Test System /

3 Units of IceZone® X Model IZ-X30 (Biozone® Scientific International) were received from PepsiCo on December 09, 2015. The units were designated BCS ID 1512151, 1512152, and 1512153. Unit 1512151 was installed onto an ICE-O-Matic ICE1506HR5 Ice maker mounted atop a beverage dispenser (Model IDC 255, Cornelius, USA). Unit 1512152 was installed directly into the ice bin of the Cornelius beverage dispenser (IDC255) that was connected to a Follett HCC1400A ice machine. Additionally, two beverage dispensers equipped each with either one of the above ice makers and no Icezone® units were also installed in an area 20 feet away from the units that were equipped with the Icezone®. The latter two units served as negative controls. The installation and setup of the beverage dispensers, Icezone® units, and ice machines was conducted at BCS by IBS Interbev Inc. (Orlando, Florida) on December 16, 2015. An Ozone gas monitor (2B Technologies, USA) was set up in the area containing the negative control units and was monitored for Ozone residual.

Study Date:

Study was initiated on December 18, 2015.

Challenge Species:

Bacteria: *Escherichia coli* C3000 (ATCC 15597), *Pseudomonas aeruginosa* ATCC 10145, *Listeria monocytogenes* ATCC 15313 stock cultures were obtained from Microbiologics® (MN,

USA) and maintained as per supplier's recommendations. Working cultures of all bacteria were propagated from purified stocks onto Tryptic Soy Agar (TSA, Neogen, MI). For the challenge study, a single colony from a pure stock plate was transferred to Tryptic Soy Broth (TSB, Neogen Inc., MI) and incubated at 36.5ºC for 18-20 hours. An aliquot of the culture was then transferred to fresh sterile TSB and grown for an additional 16-20 hours at 36.5°C prior to the day of the challenge study. This was repeated for a third time. On the day of the challenge, a 10 mL aliquot of the culture was transferred into a 15- mL sterile tube (Corning®, USA), homogenized, and centrifuged for 10 minutes at 3K x G. After centrifugation, the aliquot was discarded and pellet was reconstituted in sterile deionized water. This suspension was used for the challenge studies following settling for 15 minutes.

Molds and fungi: *Candida albicans* ATCC 10231, *Geotrichum candidum* ATCC 34614, *Saccharomyces cerevisiae* ATCC 4098, *Trichophyton Mentagrophytes* ATCC 9533, *Phialophora japonica* ATCC 60806, *Mucor racemosus* ATCC 42647, *Aspergillus niger* ATCC 64958 stock cultures were obtained from Microbiologics® (MN, USA) and maintained as per supplier's recommendations. For Spore preparation ASTM E2197 was used. Potato dextrose agar was inoculated with stock cultures and incubated at 26°C for >10 days. The spores were harvested as per the protocol. Breifly, the growth on the petri plates was flooded with sterile Phosphate Buffered Saline containing 0.05% Tween 80. The culture was scraped off the agar surface by sterile spreader, transferred to a sterile 50-mL centrifuge tube with glass beads, and homogenized

thoroughly. The suspension was passed through a 50 µM screen and stored at 4°C until used in the challenge study.

Challenge Study Description / Methodology:

Twenty uL of cultures was applied to the center surface of 12 sterile 22 mm^2 glass slides placed on sterile trays. Three inoculated glass slides per test microorganism were placed on the shelf attached to the stainless steel front panel in Ice-O-Matic or to the inside wall of the ice bin for the Follett Horizon ice machine/soda dispenser unit. Molds and fungi inoculated slides were incubated in the operating units for 70-72 hours and bacteria inoculated slides for 24 hours. After incubation, glass slides were removed from the units and each was transferred to 10 mL Buffered Peptone Water (Neogen, USA) with 0.01% Sodium Thiosulfate in a sterile 50-mL centrifuge tube (Corning®, USA). The tubes were sealed and placed on a platform shaker for ten minutes. Following, the solution was assayed for the respective bacterial, and mold and fungal species by spread plating onto Tryptic Soy Agar (TSA) and Potato Dextrose Agar, respectively as per Lab Standard Operating Procedure. Each control sample was analyzed in duplicates of 0.1 mL and 1.0 mL and each treated sample was analyzed in duplicates of 1.0 mL. TSA plates were incubated at 36.5ºC ±1 for 24 hours and Potato Dextrose Agar plates were incubated at 26.0 ºC for 5-10 days. Following incubation, the colonies on the respective plates were enumerated, and concentration (cfu/slide) was calculated. The results are presented in the following tables. The average percent reduction of each microorganism by the IceZone® equipped units vs control units is calculated.

