

**CUSTOMER SAMPLE ANALYSIS REPORT**

<b>A. CUSTOMER NAME</b>	TECHNICAL INDUSTRIAL SALES INC.
<b>B. CLIENT NUMBER</b>	548
<b>C. ADDRESS</b>	HC-01 BOX 23223 CAGUAS, PR 00725-8918
<b>D. TELEPHONE</b>	(787) 272-3345
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<b>F. CONTACT PERSON</b>	EDUARDO VARON
<b>G. DATE /TIME OF SAMPLE RECEIPT</b>	09-17-08 / 14:50
<b>H. DATE</b>	09-17-08
<b>I. QUANTITY OF SAMPLES</b>	28
<b>J. DESCRIPTION OF SAMPLES</b>	ENVIROSWAB/ EXPOSURE PLATES / MICROSCOPE SLIDES/ DISINFECTANT
<b>K. SAMPLES COLLECTOR NAME</b>	GLORIMAR VELAZCO
<b>L. DATE/TIME ANALYSIS BEGINS:</b>	09-17-08 / 15:00
<b>M. RESULTS</b>	

**PROCEDURE PERFORMED AS PER:**

SOP No. 300-021: Environmental Monitoring

**REFERENCES:**

USP <1072> METHOD: DISINFECTANT CHALLENGING TESTING USP 31/NF26 US PHARMACOPEIA,  
THE OFFICIAL COMPENDIA OF STANDARDS, VOL 1 2008.

<b><sup>1</sup>ENVIROSWABS</b>				
<b>SAMPLING SITE / ID</b>		<b>30-35°C</b>	<b>20-25°C</b>	<b>IDENTIFICATION</b>
		<b>48 h ± 3 h</b>	<b>120 h ± 4 h</b>	
		<b>Bacteria</b>	<b>Yeast / Mold</b>	
DEMO ROOM Room Door Handle	9081-1 Before Treatment 11:10	2 COLS/50CM <sup>2</sup>	0 COLS/50CM <sup>2</sup>	<u>Bacillus subtilis</u> <u>Staphylococcus auricularis</u>
	9081-7 After Treatment 11:10	0 COLS/50CM <sup>2</sup>	0 COLS/50CM <sup>2</sup>	N/A
DEMO ROOM Window next to door	9081-2 Before Treatment 11:10	2 COLS/50CM <sup>2</sup>	0 COLS/50CM <sup>2</sup>	<u>Staphylococcus hominis</u> <u>Staphylococcus auricularis</u>
	9081-8 After Treatment 11:10	0 COLS/50CM <sup>2</sup>	0 COLS/50CM <sup>2</sup>	N/A
DEMO ROOM Wall outlet	9081-3 Before Treatment 11:10	1 COLS/50CM <sup>2</sup>	0 COLS/50CM <sup>2</sup>	<u>Bacillus subtilis</u>
	9081-9 After Treatment 11:10	0 COLS/50CM <sup>2</sup>	0 COLS/50CM <sup>2</sup>	N/A

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<sup>2</sup> EXPOSURE PLATES				
SAMPLING SITE / ID	30-35°C	20-25°C	IDENTIFICATION	
	48 h ± 3 h	120 h ± 4 h		
	Bacteria	Yeast / Molds		
Demo Room Midle of the room	9081-4 Before Treatment 11:10	0	0	N/A
	9081-10 After Treatment 13:15	0	0	N/A
Demo Room Left side of the room	9081-5 Before Treatment 11:10	3	0	<u>Bacillus subtilis</u> <u>Staphylococcus auricularis</u> <u>Staphylococcus hominis</u>
	9081-11 After Treatment 13:15	2	0	<u>Staphylococcus auricularis</u> <u>Staphylococcus hominis</u>
Demo Room Right side of the room	9081-6 Before Treatment 11:10	1	0	<u>Staphylococcus hominis</u>
	9081-12 After Treatment 13:15	0	0	N/A

CULTURE SLIDES			
Organisms	cfu / mL		Percent of Reduction
	Initial Count	Count after exposure to treatment	
9095-1 C. albicans ATCC 10231	5.7 x 10 <sup>7</sup> CFU/mL	19 CFU/mL	99.99%
9095-2 S. aureus ATCC 6538	1.8 x 10 <sup>8</sup> CFU/mL	18 CFU/mL	99.99%
9082-3 S. cholerasuis ATCC 10708	1.2 x 10 <sup>7</sup> CFU/mL	10 CFU/mL	99.99%
9095-4 Ps. aeruginosa ATCC 9027	1.46 x 10 <sup>7</sup> CFU/mL	11 CFU/mL	99.99%
9095-5 E. coli ATCC 8739	2.2 x 10 <sup>8</sup> CFU/mL	24 CFU/mL	99.99%
9095-6 L. monocytogenes ATCC 7644	1.77 x 10 <sup>7</sup> CFU/mL	16 CFU/mL	99.99%

