

**REPORT 031206R**

October 28, 2004

**DEVELOPMENT OF A BIOLOGICAL INDICATOR FOR A ClorDiSys Solutions,  
Inc. CHLORINE DIOXIDE STERILIZER****Purpose:**

The purpose of this study was to develop a biological indicator (BI) for use with a ClorDiSys Solutions, Inc. chlorine dioxide (ClO<sub>2</sub>) gas generation system. This study was performed by SGM Biotech, Inc. ([www.sgmbiotech.com](http://www.sgmbiotech.com)) located in Bozeman, Montana.

**Scope:**

Two sets of spore strips and two sets of self contained BIs were manufactured and sent to ChlorDiSys, Inc. for exposures in a ClO<sub>2</sub> chamber, and returned to SGM for analysis.

**Methods and Materials:**Biological Indicators and Components

Two spore strip lots and two self-contained BIs were manufactured using *Bacillus atrophaeus* spores at a concentration of ~10<sup>6</sup> spores/unit. The specifications are presented in the following Table.

**Table I. Chlorine Dioxide BI's for CSI (ClorDiSys Solutions, Inc.)**

Lot #	Carrier	filter or cover	Amt	SS	Population	Date of Manuf
RD-2000EC	Kimguard	tyvek	162	Batr	2.4 x 10 <sup>6</sup>	1/27/04
RD-2001EC	991	tyvek	167	Batr	2.1 x 10 <sup>6</sup>	1/27/04
RD-2002SC	Kimguard	tyvek/mylar	172	Batr	2.4 x 10 <sup>6</sup>	1/27/04
RD-2003SC	991	tyvek/mylar	173	Batr	2.1 x 10 <sup>6</sup>	1/27/04

## Components of self-contained BIs:

Bodies MB-009

Caps PC008 (purple "flash cap")

Media Amps BSN 0104/1

Vent Filter TP-05

## Spore strip components:

Tyvek/mylar envelopes HPV-001

Schleicher &amp; Schuell #991 paper

Kimberly Clark KIMGUARD KC500

**Spore Crop:**

Spore Dilution Batr-073002B/E4-8 (KimGuard) and E4-10 (991)

**Primary Packaging:**

The spore strip BIs (RD-2002SC and RD-2003SC) consisted of the spore carriers packaged in Tyvek/Mylar envelopes.

The self-contained BIs (RD-2000EC and RD-2001EC) consisted of the spore carriers inserted into the bottom of plastic vials followed by a media ampoule, filter and a cap.

**Resistance determination:**

The four lots of BIs (101 units each) were sent to ChlorDiSys, Inc. in Lebanon, NJ for exposures in a ClO<sub>2</sub> chamber. Four BIs from each lot were exposed in each decontamination cycle. All parameters remained constant (5 mg/l, ambient pressure) with the exception of time. Exposure times were 10, 20, 30 and 40% of a standard cycle. (3, 6, 9 and 12 minutes) plus 30 minutes, a regular cycle time. Zero exposure controls were returned (bagged separately) with each exposure set and a final population was also determined for each lot from retains at SGM.

**Results:**

**Population Analysis:**

Populations were determined by glass bead method using four carriers per assay. Kinguard strips were sonicated for 3 minutes in 0.1% Tween 80 and vortexed for 5 minutes in glass bead tubes containing 4 beads/tube before dilution and plating. 991 paper strips were vortexed in glass bead tubes in 0.1 % Tween 80 for 3 minutes. Colony count results for each BI Lot are presented in Tables II through V. Each table pertains to one BI Lot.

**Table II. *Bacillus atrophaeus* plate counts from Self-contained BI Carrier Strips of RD-2000EC**

Dilution	Exposure Time											
	Initial		3 min		6 min		9 min		12 min		30 min	
	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	
10 <sup>-1</sup>	---	---	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	2.8	2.8	1	0
10 <sup>-2</sup>	---	---	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	1.5	1.5	0	0
10 <sup>-3</sup>	---	---	TNTC	TNTC	116	143	213	232	0	0	0	0
10 <sup>-4</sup>	---	---	69	70	13	14	27	29	0	0	---	---
10 <sup>-5</sup>	54	58	---	---	5	2	0	0	0	0	---	---