Performed by: Wei-Yea Hsu, M.S.

Analyzed by: Wei-Yea Hsu

Study Supervisor: George Lukasik, Ph.D.

Study data are summarized in the provided table(s). The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The results presented pertain only to the samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition at the time of test. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. Positive, negative, and neutralization controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our accreditation standards (ISO17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product.

Table 1a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Escherichia coli.*

Test Replicate (Slide)	*E. coli* cfu/slide from control unit*	Average *E. coli* cfu/slide recovered from control	*E. coli* cfu/slide from IceZone® equipped unit*	Average *E. coli* cfu/slide recovered from IceZone® Unit	Percent Reduction of *E. coli* over control
A	2.16×10^4	2.10×10^4	2.06×10^3	3.50×10^3	83.3%
B	3.91×10^4		1.45×10^3		
C	2.25×10^3		7.0 10^3		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the Biozone treatment or unit not equipped (i.e. control)

Table 1b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Escherichia coli*.

Test Replicate (Slide)	*E. coli* cfu/slide from control unit *	Average *E. coli* cfu/slide recovered from control	*E. coli* cfu/slide from IceZone® equipped unit*	Average *E. coli* cfu/slide recovered from IceZone® Unit	Percent Reduction of *E. coli* over control
A	1.10×10^7	1.00×10^7	2.35×10^4	7.95×10^4	99.2%
B	8.30×10^6		$5.00x\ 10^3$		
C	1.07×10^6		2.10×10^5		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 2a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Listeria monocytogenes.*

Test Replicate (Slide)	*L. monocytogenes* cfu/slide from control unit*	Average *L. monocytogenes* cfu/slide recovered from control	*L. monocytogenes* cfu/slide from IceZone® unit*	Average *L. monocytogenes* cfu/slide recovered from IceZone® unit	Percent Reduction of *L. monocytogenes* over control
A	3.70×10^2	1.03×10^3	1.00×10^1	1.30×10^2	87.4%
B	1.00×10^3		3.50×10^1		
C	1.73×10^3		3.45×10^2		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 2b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Listeria monocytogenes.*

Test Replicate (Slide)	*L. monocytogenes* cfu/slide from control unit*	Average *L. monocytogenes* cfu/slide recovered from control	*L. monocytogenes* cfu/slide from IceZone® unit*	Average *L. monocytogenes* cfu/slide recovered from IceZone® unit	Percent Reduction of *L. monocytogenes* over control
A	3.10×10^2	5.42×10^2	2.00×10^1	1.83×10^1	96.6%
B	3.05×10^2		2.00×10^1		
C	1.01×10^3		1.50×10^1		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 3a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Pseudomonas aeruginosa.*

Test Replicate (Slide)	*P. aeruginosa* cfu/slide from control unit*	Average *P. aeruginosa* cfu/slide recovered from control	*P. aeruginosa* cfu/slide from IceZone® unit*	Average *P. aeruginosa* cfu/slide recovered from IceZone® unit	Percent Reduction of *P. aeruginosa* over control
A	2.21×10^4	4.89×10^4	1.50×10^3	8.90×10^2	98.2%
B	4.64×10^4		1.05×10^3		
C	7.83×10^4		1.20×10^2		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 3b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Pseudomonas. aeruginosa.*