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TEST AGENT			
Sample ID	30-35°C	20-25°C	IDENTIFICATION
	48 h ± 3 h	120 h ± 4 h	
	Bacteria	Yeast / Mold	
9095-7 Zimek Dri-mist Optimizer	0 CFU/mL	0 CFU/mL	N/A
9095-8 Zimek QD	0 CFU/mL	0 CFU/mL	N/A
9095-9 <sup>3</sup> Zimek Dri-mist and optimizer mixture	0 CFU/mL	0 CFU/mL	N/A
9095-10 <sup>4</sup> Zimek Dri-mist and optimizer mixture	0 CFU/mL	0 CFU/mL	N/A

CULTURE SLIDES			
Sample ID	CFU/ML		Percent of Reduction
	Initial Count	After 10 min DropTtest	
9095-11 C. albicans ATCC 10231	5.7 x 10 <sup>7</sup>	0	99.99%
9095-12 S. aureus ATCC 6538	1.8 x 10 <sup>8</sup>	0	99.99%
9082-13 S. cholerasuis ATCC 10708	1.2 x 10 <sup>7</sup>	0	99.99%
9095-14 Ps. aeruginosa ATCC 9027	1.46 x 10 <sup>7</sup>	0	99.99%
9095-15 E. coli ATCC 8739	2.2 x 10 <sup>8</sup>	1	99.99%
9095-16 L. monocytogenes ATCC 7644	1.77 x 10 <sup>7</sup>	3	99.99%

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CULTURE SLIDES CONTROLS			
Sample ID	CFU/ML		Percent of Reduction
	Initial Count	Positive Controls	
9095-17 C. ALBICANS ATCC 10231	5.7 x 10 <sup>7</sup>	> 5,700 (ESTIMATED)	N/A
9095-18 S. AUREUS ATCC 6538	1.8 x 10 <sup>8</sup>	> 5,700 (ESTIMATED)	N/A
9082-19 S. CHOLERASUIS ATCC 10708	1.2 x 10 <sup>7</sup>	> 5,700 (ESTIMATED)	N/A
9095-20 Ps. AERUGINOSA ATCC 9027	1.46 x 10 <sup>7</sup>	> 5,700 (ESTIMATED)	N/A
9095-21 E. COLI ATCC 8739	2.2 x 10 <sup>8</sup>	> 5,700 (ESTIMATED)	N/A
9095-22 L. MONOCYTOGENES ATCC 7644	1.77 x 10 <sup>7</sup>	> 5,700 (ESTIMATED)	N/A

**N. COMMENTS**

<sup>1</sup>In future Zimek System treatments, Lethen Broth Envirotrans™ swab rinse kit will be used instead of Enviroswabs, as recommended in Zimek Testing Protocol 20080228.pdf.

<sup>2</sup>In future Zimek System treatments, Exposure Plates will not be used, since Zimek Technologies, LLC considers that Exposure plates do not represent real world conditions and prefer to use swab tests as primary collection source.

<sup>3</sup>This Zimek Dri-mist and optimizer mixture was used in the demo room on 09-17-08.

<sup>4</sup>This Zimek Dri-mist and optimizer mixture was used in the Ritz Carlton Hotel and San Lucas Hospital in Ponce on 09-11-08 and 09-12-08 respectively.

mL =Mililiter

CFU/mL =Colonies Forming Unit per milliliter

h = hour

Performed By:  
Glorimar Velazco / Gliselle Nieves - Laboratory Analyst

DATE 09-24-08

Reviewed By:  
Nydia Caraballo- Laboratory Supervisor

DATE 09-24-08

Approved By:  
Lizzette M. Rivera, BSMT - Laboratory Director (Lic.2015)

DATE 09-24-08

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**CASE NARRATIVE**

**CLIENT: TECHNICAL INDUSTRIAL SALES INC.  
CLENDO CONTROL NO. 9082, 9095**

**I. Background Information**

The Zimek system is a three-dimensional indoor air and surface disinfectant equipment that aims to kill viruses, bacteria, and mold. This goal is achieved with the production of a Dri-mist (a vaporous flurry of negatively charged micro-particles generated by Zimek's disinfectants).

The micro-particles are delivered wherever air flows and are deposited for a determined period of time (dwell time). This dwell time is to allow the disinfectant to effectively kill or reduce the amount of micro flora present on the treated area. Once the dwell time has pass, a Z-Vac equipment is used to evacuate the airborne contaminants and also to allow a faster re-occupation of the treated area.

The Zimek treatment is recommended for hospitals, industrial and residential facilities and can also be used in motor vehicles like ambulance, fire trucks, helicopters, etc.

