UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

September 8, 2011

MEMORANDUM

Subject: Efficacy Review for Puma; EPA Reg. No. 5813-100; DP Barcode: D390560

From: Ibrahim Laniyan, Ph.D.
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To: Wanda Henson / David Liem
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Antimicrobials Division (7510P)

Applicant: The Clorox Company
1221 Broadway
Oakland, CA 94612

Formulation from the Label:

Active Ingredient	% by wt.
Sodium Hypochlorite	8.25 %
Other Ingredients:	91.75 %
Total	100.00 %

(Yields 7.85% available chlorine)

I. BACKGROUND

The product, Puma (EPA Reg. No. 5813-100), is an EPA-approved disinfectant (bactericide, fungicide, virucide), sanitizer, sanitizing rinse, and deodorizer for use on hard, non-porous surfaces in household, commercial, institutional, food preparation, animal care, and hospital or medical environments. The product is also a laundry sanitizer. The applicant requested to amend the registration of this product to: (1) change the label dose to ½ cup of the product when sanitizing laundry in standard and high efficiency washers; (2) add a new claim for effectiveness as a disinfectant against *Clostridium difficile* – spore form; (3) add a new claim for effectiveness as a spray-applied broad spectrum disinfectant; and (4) add claims for effectiveness as a disinfectant against additional bacteria and viruses. Efficacy studies provided in the data package were conducted using the product formulated at the lower certified limit. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; and BioScience Laboratories, Inc., located at 300 N. Willson Avenue in Bozeman, MT 59715.

This data package contained a letter from the applicant to EPA (dated May 13, 2011), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), forty four studies (MRID 484848-02 through 484848-45), Statements of No Data Confidentiality Claims for all forty four studies, and the proposed label.

II. USE DIRECTIONS

The product is designed for disinfecting and sanitizing hard, non-porous surfaces. The product is also designed for use as a laundry sanitizer. The product may be used to treat hard, non-porous surfaces, including: appliances, baby bottles, baby toys, bathtubs, bicycles, bidets, brushes, car dashboards, car door handles, changing tables, combs, counter tops, crib bumpers, cutting boards, diaper pails, dishes, faucets, floors, flower pots, furniture, garbage cans, garbage disposals, glassware, golf balls and clubs, grills, handles, high chairs, litter boxes, lunchboxes, outdoor siding, painted cribs, patio furniture, pet bowls, plastic mattress covers, playground sets, play pens, pots and pans, shower curtains, shower doors, showers, sinks, sports equipment, steering wheels, thermometers, toilet bowls, toilets, toys, trash cans, trash compactors, urinals, utensils, wading pools, and walls. The label indicates that the product may be used on hard, non-porous surfaces, including: finished woodwork, glass, glazed porcelain, glazed tile, laminate, linoleum, painted woodwork, plastic (e.g., vinyl), sealed brick, sealed granite, sealed patio stone, stainless steel, and sealed stucco. The label indicates that the product is not for use on aluminum, chipped enamel, non-stainless steel, and silver. Directions on the proposed label provide the following information regarding preparation and use of the product:

> As a disinfectant: Prepare a use solution by adding 4 ounces of the product and 1 gallon of water (a 1:32 dilution). Wash surfaces or items. Soak, mop, or wipe with use solution. Allow use solution to contact surface for 5 minutes (for 10 minutes against *Pseudomonas aeruginosa*, Feline panleukopenia virus, and Canine parvovirus; for 10 minutes in hospital and healthcare environments). Rinse. Air dry.
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> As a disinfectant against *Clostridium difficile* spores: Prepare a use solution by adding 1 part product and 9 parts water (a 1:10 dilution; ~6,000 ppm). Fecal matter/waste must be thoroughly cleaned from surfaces/objects before disinfection. Cleaning is to include vigorous wiping and/or scrubbing, until visible soil is removed. Special attention is

needed for high-touch surfaces. Surfaces in patient rooms are to be cleaned in an appropriate manner, with restrooms and other "dirty" areas cleaned last. Do not reuse soiled cloths. To disinfect surfaces, apply use solution. Let stand for 10 minutes. Rinse and air dry.

As a laundry sanitizer in standard washing machines (i.e., 69 L): Sort laundry by color. Add detergent. Add ½ cup of product to wash water (i.e., a 1:585 dilution). Add clothes. Start wash.

As a laundry sanitizer in high efficiency washing machines (i.e., 69 L): Sort laundry by color. Add detergent. Add ½ cup of product to wash water (i.e., a 1:585 dilution). Add clothes. Start wash.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Virucides – Novel Virus Protocol Standards: To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

Sporicidal Disinfectant against Clostridium difficile: The Agency has established interim guidance for the efficacy evaluation of antimicrobial products (e.g., dilutable products, ready-to-use products, spray products, towelettes) that are labeled for use to treat hard, non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile*. The effectiveness of such a product must be substantiated by data derived from one of the following four test methods: Most recent version (2006) of AOAC Method 966.04 (For the AOAC Method 966.04, testing should be conducted with two separate batches of product, using 30 carriers per batch for testing of registered sterilants; and three separate batches of product (one of which is at least 60 days old), using 60 carriers per batch for testing of hospital disinfectants. For the quantitative tests, the carrier number specified in the test method should be used): AOAC Sporicidal Activity of Disinfectants Test, Method I for *Clostridium sporogenes*; AOAC Method 2008.05: Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface); ASTM E 2414-05: Standard Test Method for Quantitative Sporicidal Three Step Method (TSM) to Determine Efficacy of Liquids, Liquid Sprays, and Vapor or Gases on Contaminated Carrier Surfaces; or ASTM E 2197-02: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides. Modifications to each test method will be necessary to specifically accommodate spores of *Clostridium difficile*. Because *Clostridium difficile* is an obligate anaerobe, testing should ensure adequate incubation conditions for the recovery of viable spores. The following toxigenic strains of *Clostridium difficile* may be used for testing: ATCC 700792, ATCC 43598, or ATCC 43599. All products must carry a pre-cleaning step, thus no organic soil should be added to the spore inoculum. Results must show a minimum 6 log reduction of viable spores in 10 minutes or less. Control carrier counts must be greater than 10^6 spores/carrier.