**Table III. *Bacillus atrophaeus* plate counts from Self-contained BI Carrier Strips of RD-2001EC**

Dilution	Exposure Time											
	Initial		3 min		6 min		9 min		12 min		30 min	
	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count
10 <sup>-1</sup>	---	---	79	70	TNTC	TNTC	14	14	TNTC	TNTC	3	1
10 <sup>-2</sup>	---	---	5	7	TNTC	TNTC	4	2	TNTC	TNTC	0	1
10 <sup>-3</sup>	---	---	0	0	TNTC	TNTC	0	0	33	59	0	0
10 <sup>-4</sup>	---	---	0	0	44	46	0	0	---	---	---	---
10 <sup>-5</sup>	83	102	0	0	6	6	0	0	---	---	---	---

**Table IV. *Bacillus atrophaeus* plate counts from Carrier Strips of RD-2002SC**

Dilution	Exposure Time											
	Initial		3 min		6 min		9 min		12 min		30 min	
	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count
10 <sup>-1</sup>	---	---	TNTC	TNTC	5	5	1	0	0	0	0	0
10 <sup>-2</sup>	---	---	TNTC	TNTC	1	0	0	0	0	0	0	0
10 <sup>-3</sup>	---	---	TNTC	TNTC	0	0	0	0	---	---	---	---
10 <sup>-4</sup>	---	---	117	123	0	0	0	0	---	---	---	---
10 <sup>-5</sup>	85	80	10	9	0	0	0	0	---	---	---	---

**Table V. *Bacillus atrophaeus* plate counts from Carrier Strips of RD-2003SC**

Dilution	Exposure Time											
	Initial		3 min		6 min		9 min		12 min		30 min	
	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count	Plate Count
10 <sup>-1</sup>	---	---	107	107	1	0	1	1	0	0	0	0
10 <sup>-2</sup>	---	---	10	5	0	0	0	1	0	0	0	0
10 <sup>-3</sup>	---	---	3	1	0	0	0	0	---	---	---	---
10 <sup>-4</sup>	---	---	0	0	0	0	0	0	---	---	---	---
10 <sup>-5</sup>	87	106	0	0	0	0	0	0	---	---	---	---

Using the plate counts from Tables II through V, populations in cfu/strip were calculated at each exposure time for each BI lot. These populations are presented in Table VI.