**II. Sampling Area Conditions**

The sampling was performed in the Zimek Demonstration Room (Demo Room) in Caguas, P.R.

The room measures 12 x 9 x 9. Nothing in the room was added or changed; it did not contain any air ducts or any airflow that might influence in the efficacy of the treatment.



Photo 1. Zimek Demonstration Room in Caguas

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### **III. Sampling Procedure**

All samples were taken following SOP No. 300 -021 and USP <1072> Method, with the analyst wearing disposable gloves, hair nets, masks, disposable shoe covers, and disposable laboratory gowns.

A total of three samples were taken using enviroswabs before the treatment and three exposure plates were placed on selected areas of the room. The same amount of samples was taken after the treatment. For the samples taken with the swabs the left side of the selected sampling area was sampled before the treatment and the right side of that same area was sampled after the treatment. The Tryptic Soy Agar (TSA) plates were open and placed on the selected areas and exposed for 30 minutes; this was done before and after the treatment.

A set of six microscope slides inoculated with *Candida albicans*, *Staphylococcus aureus*, *Salmonella cholerasuis*, *Pseudomonas aeruginosa*, *Esherichia coli*, and *Listeria monocytogenes* were placed on the Demo Room and exposed to the treatment.

A sample was taken of the Zimek Dri-mist Optimizer, the QD disinfectant, the Dri-mist Optimizer + QD mixture used in the Demo Room on 09-17-08, and the Dri-mist Optimizer + QD mixture used in the Ritz Carlton and the San Luca's Hospital on 09-11-08 and 09-12-08 respectively.



Photo 2. Sampling of the Zimek Demo Room.



Photo 3. Sample handling and preparation for transport

### **IV. Zimek Treatment**

The Zimek machine was placed on a specific place in the room, which allowed the entire room to be exposed to the Zimek QD micro particles for twenty minutes. Following the ten minutes exposure the machine was turned off and the micro particles continued to treat the room for an additional twenty-five minutes dwell time. The Zimek Z Vac was used for twenty-one minutes to evacuate the micro particles that remained suspended in the room.

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The Zimek system was programmed before the treatment; there was nobody inside the room at the time of the treatment because the equipment was controlled with a remote control.

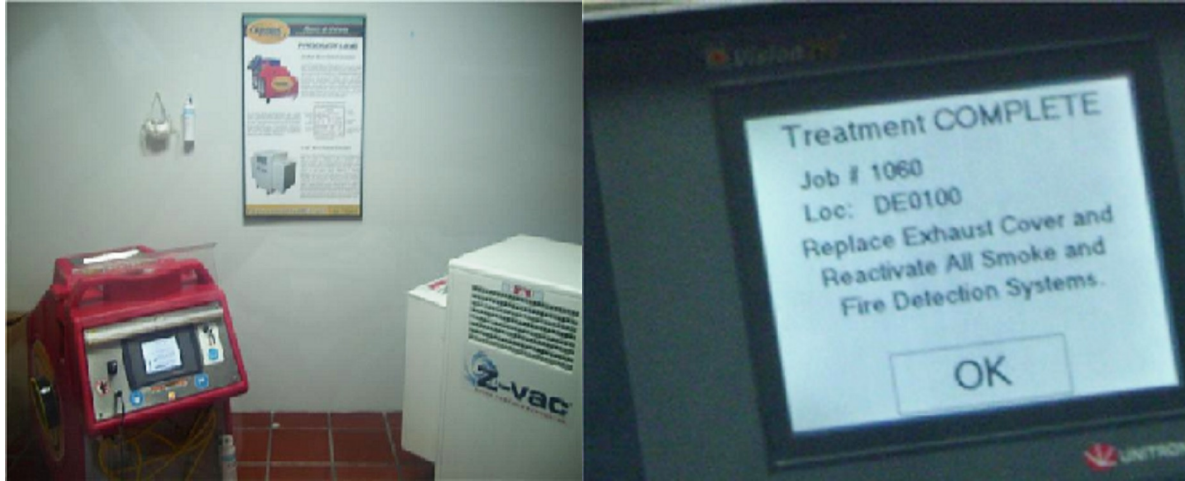


Photo 4. Actual disinfecting treatment seen thru the window in the Zimek Demonstration Room.

Photo 5. Zimek equipment display after completion of treatment.

**V. Sample Receiving / Custody**

The samples were received and processed by the Control Room Section of the Laboratory. There were no significant logistics or quality problems unless noted below.

**VI. Analysis Procedure**

The samples were analyzed according to Clendo Industrial Laboratory Standard Operating Procedures for the methodologies requested. After the analysis and the incubation period the results were obtained and the data was analyzed.