Laundry Sanitizer: The effectiveness of laundry sanitizers must be supported by data that show that the product will substantially reduce the numbers of test bacteria on fabric and in laundry water. Laundry additives may either be used as soaking treatments prior to laundering or as treatments added during laundry operations. The label must specify the type of use. Laundry additives may be recommended for household/coin-operated machine use or commercial-industrial-institutional use. The label must specify the type of use. There is a significant difference in the water to fabric ratio between these two uses, which may affect the efficacy of the product. Tests should be conducted using a simulated-use procedure such as Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives" or a simulated use study involving washing machines. Tests should be performed with each of 3 product samples, representing 3 different lots, one of which is at least 60 days old. Tests should be conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Products labeled as being suitable for hospital use must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). Each product lot must be tested with 3 fabrics swatches against each of the test organisms. The method employed must include subculturing of both the fabric and the laundry water. The laundry water to media volume ratio must not exceed 1:40. Testing of a 0.5 mL sample of laundry water from the simulated washing device (or a 5 mL sample from the automatic washer) is recommended. Results from a quantitative bacteriological assay must be reported. Results must show a bacterial reduction of 99.9% over the control count for both fabric and laundry water for each organism tested. The label directions for use of laundry additives should specify the machine cycle in which the product is to be

added, as well as water level, temperature, and treatment time. Compatibility of the treatment with other laundry additives should be determined in testing and addressed in labeling, when applicable. These Agency standards are presented in DIS/TSS-13, and do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones, or trichloro-s-triazinetrione.

Note: The water to fabric ratio for household laundry operations is about 10:1. The water to fabric ratio in industrial laundering operations is about 5:1. The water to fabric ratio for high efficiency operations is about 4:1.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 484848-02 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: Methicillin Resistant *Staphylococcus aureus* (ATCC 33592), for Puma, by Becky Lien. Study conducted at ATS Labs. Study completion date – May 25, 2010. Project Number A09310.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592). Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested using ATS Protocol No. CX18032410.LSAN.4 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Testing was conducted on November 11, 2009 and February 2, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 150.0 grams of the prepared use solution. The carriers for this test were prepared by boiling 650 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 3.3 grams of Na_2CO_3, 3.3 grams of Triton X-100, and 6.5 liters of deionized water for 60 minutes on November 11, 2009, or by boiling 597 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 3.0 grams of Na_2CO_3, 3.0 grams of Triton X-100, and 6.0 liters of deionized water for 60 minutes on February 2, 2010. The fabric then was rinsed in boiling water for 5 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were steam sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizing subculture medium (10^0 dilution) was transferred to 9.0 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 47.25 hours at 35-37°C. Following incubation, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability,

neutralization confirmation, and antibiotic resistance. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 6 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) to oxacillin. See page 9 and Table 8 of the laboratory report.

Note: Adding a ca. 15-gram cloth strip to 150.0 grams of product use solution yields a 1:10 w/w ratio of simulated laundry to wash water, the appropriate ratio for household laundry operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 484848-03 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Puma, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – July 8, 2010. Project Number A09429.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested using ATS Protocol No. CX18032410.LSAN.2 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." At least one of the product lots tested (i.e., Lot No. 09PUMA1) was at least 60 days old at the time of testing. Testing was conducted on September 18, 2009 and April 1, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 103 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 150.0 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 300.00 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5275 grams of Na_2CO_3, 1.5000 grams of Triton X-100, and 3.0 liters of deionized water for 60 minutes on April 1, 2010. The fabric then was rinsed in boiling water for 6 minutes and then rinsed in cold water for 5-7 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10.0 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizing subculture medium (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4}

dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 49.5 hours at 35-37°C. Following incubation, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 150.0 grams of product use solution yields a 1:10 w/w ratio of simulated laundry to wash water, the appropriate ratio for household laundry operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

3. MRID 484848-04 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Klebsiella pneumoniae* (ATCC 4352), for Puma, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – July 8, 2010. Project Number A09427.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested using ATS Protocol No. CX18032410.LSAN.1 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." At least one of the product lots tested (i.e., Lot No. 09PUMA1) was at least 60 days old at the time of testing. Testing was conducted on September 18, 2009 and April 1, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 150.00 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 300.00 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5275 grams of Na_2CO_3, 1.5000 grams of Triton X-100, and 3.0 liters of deionized water for 60 minutes on April 1, 2010. The fabric then was rinsed in boiling water for 6 minutes and then rinsed in cold water for 5-7 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 30.0 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 20°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10.0 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizing subculture medium (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 47 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination.

