ClorDiSys

"The Chlorine Dioxide People"

Application Note:

Neutralization of chlorine dioxide gas from biological indicators is NOT necessary

Chlorine dioxide (CD) gas can absorb into paper. Biological indicators (BI's) used by ClorDiSys utilize paper substrate. So the question comes, does CD gas absorption into paper provide any residual kill. The answer is NO. If this did happen (residual kill), BI results could lead to false negatives. False negatives could indicate sterile product or environments when the opposite is true. So to prove that CD gas absorbed into paper substrate BI's does not lead false results, tests were performed.

The tests that performed were referenced from the US-EPA
Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD
Standard Operating Procedure for AOAC Sporicidal Activity of Disinfectants Test (Bacillus × porcelain component only)
SOP Number: MB-15-03

This protocol (MB-15-03) defines steps to determine sterilization neutralization efficacy to ensure there is no residual kill of the biological indicator after exposure is completed. For a neutralizer to be deemed effective, or to be determined as not required, growth must occur in the tubes which received low levels of inoculum (e.g., 5 - 100 CFU/mL). Growth in tubes inoculated with this inoculum verifies the presence of the spores, performance of the media, and lack of residual kill.

Conclusions

Based on the results of the experiment performed, it was proven that neutralization of biological indicators exposed to chlorine dioxide gas is not required before aseptically transferring to growth media. Any miniscule amounts of chlorine dioxide left on the spore strips is not adequate enough to provide residual kill on microorganisms during the incubation period.

The biological indicators pulled from the test chamber containing 5 mg/L of chlorine dioxide gas post-exposure/pre-aeration would have contained the maximum amount of CD gas absorbed into the spore strips. These negative test strips, exposed to extended CD gas cycle, were not capable of providing residual kill on the microorganisms added to the test tubes prior to incubation. All other negative test strips removed after increasing lengths of aeration would have contained far less CD gas absorbed into the test strips, and as a result, were also unable to provide any residual kill during incubation. In addition, negative test strips exposed to the more typical concentration of 1 mg/L, 2 hour exposure showed identical results to the over exposed 5mg/L, 1 hour exposure cycle.

The positive control biological indicator not treated with CD gas grew out as expected thus confirming the spores used were in fact viable. All tubes containing just the quality control suspension added to media also turned positive during the incubation period indicating the spore suspension used was also viable. The cycle was validated with 3 biological indicators that were gassed along with the regular samples and dropped in media containing NO quality control suspension. These samples remained negative, indicating a successful decontamination cycle was achieved.

CD gas may absorb into paper and materials comprised of cellulose; however the amount absorbed is not capable of providing residual kill on a spore suspension of <100 colony forming units/0.1mL added to the same media tube as the exposure spore strip. It was proven that even without aerating the gas before removing biological indicators no residual kill could be achieved. The neutralization step used to neutralize any chlorine dioxide gas still absorbed onto a biological indicator spore strip is therefore not required before aseptically transferring to media tubes for incubation.

STUDY MATERIALS

EQUIPMENT

- ClorDiSys Solutions, Inc Minidox-M Chlorine Dioxide gas generator
- Test Chamber - 17 cu ft (0.48 cu m) Isolator
- EBM PAPST 4600Z compact axial fan, 105.9 CFM, fan diameter 4.68"
- Incubator set to 57°C
- Biological Indicators: NAMSA *Geobacillus stearothermophilus (GS),* Lot# 95106, Population 2.1 x 106 per 6mm x 30mm strip, Expiration: 08 Dec 2017

- Growth Media: NAMSA Tryptic Soy Broth – Lot# GM004858, Expiration: 05 Jan 2016
- Negative Control Strip: Strip treated with Ethylene Oxide wrapped in Tyvek/Tyvek, Lot# NC1015, Expiration: 30 Oct 2016
- Microbial Suspension: Quality Control Microbial Suspension – Lot# S89504, *Geobacillus stearothermophilus* – 10 mL volume, < 100 Colony Forming Units/0.1 mL, Expiration: 09 Sep 2017

TEST METHOD

PREPARATION OF TEST CHAMBER

A total of 15 biological indicator samples (12 Negative control, 3 GS spore strips) to be treated with CD gas were placed in the test chamber for each run. The Minidox-M was connected to the test chamber and a total of 2 cycles were run.

EXPOSURE OF BIOLOGICAL INDICATORS AND NEGATIVE CONTROL STRIPS

CD gas generated by the Minidox-M was injected into the test chamber on the right side while a sample was taken from the left side, measured by the Minidox-M photometer.

- Cycle 1: 65%RH, 5 minute condition time, 1 mg/L, 2 hours of exposure (standard room cycle)
- Cycle 2: 65%RH, 5 minute condition time, 5 mg/L, 1 hour of exposure (extended cycle)

POST EXPOSURE SAMPLE TREATMENT

At the end of exposure and prior to aeration initiation, 3 negative control strips were removed from the test chamber.
At the end of aeration (<0.1ppm) 3 negative control strips and 3 GS spore strips were removed from the test chamber.
30 Minutes post aeration completion 3 negative control strips were removed from the test chamber.
60 Minutes post aeration completion 3 negative control strips were removed from the test chamber.
When negative control test strips were removed, they were immediately dropped into growth media tube. Into this media tube 0.1ml of the Quality Control Microbial Suspension was added then incubated.
When the GS spore strips were removed they were dropped into growth media tubes and incubated. At the same time the positive control GS spore strips were dropped into growth media tubes and incubated.
Finally 3 media tubes were inoculated with 0.1ml of the Quality Control Microbial Suspension and incubated.

SUMMARY OF RUNS

Two cycles were run with 15 strips inside (30 total). Cycle 1 had a concentration of 1 mg/L for 2 hours of exposure and Cycle 2 had a concentration of 5 mg/L for 1 hour of exposure (this exposure is an extended cycle).
For each run:

- All 12 negative control test strips incubated with the addition of quality control suspension grew out within 36 hours of incubation at 55-60C.
- The positive control biological indicators not exposed to chlorine dioxide gas were positive.
- The media tubes into which 0.1mL of quality control suspension was added were positive.
- Biological indicators exposed to the cycle and incubated as per normal manufacturer instructions without the addition of any quality control suspension remained negative throughout the incubation period.

Results

The table below documents the results of the biological indicators after incubation, G= Growth and NG = No Growth

BI #	Description	Result: 1mg/L	Result: 5mg/L	BI #	Description	Result: 1mg/L	Result: 5mg/L
1	Post Exposure / Pre Aeration	G	G	PC1	Positive Control 1	G	G
2	Post Exposure / Pre Aeration	G	G	PC2	Positive Control 2	G	G
3	Post Exposure / Pre Aeration	G	G	PC3	Positive Control 3	G	G
4	Immediate Post Aeration	G	G	SC1	Quality Control Test Suspension Controls 1	G	G
5	Immediate Post Aeration	G	G	SC2	Quality Control Test Suspension Controls 2	G	G
6	Immediate Post Aeration	G	G	SC3	Quality Control Test Suspension Controls 3	G	G
7	30 Minutes Post Aeration	G	G	GS1	GS Exposed 1	NG	NG
8	30 Minutes Post Aeration	G	G	GS2	GS Exposed 2	NG	NG
9	30 Minutes Post Aeration	G	G	GS3	GS Exposed 3	NG	NG
10	60 Minutes Post Aeration	G	G				
11	60 Minutes Post Aeration	G	G				
12	60 Minutes Post Aeration	G	G				