Swabs - Twenty mL of Tryptic Soy Broth (TSB) was added to the enviroswabs and they were vortex for 30 secs to one minute; right after a TSA pour plate of 1.0 mL and 0.1mL was made in duplicates. They were left to solidify and then incubated at 35.5°C for 48 h; after which they were moved to an incubator at 20 -25°C for 120 ± 4 h to verify for Yeast/Mold growth.

Exposure plates – The TSA exposure plates were incubated at 35.5 °C for 48 h to verify for bacterial growth, after which were moved to an incubator at 20 -25°C for 120 h ± 4 h to verify for Yeast/Mold growth.

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Slides – The three sets of six slides were made; each with *Candida albicans*, *Staphylococcus aureus*, *Salmonella cholerasuis*, *Pseudomonas aeruginosa*, *Eshierichia coli*, and *Listeria monocytogenes*. The slides were transported to the sampling site.

The first set was placed on selected areas of the demonstration room and was exposed to the treatment with the Zimek systems. The second set was moved to the laboratory and treated with Dri-mist optimizer + QD mixture (used in the Demo Room) in a ten minutes drop test after which it was swabbed and analyzed as mentioned above for the swabs. The third set subject to the transport conditions was used as a control to verify the colony count of the inoculated organisms. The analysis was then performed as mentioned above for the swabs.

All sets of slides were transported from the laboratory to the sampling site to allow them to undergo the same conditions.

This procedure will test the efficacy and effect of the disinfectant of the Zimek Dri -mist optimizer + QD disinfectant.

Disinfectant – A sample of the Dri-mist Optimizer, the QD disinfectant, the Dri-mist optimizer + QD mixture used in the Demo Room on 09-16-08, and the Dri-mist optimizer + QD mixture used in the Ritz Carlton and the San Luca' s Hospital on 09-11-08 and 09-12-08 respectively. One mL of the disinfectant was added to a test tube containing 4 mL of TSB. The sample was then vortexed and a TSA pour plate of 1.0 mL and 0.1 mL was made in triplicate. They were left to solidify and then incubated at 35.5 °C for 48 h after which they were moved to an incubator at 20 -25°C for five days to verify for Yeast/Mold growth. This procedure will test de efficacy and effect of the disinfectant of the Zimek Dri-mist optimizer + QD disinfectant individually and in mixture.