Following incubation and storage, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 150.0 grams of product use solution yields a 1:10 w/w ratio of simulated laundry to wash water, the appropriate ratio for household laundry operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

4. MRID 484848-05 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Puma, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – July 8, 2010. Project Number A09428.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested using ATS Protocol No. CX18032410.LSAN.3 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." At least one of the product lots tested (i.e., Lot No. 09PUMA1) was at least 60 days old at the time of testing. Testing was conducted on September 18, 2009 and April 1, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 150.0 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 300.00 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5275 grams of Na_2CO_3, 1.5000 grams of Triton X-100, and 3.0 liters of deionized water for 60 minutes on April 1, 2010. The fabric then was rinsed in boiling water for 6 minutes and then rinsed in cold water for 5-7 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10.0 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizing subculture medium (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 44 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, standard plate count procedures were used to determine the average colony forming

units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 150.0 grams of product use solution yields a 1:10 w/w ratio of simulated laundry to wash water, the appropriate ratio for household laundry operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

5. MRID 484848-06 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Puma, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – February 8, 2011. Project Number A10702.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 10PUMA12) of the product, Puma, was tested using ATS Protocol No. CX18121410.LSAN.1 (copy provided). The product lot was at least 60 days old at the time of testing. The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Testing was conducted on September 18, 2009 and November 3, 2010. A use solution was prepared by adding 2.00 mL of the product and 1,166 mL of 100 ppm AOAC synthetic hard water (titrated at 102 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile Nalgene jar was filled with 150.00 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 598.86 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 2.9904 grams of Na_2CO_3, 3.0429 grams of Triton X-100, and 6.0 liters of deionized water for 60 minutes on November 3, 2010. The fabric then was rinsed in boiling water for 5-6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches for the single product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizer (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count,

carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for the single product lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 150.0 grams of product use solution yields a 1:10 w/w ratio of simulated laundry to wash water, the appropriate ratio for household laundry operations.

6. MRID 484848-07 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: Methicillin Resistant *Staphylococcus aureus* (ATCC 33592), for Puma, by Becky Lien. Study conducted at ATS Labs. Study completion date – February 2, 2011. Project Number A10645.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592). Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested using ATS Protocol No. CX18112410.LSAN.6 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Testing was conducted on September 18, 2009 and November 3, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 60.00 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 598.86 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 2.9904 grams of Na_2CO_3, 3.0429 grams of Triton X-100, and 6.0 liters of deionized water for 60 minutes on November 3, 2010. The fabric then was rinsed in boiling water for 5-6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizing subculture medium (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 44.25 hours at 35-37°C. Following incubation, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 60.0 grams of product use solution yields a 1:4 w/w ratio of simulated laundry to wash water, the appropriate ratio for high efficiency operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 6 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) to oxacillin. See page 9 and Table 9 of the laboratory report.

7. MRID 484848-08 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Puma, by Becky Lien. Study conducted at ATS Labs. Study completion date – February 2, 2011. Project Number A10642.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested using ATS Protocol No. CX18112410.LSAN.3 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Testing was conducted on September 18, 2009 and November 3, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 60.00 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 598.86 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 2.9904 grams of Na_2CO_3, 3.0429 grams of Triton X-100, and 6.0 liters of deionized water for 60 minutes on November 3, 2010. The fabric then was rinsed in boiling water for 5-6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizer (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 46.75 hours at 35-37°C. Following incubation, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 60.0 grams of product use solution yields a 1:4 w/w ratio of simulated laundry to wash water, the appropriate ratio for high efficiency operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

8. MRID 484848-09 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Klebsiella pneumoniae* (ATCC 4352), for Puma, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – February 8, 2011. Project Number A10643.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested using ATS Protocol No. CX18112410.LSAN.4 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Testing was conducted on September 18, 2009 and November 3, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile Nalgene jar was filled with 60.00 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 598.86 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 2.9904 grams of Na_2CO_3, 3.0429 grams of Triton X-100, and 6.0 liters of deionized water for 60 minutes on November 3, 2010. The fabric then was rinsed in boiling water for 5-6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizer (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 60.0 grams of product use solution yields a 1:4 w/w ratio of simulated laundry to wash water, the appropriate ratio for high efficiency operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

9. MRID 484848-10 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Puma, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – February 8, 2011. Project Number A10644.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested using ATS Protocol No. CX18112410.LSAN.5 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Testing was conducted on September 18, 2009 and November 3, 2010. Use solutions were prepared by adding 1.00 mL of the product and 583 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile Nalgene jar was filled with 60.00 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 598.86 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 2.9904 grams of Na_2CO_3, 3.0429 grams of Triton X-100, and 6.0 liters of deionized water for 60 minutes on November 3, 2010. The fabric then was rinsed in boiling water for 5-6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizer (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation (both product lots). Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 60.0 grams of product use solution yields a 1:4 w/w ratio of simulated laundry to wash water, the appropriate ratio for high efficiency operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

10. MRID 484848-11 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Puma, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – February 8, 2011. Project Number A10703.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 10PUMA12) of the product, Puma, was tested using ATS Protocol No. CX18121410.LSAN.2 (copy provided). The product lot was at least 60 days old at the time of testing. The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Testing was conducted on September 18, 2009 and November 3, 2010. A use solution was prepared by adding 2.00 mL of the product and 1,166 mL of 100 ppm AOAC synthetic hard water (titrated at 102 ppm; a 1:583 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile Nalgene jar was filled with 60.00 grams of the prepared use solution. The carriers for this test were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 liters of deionized water for 60 minutes on September 18, 2009, or by boiling 598.86 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 2.9904 grams of Na_2CO_3, 3.0429 grams of Triton X-100, and 6.0 liters of deionized water for 60 minutes on November 3, 2010. The fabric then was rinsed in boiling water for 5-6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches for the single product lot were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizer (10^0 dilution) was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^0 to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for the single product lot of use solution were measured and reported.

