolator during exposure for the reduced incubation time study runs. BI's used were tyvek on both sides and placed on metal screen racks such that chlorine dioxide gas could contact all surfaces.