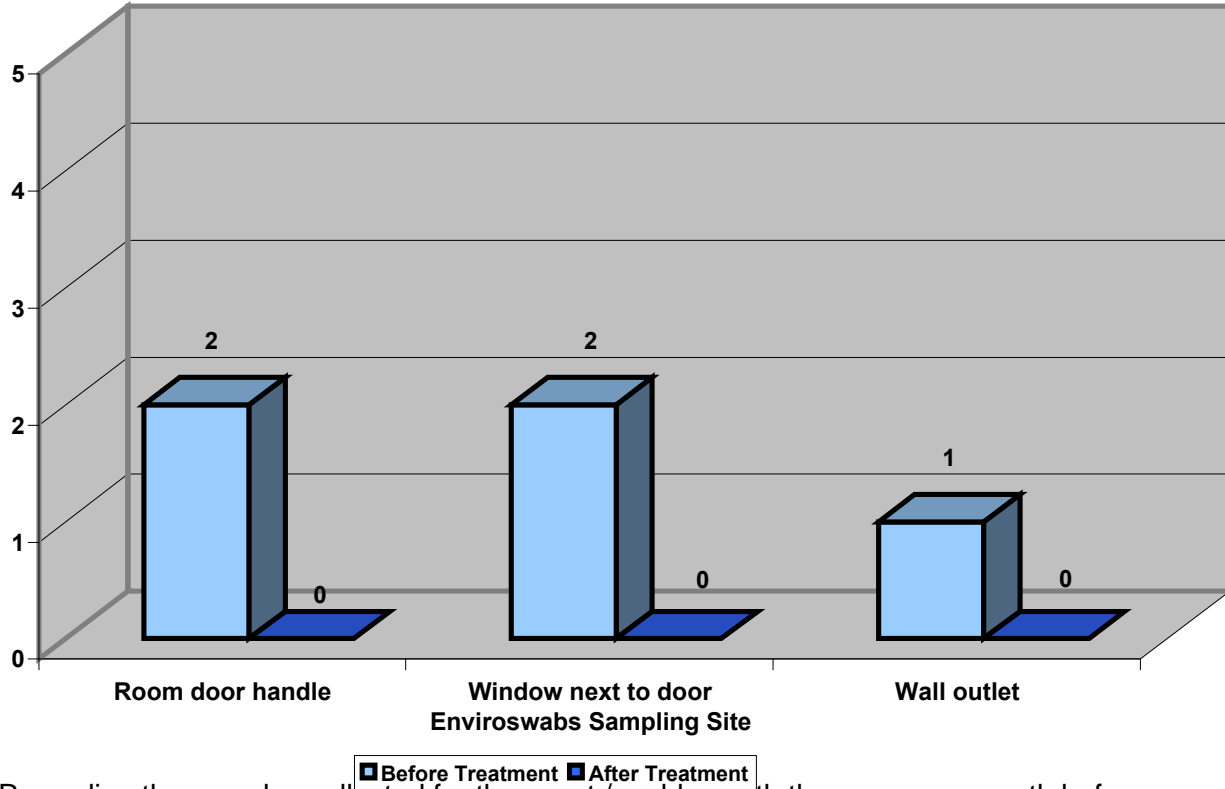
**VII. Results**

After each incubation period has pass a count was performed to obtain the data. An extremely low level count of bacteria, yeast / mold was found on both the exposure plates and the enviroswabss used for subject room, said low level is attributed to the fact that the subject room has been receiving a series of ZIMEK' s maintainance treatments.

The samples collected with the enviroswab presented a reduce amount of bacterial growth before the treatment, however a complete reduction of bacterial growth after the treatment. This can be seen in graph 1.

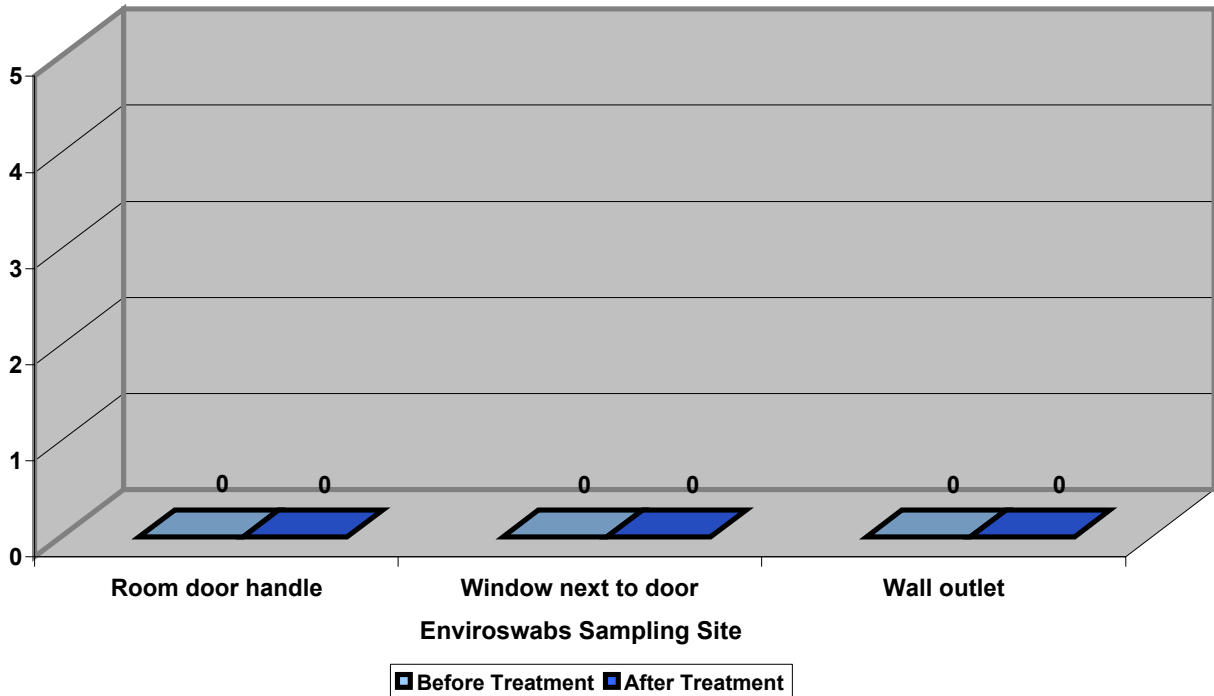
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**Graph 1. Bacterial Count Before Treatment vs. After Treatment**



Regarding the samples collected for the yeast / mold growth there was no growth before or after the treatment with the disinfectant. This can be seen in graph 2.