Note: Adding a ca. 15-gram cloth strip to 60.0 grams of product use solution yields a 1:4 w/w ratio of simulated laundry to wash water, the appropriate ratio for high efficiency operations.

11. MRID 484848-12 "AOAC Germicidal Spray Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Puma, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – July 20, 2010. Project Number A09656.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18060110.GS.3 (copy provided). At least one of the product lots tested (i.e., Lot No. 09PUMA1) was at least 60 days old at the time of testing. Use solutions were prepared by adding 30.0 mL of the product and 960 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 38.54% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution from a distance of 4-6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 4.5 minutes at 20°C at 64% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

12. MRID 484848-13 "AOAC Germicidal Spray Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Puma, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – November 3, 2010. Project Number A10269.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 10PUMA12) of the product, Puma, was tested using ATS Laboratory Protocol No. CX18090810.GS.3 (copy provided). The product lot tested was at least 60 days old at the time of testing. A use solution was prepared by adding 60.0 mL of the product and 1,920 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of a 48 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 40% relative humidity. For the single product lot, separate carriers were sprayed (4 pumps) with the use solution from a distance of 4-6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 4.5 minutes at 21°C at 41% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. All subcultures were incubated for 44 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for the single product lot of use solution were measured and reported.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

13. MRID 484848-14 "AOAC Germicidal Spray Method," Test Organism: *Salmonella enterica* (ATCC 10708), for Puma, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – July 20, 2010. Project Number A09657.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18060110.GS.4 (copy provided). At least one of the product lots tested (i.e., Lot No. 09PUMA1) was at least 60 days old at the time of testing. Use solutions were prepared by adding 30.0 mL of the product and 960 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 38.54% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution from a distance of 4-6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 4.5 minutes at 20°C at 65% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 46.0 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

14. MRID 484848-15 "AOAC Germicidal Spray Method," Test Organism: *Salmonella enterica* (ATCC 10708), for Puma, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – October 26, 2010. Project Number A10267.

This study was conducted against *Salmonella enterica* (ATCC 10708). One lot (Lot No. 10PUMA12) of the product, Puma, was tested using ATS Laboratory Protocol No. CX18090810.GS.1 (copy provided). The product lot tested was at least 60 days old at the time of testing. A use solution was prepared by adding 60.0 mL of the product and 1,920 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of a 48 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 40% relative humidity. For the single product lot, separate carriers were sprayed (4 pumps) with the use solution from a distance of 4-6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 4.5 minutes at 21°C at 41% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. All subcultures were incubated for 44 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population,

purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for the single product lot of use solution were measured and reported.

15. MRID 484848-16 "AOAC Germicidal Spray Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Puma, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – August 18, 2010. Project Number A09655.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18060110.GS.2 (copy provided). At least one of the product lots tested (i.e., Lot No. 09PUMA1) was at least 60 days old at the time of testing. Testing was conducted on June 21, 2010 and July 21, 2010. Use solutions were prepared by adding 30.0 mL of the product and 960 mL of 100 ppm AOAC synthetic hard water, or the equivalent (titrated at 96-100 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30-40 minutes at 35-37°C at 38.54-42% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution from a distance of 4-6 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 4.5 minutes at 20-21°C at 55-65% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Testing conducted on June 21, 2010 against *Pseudomonas aeruginosa* showed growth in 2 of the 60 subculture tubes for one product lot (Lot No. 09PUMA2). Testing was repeated to rule out the possibility of false positives.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

16. MRID 484848-17 "AOAC Germicidal Spray Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Puma, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – October 26, 2010. Project Number A10268.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 10PUMA12) of the product, Puma, was tested using ATS Laboratory Protocol No. CX18090810.GS.2 (copy provided). The product lot tested was at least 60 days old at the time of testing. A use solution was prepared by adding 60.0 mL of the product and 1,920 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of a 48 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 40% relative humidity. For the single product lot, separate carriers were sprayed (4 pumps) with the use solution from a distance of 4-6 inches from the carrier surface

until thoroughly wet. The carriers were allowed to remain wet for 4.5 minutes at 21°C at 41% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. All subcultures were incubated for 44 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for the single product lot of use solution were measured and reported.

17. MRID 484848-18 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Feline Panleukopenia virus, Strain Philips-Roxane, ATCC VR-648" for Puma, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – July 1, 2010. Project Number A09390.

This study was conducted against Feline panleukopenia virus (Strain Philips-Roxane; ATCC VR-648), using CRFK cells (feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.FPLV (copy provided). Use solutions were prepared by adding 2.00 mL of the product and 64.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution. The carriers were allowed to remain wet for 9.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 14 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. In the final day of incubation, a hemagglutination assay was performed on the cultures. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

18. MRID 484848-19 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes simplex virus type 2, Strain G, ATCC VR-734" for Puma, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – July 1, 2010. Project Number A09383.

This study was conducted against Herpes simplex virus type 2 (Strain G; ATCC VR-734), using RK cells (rabbit kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.HSV2 (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate

dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 5% (v/v) heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

19. MRID 484848-20 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Hepatitis A virus, Strain HM-175" for Puma, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – May 28, 2010. Project Number A09363.

This study was conducted against Hepatitis A virus (Strain HM-175; obtained from Apptec Laboratory Services, Camden, NJ), using FRhK-4 cells (fetal Rhesus monkey kidney cells; ATCC CRL-1688; propagated in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.HAV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, and 2.0 mM L-glutamine. FRhK-4 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The inoculum was allowed to adsorb for at least 90 minutes at 36-38°C in a humidified atmosphere of 5-7% CO_2. Following adsorption, the cultures were re-fed and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 15 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

20. MRID 484848-21 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Respiratory syncytial virus, Strain Long, ATCC VR-26" for Puma, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – May 28, 2010. Project Number A09368.