Test Replicate (Slide)	*P. aeruginosa* cfu/slide from control unit*	Average *P. aeruginosa* cfu/slide recovered from control	*P. aeruginosa* cfu/slide from IceZone® unit*	Average *P. aeruginosa* cfu/slide recovered from IceZone® unit	Percent Reduction of *P. aeruginosa* over control
A	3.65×10^4	5.51×10^4	< 2 (not detected)	< 2 (not detected)	> 99.996%
B	3.71×10^4		< 2 (not detected)		
C	9.16×10^4		< 2 (not detected)		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 4a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Candida albicans.*

Test Replicate (Slide)	*C. albicans* cfu/slide from control unit*	Average *C. albicans* cfu/slide recovered from control	*C. albicans* cfu/slide IceZone® equipped unit*	Average *C. albicans* cfu/slide recovered from IceZone® unit	Percent Reduction of *C. albicans* over control
A	1.35×10^3	4.18×10^3	2.00×10^1	$< 4.23 \times 10^1$	> 99.0%
B	5.30×10^3		1.05×10^2		
C	5.90×10^3		< 2 (not detected)		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 4b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Candida albicans.*

Test Replicate (Slide)	*C. albicans* cfu/slide from control unit*	Average *C. albicans* cfu/slide recovered from control	*C. albicans* cfu/slide IceZone® equipped unit*	Average *C. albicans* cfu/slide recovered from IceZone® unit	Percent Reduction of *C. albicans* over control
A	1.95×10^3	2.04×10^3	3.00×10^2	1.27×10^2	93.8%
B	3.00×10^2		3.00×10^1		
C	3.88×10^3		5.00×10^1		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 5a. The efficacy of Biozone equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Saccharomyces cerevisiae.*

Test Replicate (Slide)	*S. cerevisiae* cfu/slide from control unit	Average *S. cerevisiae* cfu/slide recovered from control	*S. cerevisiae* cfu/slide IceZone® equipped unit*	Average *S. cerevisiae* cfu/slide recovered from IceZone® unit	Percent Reduction of *S. cerevisiae* over control
A	2.00×10^5	1.57×10^5	5.05×10^4	6.07×10^4	61.3%
B	1.60×10^5		6.40×10^4		
C	1.10×10^5		6.75×10^4		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 5b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *S. cerevisiae.*

Test Replicate (Slide)	*S. cerevisiae* cfu/slide from control unit	Average *S. cerevisiae* cfu/slide recovered from control	*S. cerevisiae* cfu/slide IceZone® equipped unit*	Average *S. cerevisiae* cfu/slide recovered from IceZone® unit	Percent Reduction of *S. cerevisiae* over control
A	2.05×10^5	2.25×10^5	1.50×10^5	1.43×10^5	36.4%
B	2.90×10^5		1.50×10^5		
C	1.80×10^5		1.30×10^5		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 6a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Trichophyton mentagrophytes.*

Test Replicate (Slide)	*T. mentagrophytes* cfu/slide from control unit	Average *T. mentagrophytes* cfu/slide recovered from control	*T. mentagrophytes* cfu/slide IceZone® equipped unit*	Average *T. mentagrophytes* cfu/slide recovered from IceZone® unit	Percent Reduction of *T. mentagrophytes* over control
A	3.99×10^4	5.39×10^4	2.05×10^2	1.50×10^2	99.7%
B	2.78×10^4		7.00×10^1		
C	9.40×10^4		1.75×10^2		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 6b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Trichophyton mentagrophytes.*

Test Replicate (Slide)	*T. mentagrophytes* cfu/slide from control unit	Average *T. mentagrophytes* cfu/slide recovered from control	*T. mentagrophytes* cfu/slide IceZone® equipped unit*	Average *T. mentagrophytes* cfu/slide recovered from IceZone® unit	Percent Reduction of *T. mentagrophytes* over control
A	8.20×10^4	8.91×10^4	1.78×10^3	5.20×10^3	94.2%
B	8.60×10^4		8.60×10^3		
C	9.94×10^4		5.23×10^3		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 7a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Aspergillus niger*.

Test Replicate (Slide)	*A. niger* cfu/slide from control unit	Average *A. niger* cfu/slide recovered from control	*A. niger* cfu/slide IceZone® equipped unit	Average *A. niger* cfu/slide recovered from IceZone® unit	Percent Reduction of *A. niger* over control
A	1.01×10^4	1.24×10^4	3.90×10^3	3.31×10^3	73.3%
B	1.37×10^4		4.25×10^3		
C	1.34×10^4		1.77×10^3		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 7b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Aspergillus niger*.