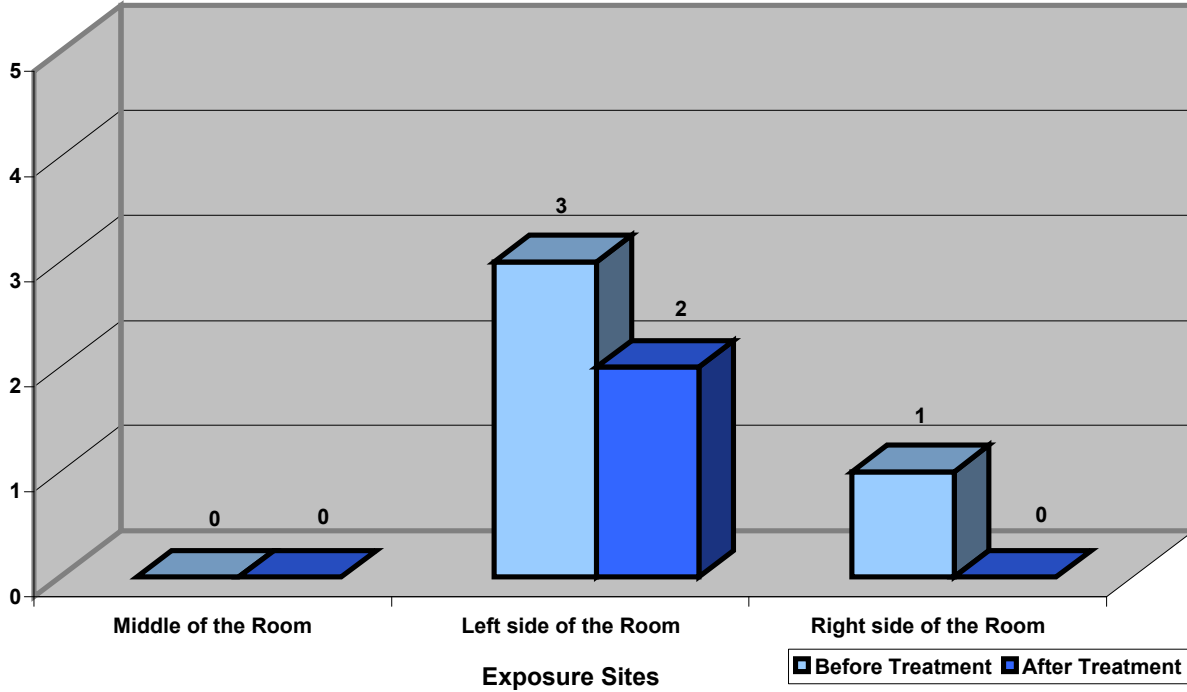
**Graph 2. Yeast and Molds Counts Before Treatment vs After Treatment**



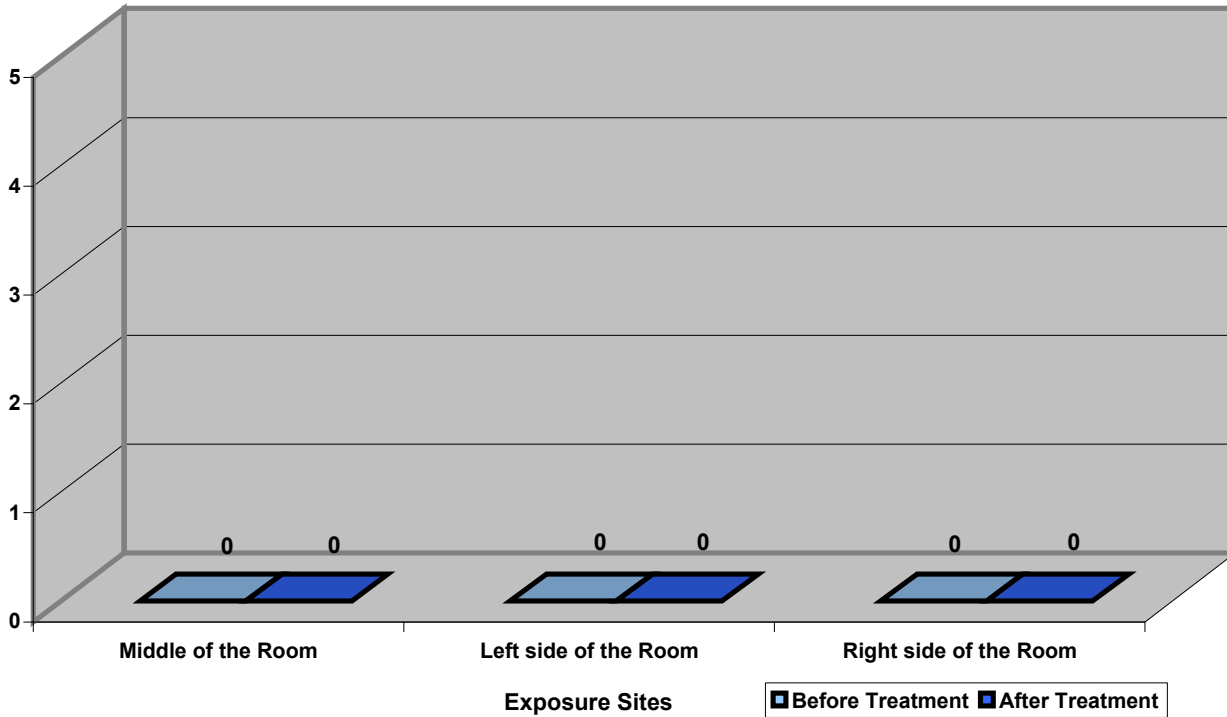
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The exposure plate presented little to no growth. However a reduction in the bacterial growth can be seen from the samples collected before the treatment to the samples collected after the treatment; this can be appreciated in the following graph 3. In reference to the samples collected for yeast / mold count no growth was recovered before or after the treatment. And this can be seen in graph 4.