This study was conducted against Respiratory syncytial virus (Strain Long; ATCC VR-26), using Hep-2 cells (human larynx carcinoma cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.RSV (copy provided). Use solutions were prepared by adding 1.00 mL of the

product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 96 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, 10 µg/mL vancomycin, 2 mM L-glutamine, and 10 mM Hepes. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

21. MRID 484848-22 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Immunodeficiency Virus type 1, Strain HTLV-III_B" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 27, 2010. Project Number A09344.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using MT-2 cells (human T-cell leukemia cells; obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.HIV.1 (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 22.0°C at 27.2% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 22.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in RPMI-1640 with 10% (v/v) heat-inactivated fetal bovine serum, 2.0 mM L-glutamine, and 50 µg/mL gentamicin. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 8 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

22. MRID 484848-23 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Immunodeficiency Virus type 1, Strain HTLV-III_B" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – June 28, 2010. Project Number A09382.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using MT-2 cells (human T-cell leukemia cells; obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.HIV.2 (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 50% whole human blood as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 24.0°C at 30.2% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in RPMI-1640 with 10% (v/v) heat-inactivated fetal bovine serum, 2.0 mM L-glutamine, and 50 µg/mL gentamicin. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 8 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

23. MRID 484848-24 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Immunodeficiency Virus type 1, Strain HTLV-III_B" for Puma, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 16, 2010. Project Number A09700.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using MT-2 cells (human T-cell leukemia cells; obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; maintained in-house) as the host system. Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18060410.HIV (copy provided). Use solutions were prepared by adding 5.0 mL of the product and 160.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 24.0°C at 55.4% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed (3 seconds) with the use solution from a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 4.5 minutes at 22.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in RPMI-1640 with 10% (v/v) heat-inactivated fetal bovine serum, 2.0 mM L-glutamine, and 50 µg/mL gentamicin. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

24. MRID 484848-25 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Adenovirus type 2, Strain Adenoid 6, ATCC VR-846" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 28, 2010. Project Number A09362.

This study was conducted against Adenovirus type 2 (Strain Adenoid 6; ATCC VR-846), using A-549 cells (human lung carcinoma cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minneapolis, MN; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.ADV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, and 10 mM HEPES. A-549 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

25. MRID 484848-26 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – July 21, 2010. Project Number A09625.

This study, under the direction of Study Director Mary J. Miller, was conducted against Duck hepatitis B virus (Strain 7/31/07; obtained from HepadnaVirus Testing, Inc., Palo Alto, CA), using primary duck hepatocytes (cultures prepared by Valley Research Institute personnel using hatchling ducks received from Metzer Farms) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to an ATS Protocol No. CX18031510.DHBV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum in addition to 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 30 minutes at 20.0°C at 40% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Leibovitz L-15 Medium with 0.1%

glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with 1.0 mL of the dilutions. Test medium was added to each cell culture well prior to inoculation. The inoculum was allowed to adsorb overnight at 36-38°C in a humidified atmosphere of 5-7% CO_2. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for a total of 9 days at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were re-fed, as necessary. On the final day of incubation, the cultures were scored microscopically for cytotoxicity. An indirect immunofluorescence assay was then performed. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

26. MRID 484848-27 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus" for Puma, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – July 21, 2010. Project Number A09626.

This study, under the direction of Study Director Kelleen Gutzmann, was conducted against Duck hepatitis B virus (Strain 7/31/07; obtained from HepadnaVirus Testing, Inc., Palo Alto, CA), using primary duck hepatocytes (cultures prepared by Valley Research Institute personnel using hatchling ducks received from Metzer Farms) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to an ATS Protocol No. CX18031610.DHBV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 96 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum in addition to 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 30 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Leibovitz L-15 Medium with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with 1.0 mL of the dilutions. Test medium was added to each cell culture well prior to inoculation. The inoculum was allowed to adsorb overnight at 36-38°C in a humidified atmosphere of 5-7% CO_2. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for a total of 9 days at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were re-fed, as necessary. On the final day of incubation, the cultures were scored microscopically for cytotoxicity. An indirect immunofluorescence assay was then performed. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

27. MRID 484848-28 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Cytomegalovirus, Strain AD-169, ATCC VR-538" by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 12, 2010. Project Number A09387.

This study was conducted against Cytomegalovirus (Strain AD-169; ATCC VR-538), using MRC-5 cells (human embryonic lung cells; ATCC CCL-171; propagated in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested

according to ATS Labs Protocol No. CX18031510.CMV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 40% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, 2.00 mL of neutralizer was added to each film, and the plates were scraped with a cell scraper and the contents were transferred to individual conical tubes ($10^{-1.3}$ dilution). The $10^{-1.3}$ dilutions were diluted serially in Minimum Essential Medium with 10% (v/v) heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Following titration, the $10^{-1.3}$ dilutions were passed through individual Sephadex columns to aid in removing the cytotoxic effects of the test substance to the host system. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 27 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: The laboratory reported a failed study set up on April 28, 2010. In the study, a recoverable virus titer of at least 10^4 was not achieved for each of the five dried virus control replicates. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on June 30, 2010. See page 8 and Attachment I of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

28. MRID 484848-29 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Canine Parvovirus, Strain Cornell, ATCC VR-2017" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – June 29, 2010. Project Number A09389.