Test Replicate (Slide)	*A. niger* cfu/slide from control unit	Average *A. niger* cfu/slide recovered from control	*A. niger* cfu/slide IceZone® equipped unit	Average *A. niger* cfu/slide recovered from IceZone® unit	Percent Reduction of *A. niger* over control
A	1.48×10^4	1.76×10^4	6.50×10^3	7.58×10^3	56.9%
B	1.71×10^4		9.25×10^3		
C	2.10×10^4		7.00×10^3		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 8a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Mucor racemosus.*

Test Replicate (Slide)	*M. racemosus* cfu/slide from control unit	Average *M. racemosus* cfu/slide recovered from control	*M. racemosus* cfu/slide IceZone® equipped unit	Average *M. racemosus* cfu/slide recovered from IceZone® unit	Percent Reduction of *M. racemosus* over control
A	3.80×10^3	3.67×10^3	3.30×10^3	3.58×10^3	2.5%
B	3.30×10^3		3.85×10^3		
C	3.90×10^3		-		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 8b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Mucor racemosus.*

Test Replicate (Slide)	*M. racemosus* cfu/slide from control unit	Average *M. racemosus* cfu/slide recovered from control	*M. racemosus* cfu/slide IceZone® equipped unit	Average *M. racemosus* cfu/slide recovered from IceZone® unit	Percent Reduction of *M. racemosus* over control
A	4.25×10^3	3.60×10^3	3.80×10^3	3.33×10^3	7.5%
B	3.75×10^3		2.55×10^3		
C	2.80×10^3		3.65×10^3		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 9a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Geotrichum candidum.*

Test Replicate (Slide)	*G. candidum* cfu/slide from control unit	Average *G. candidum* cfu/slide recovered from control	*G. candidum* cfu/slide IceZone® equipped unit	Average *G. candidum* cfu/slide recovered from IceZone® unit	Percent Reduction of *G. candidum* over control
A	<10	<10	<10	<10	NA
B	<10		<10		
C	<10		<10		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 9b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Geotrichum candidum.*

Test Replicate (Slide)	*G. candidum* cfu/slide from control unit	Average *G. candidum* cfu/slide recovered from control	*G. candidum* cfu/slide IceZone® equipped unit	Average *G. candidum* cfu/slide recovered from IceZone® unit	Percent Reduction of *G. candidum* over control
A	<10	<10	<10	<10	NA
B	<10		<10		
C	<10		<10		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 10a. The efficacy of IceZone® equipped Ice-O-Matic ice machine/soda dispenser on the inactivation of *Phialophora japonica.*

Test Replicate (Slide)	*P. Japonica* cfu/slide from control unit	Average *P. japonica* cfu/slide recovered from control	*P. japonica* cfu/slide IceZone® equipped unit	Average *P. japonica* cfu/slide recovered from IceZone® unit	Percent Reduction of *P. japonica* over control
A	5.15×10^3	4.28×10^3	2.65×10^3	2.38×10^3	44.4%
B	4.90×10^3		1.75×10^3		
C	2.80×10^3		2.75×10^3		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)

Table 10b. The efficacy of IceZone® equipped Follett Horizon Series ice machine/soda dispenser on the inactivation of *Phialophora japonica.*

Test Replicate (Slide)	*P. Japonica* cfu/slide from control unit	Average *P. japonica* cfu/slide recovered from control	*P. japonica* cfu/slide IceZone® equipped unit	Average *P. japonica* cfu/slide recovered from IceZone® unit	Percent Reduction of *P. japonica* over control
A	9.10×10^3	6.72×10^3	5.15×10^3	4.85×10^3	27.8%
B	6.00×10^3		4.80×10^3		
C	5.05×10^3		4.60×10^3		

*Value represents the colony forming units recovered from glass slides inoculated with the indicated species and placed in the unit equipped with the IceZone® treatment or unit not equipped (i.e. control)