**Graph 3. Bacterial Count Before Treatment vs. After Treatment**



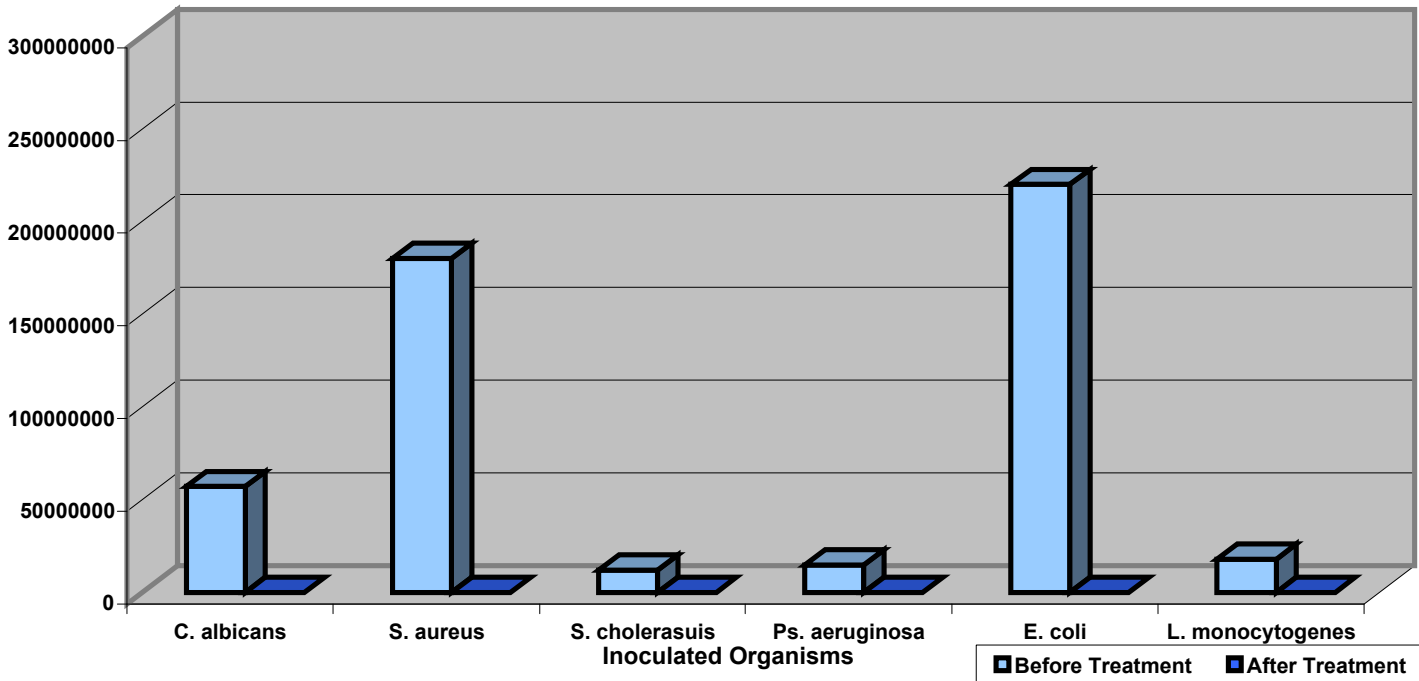
**Graph 4. Yeast and Molds Count Before Treatment vs. After Treatment**



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The set of culture slides that was exposed to the treatment was inoculated with approximately a population of  $x 10^7$  to  $x 10^8$ . After they were exposed to the treatment they presented a 99.99% percent of reduction of the population inoculated. The percent reduction or Kill rate found of the culture slide containin g “live” bacteria and yeast/mold shows very impressive results since achieved a 99.99% in all samples taken. This can be seen in the graph 5.