This study was conducted against Canine parvovirus (Strain Cornell; ATCC VR-2017), using A-72 cells (canine tumor cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.CPV (copy provided). Use solutions were prepared by adding 2.00 mL of the product and 64.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 9.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, and 10 mM HEPES. A-72 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7%

CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. On the final day of incubation, a hemagglutination assay was performed on the culture using swine red blood cells at 2-8°C. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

29. MRID 484848-30 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: 2009-H1N1 Influenza A virus (Novel H1N1), Strain A/Mexico/4108/2009 CDC #2009712192" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 28, 2010. Project Number A09419.

This study was conducted against 2009-H1N1 Influenza A virus (Novel H1N1) (Strain A/Mexico/4108/2009; CDC #2009712192), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.FLUA (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 22.0°C at 19.7% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 22.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

30. MRID 484848-31 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza B virus, Strain B/Hong Kong/5/72, ATCC VR-823" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 28, 2010. Project Number A09412.

This study was conducted against Influenza B virus (Strain B/Hong Kong/5/72; ATCC VR-823), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.FLUB (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 40% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the

contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

31. MRID 484848-32 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Coronavirus, Strain 229E, ATCC VR-470" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – June 29, 2010. Project Number A09388.

This study was conducted against Human coronavirus (Strain 229E; ATCC VR-740), using WI-38 cells (human lung cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.COR (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. WI-38 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

32. MRID 484848-33 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Poliovirus type 1, Strain Chat, ATCC VR-1562" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – June 1, 2010. Project Number A08918.

This study was conducted against Poliovirus type 1 (Strain Chat; ATCC VR-1562), using Vero cells (obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minneapolis, MN; maintained in-house) as the host system. Three lots (Lot Nos. 09PUMA1, 09PUMA2, and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18111709.POL (copy provided). Use solutions were prepared by adding 5.0 mL of the product and 160.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 15.5°C at

55% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, and 10 mM HEPES. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. Sodium hypochlorite levels for each lot of use solution were measured and reported.

Note: The laboratory reported a failed study set up on February 11, 2010. In the study, a recoverable virus titer of at least 10^4 was not achieved for each of the five dried virus control replicates. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on March 1, 2010. See page 8 and Attachment I of the laboratory report.

Note: The study set up on March 1, 2010 also failed. In the study, a recoverable virus titer of at least 10^4 was not achieved for any of the five dried virus control replicates. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on March 19, 2010. See page 8 and Attachment II of the laboratory report.

33. MRID 484848-34 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A (H3N2) virus (Avian Reassortant), Strain A/Washington/897/80 X A/Mallard/New York/6750/78, ATCC VR-2072" for Puma, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – May 28, 2010. Project Number A09375.

This study was conducted against Avian Influenza A (H3N2) virus (Avian Reassortant) (Strain A/Washington/897/80 X A/Mallard/New York/6750/78; ATCC VR-2072), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested according to ATS Labs Protocol No. CX18031510.AFLU (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% (v/v) heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count,

cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

34. MRID 484848-35 "GLP Hard Surface Disinfection Evaluation Versus Rubella and Varicella-Zoster Viruses" for Puma, by Volha Dzyakanava. Study conducted at BioScience Laboratories, Inc. Study completion date – January 11, 2011. Laboratory Study Number 100828-404.

This study was conducted against Rubella virus (Strain M-33; ATCC VR-315) and against Varicella-Zoster virus (Strain Ellen; ATCC VR-1367), using BS-C-1 cells (African green monkey kidney cells; ATCC CCL-26); and/or LLC-MK2 cells (rhesus monkey epithelial cells; ATCC CCL-71; maintained in-house) as the host system. Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested according to BioScience Laboratories, Inc.'s Protocol #100828-404 (copy provided). Use solutions were prepared by adding 1 part of the product and 32 parts of 100 ppm AOAC synthetic hard water (titrated at 104 ppm; a 1:32 dilution). The stock virus cultures were adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 24 minutes at 24-27°C. Five replicates per product lot per virus were tested. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 4.5 minutes at 24-27°C. Following exposure, the plates were treated with Dey-Engley Broth with 0.5% sodium thiosulfate. Then, the plates were scraped with a cell scraper to re-suspend the contents. The neutralized virus-disinfectant mixtures were diluted serially in EMEM with 2% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Host cells in multi-well culture dishes were inoculated in quadruplicate with 1.0 mL of the dilutions. Varicella-Zoster virus cultures were incubated for 11 days at 37±2°C in a CO_2 incubator. Rubella virus cultures were incubated for 12 days at 37±2°C in a CO_2 incubator. The cultures were scored for the presence or absence of unspecified cytopathic effects and cytotoxicity. Controls included those for initial population, virus count, cell culture viability, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

35. MRID 484848-36 "AOAC Use-Dilution Method, Test Organism: *Streptococcus pyogenes* (ATCC 19615)" for Puma, by Anne Stemper. Study conducted at ATS Labs. Study completion date – May 13, 2010. Project Number A08926.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18010610.UD.1 (copy provided). Use solutions were prepared by adding 8.0 mL of the product and 256 mL of 100 ppm AOAC synthetic hard water, or the equivalent (titrated at 100 ppm; a 1:32 dilution). Testing was conducted on February 5, 2010, March 8, 2010, and April 2, 2010. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 25-30°C at 62-67.5% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Brain Heart Infusion Broth at least 30 minutes following the first transfer. All subcultures were incubated for 48±4 hours at 35-37°C in CO_2. The subcultures prepared on February 5, 2010 and April 2, 2010 were stored for 1 day at 2-8°C prior to examination. Following incubation, or incubation and storage, the subcultures were examined for the

presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: The laboratory reported a failed study set up on January 25, 2010. In the study, unacceptable growth was seen in the secondary subculture of 1 of 10 carriers for one product lot (i.e., Lot No. 09PUMA3). The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on February 5, 2010. On February 5, 2010, it was discovered that the hard water used on January 25, 2010 was 64 ppm, instead of 100 ppm. For this reason, all data generated on January 25, 2010 is considered invalid (i.e., Lot Nos. 09PUMA2 and 09PUMA3). See page 8 and Attachment I of the laboratory report.