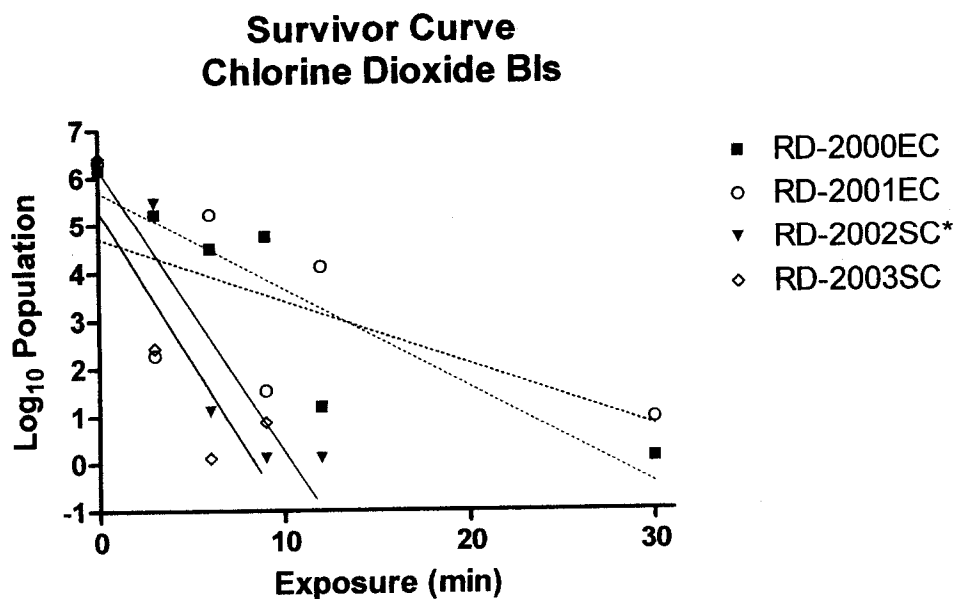
**Table VI. Populations**

Exposure	RD-2000EC	RD-2001EC	RD-2002SC	RD-2003SC
0	1.5e+006	2.1e+006	2.1e+006	2.6e+006
3	1.7e+005	1.9e+002	3.0e+005	2.7e+002
6	3.2e+004	1.7e+005	1.3e+001	1.3e+000
9	5.6e+004	3.5e+001	1.3e+000	7.5e+000
12	1.6e+001	1.3e+004	1.3e+000	0.0
30	1.3e+000	8.8e+000	0.0	0.0

The twelve minute exposure was repeated due to differences in BI numbers in the first run. Although the first run gave the most suitable values the values reported in this table are averages of the two runs.

D-values:

Survivor curves were plotted from the population data and subsequent regression analyses leading to calculation of D-values were determined as described in Attachment 1. Results are presented and in Table VII and graphically in Figure 1.



**Figure 1. Survivor Curves of Four BI Lots Upon Exposure to ClO<sub>2</sub> (5 mg/l, ambient pressure)**


**Table VII. D-values and Coefficients of Determination ( $r^2$ ) for ClO<sub>2</sub> BIs**


BI	RD-2000EC	RD-2001EC	RD-2002SC	RD-2003SC
D-value	4.9 min	7.7 min	1.7 min	1.6 min
$r^2$	0.812	0.421	0.861	0.758

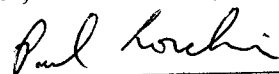
**Conclusions:**

The BI lot with the most predictable response to ClO<sub>2</sub> (5 mg/ml, ambient pressure) is the spore strip BI containing a KIMGUARD500 carrier strip (RD-2002SC). Although the D-value calculated for the 991 paper spore strip (RD-2003SC) is equivalent, the coefficient of determination is too low to be acceptable. The self-contained BI with a KIMGUARD strip (RD-2000EC) was also precise in response but much more resistant with survivors present even at 100% of a cycle (30 minutes). This is likely due to increased difficulty of penetration by the sterilant into the BI.

The use of Kimguard as a carrier material appears to be preferable as the BIs constructed with 991 paper were more variable in response (lower  $r^2$  values).

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## Attachment 1

### ANNEX B ISO 11138-1:1994(E) (normative)

#### Survivor curve method

NOTE 10: The ideal survivor curve is linear over the full range of inactivation. In practice deviations from this ideal occur but linearity must be maintained within acceptable limits. Construction of a survivor curve by direct enumeration establishes the resistance for surviving populations greater than  $5 \times 10^1$  whereas the MPN method establishes the resistance for surviving populations below  $5 \times 10^0$ . Good correlation of the D values obtained by the two methods can therefore be used to establish that there are no serious deviations from a linear survivor curve.

B.1 Test samples shall be subjected at graded exposures to the defined exposure conditions. The range of exposures shall be stated. Each exposure period or dose shall differ from the previous exposure period or dose by a constant interval.

NOTE 11 Details of the performance requirements for exposure apparatus are given in ISO 11138-2 and ISO 11138-3, as appropriate.

B.2 A minimum of five exposures shall be used and shall include:

- a) one exposure in which the sample is not subjected to the sterilant (The sterilant may be absent, or replaced by a non-lethal gas.);
- b) reduction of the viable population to not more than 0.01% of the original inoculum with at least one exposure employed.

B.3 Not less than four inoculated carriers shall be used for each exposure in each determination. The same number of replicates shall be used for each exposure.

B.4 Within 2 h after each exposure, the test samples shall be treated to remove the test organisms from the carrier and a viable count assay performed using the specified culture conditions and methods stated by the manufacturer.

B.5 Using all the data obtained, plot the  $\log_{10}$  of the surviving population against time in minutes or dose level and determine the best fit rectilinear curve by regression analysis using the method of least squares. Survivor data points within 0.5-logarithm of the initial population shall not be included in the regression analysis. Calculate the negative reciprocal of the slope of the line obtained, which is equal to the D-value in minutes or absorbed irradiation dose.

B.6 The value obtained for the correlation coefficient for the linearity of the survivor curve shall be not less than 0.8.