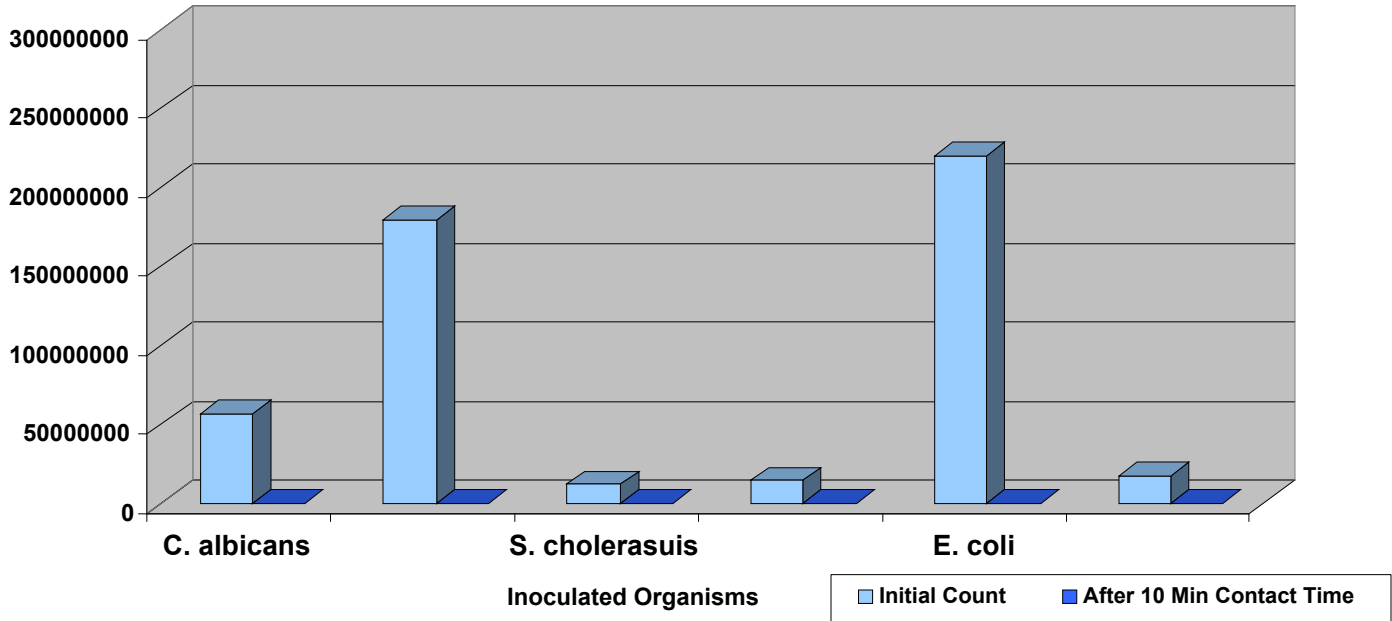
**Graph 5. CFU/mL Count Before Treatment vs. CFU/mL Count After Treatment**



A sample of the Dri-mist Optimizer, the QD disinfectant, the Dri-mist optimizer + QD mixture used in the Demo Room on 09-16-08, and the Dri -mist optimizer + QD mixture used in the Ritz Carlton and the San Luca’s Hospital on 09-11-08 and 09-12-08 respectively, was uses to determine the effect over the inoculated organisms. After the incubation period no growth was present in any of the samples, which proves the efficacy of each of the disinfectant by them selves of in a mixture.

A set of culture slides also inoculated with approximately a population of  $x 10^7$  to  $x 10^8$  was exposed to the ten-minute drop test with the Dri-mist optimizer + QD mixture (1:3) used in the Demo Room on 09-16-08. After they were exposed to the treatment and the incubation period elapsed they presented an outstanding percent of reduction kill rate around a 99.99% average was achieved. This can be seen in the graph 6.

**Graph 6. CFU/mL Initial Count vs. CFU/mL Count After 10 min. Contact Time**



A set of positive controls was made to verify the viability and the amount of the organisms inoculated according to the manufacturer. The results were as expected and as assured by the manufacturer.

For detailed information on the results see Clendo's report # 9082 and 9095.

### **VIII. Conclusion**

In this entire procedure we have to give emphasize that the selected test area is a control room where no air turbulence (Supply and/or return A/C diffusers) was allowed; permitting the Dri-Mist unit to generate the micro-particles during the Dri-stage filling the room without affecting its intended forced air flow pattern and then causing good and even adherence to all the room surfaces during the dwell and vacuum stages.

Also we have to indicate Based on the results obtained after all this test has been performed and the data has been analyzed we can conclude, with the above supporting evidence, that the ionized Dri-Mist method used by ZIMEK shows to be very effective eliminating even critical amount of micro flora present in the treated area.

### **IX. Quality Control**

For each analysis the analysts used the personal protective equipment: [laboratory coat (completely buttoned); sterile sleeves; sterile gloves (covering the whole hand and mid-arm); hair nets (covering ears and all hair) and face mask (covering the nose and the mouth) according to SOP No. 100-04 and SOP No. 100-05.

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During the transport and handling of the samples in the laboratory all aseptic techniques were taken to assure the quality of the samples and the quality of the results to be obtained after the processing of the samples.

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**Appendix A Microbial Isolates and Identification of Organisms Present**

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**Appendix B Test Area Location Outline**