Note: Repeat testing for Lot No. 09PUMA2 was intended to be repeated on March 8, 2010; however, Lot No. 09PUMA3 was inadvertently tested resulting in two sets of valid test results for Lot No. 09PUMA3. Testing for Lot No. 09PUMA2 was performed on April 2, 2010. See page 8 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

36. MRID 484848-37 "AOAC Use-Dilution Method, Test Organism: *Legionella pneumophila* (ATCC 33153)" for Puma, by Anne Stemper. Study conducted at ATS Labs. Study completion date – April 16, 2010. Project Number A08920.

This study was conducted against *Legionella pneumophila* (ATCC 33153). Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18010610.UD.3 (copy provided). Use solutions were prepared by adding 14.0 mL of the product and 448 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 25-30°C at 66% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Buffered Charcoal Yeast Extract Medium to neutralize. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Buffered Charcoal Yeast Extract Medium at least 30 minutes following the first transfer. All subcultures were incubated for 48 hours and 10 minutes at 35-37°C in 6.0% CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: The laboratory reported a failed study set up on January 25, 2010. Upon review of the raw data, it was discovered that the hard water used on January 25, 2010 was 64 ppm, instead of 100 ppm. For this reason, all data generated on January 25, 2010 are considered invalid. Testing was repeated on March 8, 2010. See page 8 and Attachment I of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

37. MRID 484848-38 "AOAC Use-Dilution Method, Test Organism: *Escherichia coli* O157:H7 (ATCC 35150)" for Puma, by Anne Stemper. Study conducted at ATS Labs. Study completion date – March 19, 2010. Project Number A08919.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two lots (Lot Nos. 09PUMA2 and 09PUMA3) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18010610.UD.2 (copy provided). Use solutions were prepared by adding 14.0 mL of the product and 448 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 35-37°C at 46.19% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: The laboratory reported a failed study set up on January 25, 2010. Upon review of the raw data, it was discovered that the hard water used on January 25, 2010 was 64 ppm, instead of 100 ppm. For this reason, all data generated on January 25, 2010 are considered invalid. Testing was repeated on March 8, 2010. See page 8 and Attachment I of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

38. MRID 484848-39 "A Quantitative Hard Surface Disinfection Evaluation of One Test Formulation Versus Spores of *Clostridium difficile* (ATCC 43598)" for Puma, by Terri Eastman. Study conducted at BioScience Laboratories, Inc. Study completion date – March 3, 2011. Laboratory Study Number 100827-204.

This study was conducted against spores of *Clostridium difficile* (ATCC 43598; spore suspension; obtained from the Centre for Research on Environmental Microbiology, University of Ottawa, Ottawa, Ontario, Canada). Three lots (Lot Nos. 10PUMA12, 10PUMA13, and 10PUMA14) of the product, Puma, were tested using the ASTM E 2197-02, Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides (modified). At least one of the product lots tested (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. Use solutions were prepared by adding 1.0 mL of the product and 8.0 mL of 100±10 ppm AOAC synthetic hard water (a 1:9 dilution). The culture of the challenge microorganism, obtained from the Centre for Research on Environmental Microbiology, was certified to contain 1.79×10^9 CFU/mL of spores. Use solutions were not tested in the presence of an organic soil load. Ten (10) sterile stainless steel disk carriers (1 cm in diameter; thickness not reported) per product lot were inoculated with 10 µL of a culture of the test organism. The carriers were dried in a desiccator containing anhydrous calcium chloride, under vacuum, for ~21.5 hours at room temperature. Each carrier was transferred, inoculated side up, to a sterile 15 mL vial, to which 50 µL of the use solution was added. In addition, three carriers were treated with 50 µL of a control solution (i.e., phosphate buffered saline with 0.1% (v/v) Tween-80 [PBSP80]). The test and control carriers remained exposed to the appropriate solution for 4 minutes at 21.4-24.1°C. Following exposure, 9.95 mL of phosphate buffered saline with 1% (w/v) sodium thiosulfate and

0.1% Tween-80 was added to each vial to neutralize. The contents of each vial were vortexed for a minimum of 90 seconds. The eluate from each vial was processed within 30 minutes of the addition of the neutralizer. The contents of each vial containing a carrier treated with the use solution were vacuum-filtered using separate, sterile analytical filter units with 0.45 µm pore-size membranes. Each vial was rinsed 3 times with 10 mL of PBSP80, with each rinse poured through the same membrane filter. Each filter unit was also rinsed with ~30 mL of PBSP80, and the rinsate was filtered. [The contents of each vial containing a carrier treated with the control solution were also filtered and plated.] Each membrane filter was plated on Brain Heart Infusion Agar modified for *Clostridium* species and incubated for 5 days at 35±2°C under anaerobic conditions. Following incubation, the subcultures were examined for the presence or absence of visible growth and colonies were counted manually using a hand-tally counter. Controls included those for carrier count, purity, sterility, carrier viability, neutralizer efficacy verification, and acid resistance at 2, 5, and 10 minutes. Total chlorine content for each lot of use solution was measured and reported.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

39. MRID 484848-40 "AOAC Use-Dilution Method, Test Organism: Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575)" for Puma, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – March 16, 2011. Project Number A10890.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575). Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18012111.UD.3 (copy provided). Use solutions were prepared by adding 13.0 mL of the product and 416 mL of 100 ppm AOAC synthetic hard water (titrated at 102 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 25-30°C at 65% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

Note: Antibiotic resistance of Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 6 mm) confirmed antibiotic resistance of Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575) to vancomycin. See page 9 and Table 6 of the laboratory report.

