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A FOOD TESTING LABORATORY SINCE 1967

April 20, 2006

Ms. Ruwani Rajakaruna
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Dear Ruwani,

Please find enclosed a proposal for the project entitled "Evaluation of the Antibacterial Efficacy of a Foam Rubber Product Against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*."

Please let me know if you have any questions. We at ABC Research appreciate this opportunity to work with you and Richard Pieris & Co. again.

Sincerely,

James E. (Ken) Kennedy, Jr., Ph.D.
Vice President, Research Microbiology
ABC Research Corp.

Enclosure:

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PROJECT REPORT
RESEARCH MICROBIOLOGY DEPARTMENT

DATE: April 20, 2006

PREPARED FOR: Richard Pieris & Co. Ltd.

CLIENT CONTACT: Ms. Ruwani Rajakaruna

TITLE: Evaluation of the Antibacterial Efficacy of a Foam Rubber Product Against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*.

EXPERIMENTAL APPROACH:

A. TEST MATERIALS

The client provided an approximately 3 ft square sample of the subject foam rubber product (manufactured by Richard Pieris Natural Foams, Ltd.) for testing. The sample was sealed in a plastic bag that was packaged in a box for shipment.

B. TEST MICROORGANISMS

Staphylococcus aureus (ATCC #8095), *E. coli* (ATCC #25992) and *Pseudomonas aeruginosa* (ATCC #15442) were used in the study. Each bacterial culture was individually propagated via at least two serial transfers Trypticase Soy broth (TSB) and incubated at 35°C for 24 h before the experiment. Each culture was diluted in sterile 0.1% peptone buffer to obtain a cell suspension having approx. 10^6 CFU/mL. The inoculum suspension for each culture was enumerated at time-0 (see section D). The working inoculum concentrations for *E. coli*, *S. aureus* and *P. aeruginosa* were 1.1 x 10^6, 1.2 x 10^6 and 9.3 x 10^4, respectively.

C. TEST PROCEDURES:

The foam sample was cut into square pieces (i.e., approximate L x W x H = 2 x 2 x 0.5 in.) for the antimicrobial efficacy tests. A method similar to that described in a paper by W. N. Poh (Elastomerics, 1989) was used. For each bacterial inoculum, four foam pieces were each placed in a separate sterile petri dish. Each foam piece was inoculated by adding 0.5 ml of the inoculum suspension to the foam surface in a series of small droplets to obtain an inoculum of approximately 5 x 10^5 bacterial cells per piece. Two foam pieces for each inoculum were analyzed immediately after inoculation to establish initial (Time-0) counts. Two empty plastic petri dishes were also inoculated with 0.5 ml each of the inoculum suspension to serve as inoculum (positive) controls. The foam samples along with the control petri dishes were placed in covered containers along with water to maintain humidity during incubation at 35°C for 24 h. The samples were analyzed after 24 h for surviving microorganisms.

D. MICROBIAL ANALYSES

For the inoculum counts at time-0, each working inoculum was serially diluted as required and plated in duplicate on Tryptic Soy Agar (TSA) plates. For foam pieces, the samples were aseptically removed from the petri dish and placed in a sterile stomacher bag along with 9.5 ml of sterile diluent (Butterfield's phosphate buffer, BPB). Samples were stomached for 1 min. and the foam sample rinsate serially diluted as required. For the control dishes, sterile diluent (9.5 ml) was added to the petri dish and the diluent suspension thoroughly rinsed. The control (petri dish) rinsate was serially diluted in BPB as required. The rinsates were analyzed via duplicate surface plating aliquots of appropriate serial dilutions onto pre-poured plates of TSA with incubation at 35°C for 24 h.

RESULTS:

Results of the antimicrobial efficacy evaluation of the natural latex foam rubber product manufactured by "Richard Pieris Natural Foams Ltd." are presented in Table1.

The mean *E. coli* level on the foam was reduced from 5.78 to <0.70 $\log_{10}$ CFU/sample after 24 h exposure at 35°C. This represents an *E. coli* reduction of greater than 5.08 $\log_{10}$ units (or >99.9992%). In contrast, the *E. coli* inoculum did not change significantly on the control sample (plastic surface of petri dish) over 24 h at 35°C.

The mean *S. aureus* level on the foam was reduced from 6.44 to <1.85 $\log_{10}$ CFU/sample after 24 h exposure at 35°C. This represents a *S. aureus* reduction of greater than 4.59 $\log_{10}$ units (or >99.997%). In contrast, the *S. aureus* inoculum did not change significantly on the control sample over 24 h at 35°C.

The mean *P. aeruginosa* level on the foam was reduced from 6.06 to <0.70 $\log_{10}$ CFU/sample after 24 h exposure at 35°C. This represents a *P. aeruginosa* reduction of greater than 5.36 $\log_{10}$ units (or >99.9996%). In contrast, the *P. aeruginosa* inoculum did not change significantly on the control sample over 24 h at 35°C.

In summary, the natural latex foam rubber sample product manufactured by "Richard Pieris Natural Foams Ltd." demonstrated a significant antimicrobial property in reducing the viable levels of each microorganism (i.e., *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) by more than 99.99% within a 24 h exposure time at 35°C.

PREPARED BY: _____

James E. (Ken) Kennedy, Ph.D.
Vice President, Research Microbiology
ABC Research Corp.

Table 1. Antimicrobial Efficacy Evaluation of Natural Latex Foam Rubber Product.

Sample (Time)		***E. coli* Counts**		***S. aureus* Counts**		***Ps. aeruginosa* Counts**	
		CFU/sample	Log_{10} /sample	CFU/sample	Log_{10} /sample	CFU/sample	Log_{10} /sample
Foam	Rep 1	660,000	5.82	790,000	5.90	1,100,000	6.04
(Time 0)	Rep 2	560,000	5.75	9,400,000	6.97	1,200,000	6.08
	Mean		**5.78**		**6.44**		**6.06**
	Std. Dev.		0.05		0.76		0.03
Foam	Rep 1	<5	<0.70	<5	<0.70	<5	<0.70
(Time-24 h)	Rep 2	<5	<0.70	1,000	3.00	<5	<0.70
	Mean		<0.70		**1.85**		<0.70
	Std. Dev.		0.00		1.63		0.00
	Reduction		**>5.08**		**>4.59**		**>5.36**
	% Reduction		**>99.9992**		**>99.997**		**>99.9996**
Control	Rep 1	500,000	5.70	710,000	5.85	380,000	5.58
(Time-0)	Rep 2	580,000	5.76	500,000	5.70	550,000	5.74
	Mean		**5.73**		**5.78**		**5.66**
	Std. Dev.		0.05		0.11		0.11
Control	Rep 1	610,000	5.79	990,000	6.00	720,000	5.86
(Time-24 h)	Rep 2	660,000	5.82	910,000	5.96	650,000	5.81
	Mean		**5.80**		**5.98**		**5.84**
	Std. Dev.		0.02		0.03		0.03
	Change		**+0.07**		**+0.20**		**+0.18**

Notes: 1) Reduction = {Foam (time-0) mean log_{10} CFU/sample} - {Foam (time-24 h) mean log_{10} CFU/sample}; percentage reductions based upon log mean counts.

2) Change = {Control (time-24 h) mean log_{10} CFU/sample} - {Control (time-0) mean log_{10} CFU/sample}

3) <5 CFU/sample indicates no recovery at the limit of sensitivity (5 CFU/sample)