40. MRID 484848-41 "AOAC Use-Dilution Method, Test Organism: *Acinetobacter baumannii* (ATCC 19606)" for Puma, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – March 16, 2011. Project Number A10888.

This study was conducted against *Acinetobacter baumannii* (ATCC 19606). Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18012111.UD.1 (copy provided). Use solutions were prepared by adding 7.0

mL of the product and 224.0 mL of 100 ppm AOAC synthetic hard water (titrated at 98 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 60% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

41. MRID 484848-42 "AOAC Use-Dilution Method, Test Organism: *Streptococcus pneumoniae* (ATCC 6305)" for Puma, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – March 16, 2011. Project Number A10889.

This study was conducted against *Streptococcus pneumoniae* (ATCC 6305). Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested using ATS Laboratory Protocol No. CX18012111.UD.2 (copy provided). Use solutions were prepared by adding 13.0 mL of the product and 416 mL of 100 ppm AOAC synthetic hard water (titrated at 102 ppm; a 1:32 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 3 day old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 25-30°C at 65% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Brain Heart Infusion Broth at least 30 minutes following the first transfer. All subcultures were incubated for 48±4 hours at 35-37°C in 6.0% CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite levels and pH for each lot of use solution were measured and reported.

42. MRID 484848-43 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C virus – Confirmatory Assay," for Puma, by Shanen Conway. Study conducted at ATS Labs. Study completion date – March 18, 2011. Project Number A10878.

This confirmatory study, under the direction of Study Director Shanen Conway, was conducted against Bovine viral diarrhea virus (Strain Oregon C24v-genotype 1; obtained from the National Veterinary Services Laboratories, Ames, IA), using BT cells (bovine turbinate cells; ATCC CRL-1390; propagated in-house) as the host system. One lot (Lot No. 10PUMA13) of the product, Puma, was tested according to ATS Protocol No. CX18012011.BVD.2 (copy provided). A use solution was prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture contained 5% horse serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. Two replicates were tested. For the single product lot, separate dried virus films were exposed to 2.00 mL of the use

solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns. This initial dilution was considered the 10^{-1} dilution. A 0.2 mL aliquot of the test virus was re-suspended in 2.00 mL of the test substance, which equals a 1:10 dilution. The filtrates were diluted serially in Minimum Essential Medium with 5% (v/v) non-heat inactivated horse serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. BT cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., rounded, dark, with a granular appearance), cytotoxicity, and viability. On the final day of incubation, infectious virus was assayed by a direct immunofluorescence assay to verify the CPE reading. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

43. MRID 484848-44 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – March 21, 2011. Project Number A10892.

This study, under the direction of Study Director Mary J. Miller, was conducted against Bovine viral diarrhea virus (Strain Oregon C24v-genotype 1; obtained from the National Veterinary Services Laboratories, Ames, IA), using BT cells (bovine turbinate cells; ATCC CRL-1390; propagated in-house) as the host system. Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested according to ATS Protocol No. CX18012611.BVD (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture contained 5% horse serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns. This initial dilution was considered the 10^{-1} dilution. A 0.2 mL aliquot of the test virus was re-suspended in 2.0 mL of the test substance, which equals a 1:10 dilution. The filtrates were diluted serially in Minimum Essential Medium with 5% (v/v) non-heat inactivated horse serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. BT cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., rounded, dark, with a granular appearance), cytotoxicity, and viability. On the final day of incubation, infectious virus was assayed by a direct immunofluorescence assay to verify the CPE reading. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

44. MRID 484848-45 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Parainfluenza virus type 3, Strain C243, ATCC VR-93" for Puma, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – April 18, 2011. Project Number A10933.

This study was conducted against Parainfluenza virus type 3 (Strain C243; ATCC VR-93), using MDBK cells (bovine kidney cells; ATCC CCL-22; propagated in-house) as the host system. Two lots (Lot Nos. 10PUMA13 and 10PUMA14) of the product, Puma, were tested according to ATS Labs Protocol No. CX18012611.PFLU (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 32.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:32 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 40.0% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% (v/v) heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MDBK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: The laboratory reported a failed study set up on February 16, 2011. In the study, a disruption in the cell monolayer made it impossible to evaluate infectivity and/or cytotoxicity. No data were obtained. Testing was repeated on March 16, 2011. See page 8 and Attachment I of the laboratory report.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ carrier)
		Lot No. 09PUMA1	Lot No. 09PUMA2	Lot No. 09PUMA3	
4.5-Minute Exposure Time					
484848-12	*Staphylococcus aureus*	0/60	0/60	0/60	3.8×10^6
484848-14	*Salmonella enterica*	0/60	0/60	0/60	1.20×10^5
484848-16	*Pseudomonas aeruginosa* Test Date: 6/21/2010 Test Date: 7/21/2010	1/60 ---	2/60 0/60	0/60 ---	1.98×10^6 1.95×10^6
484848-36	*Streptococcus pyogenes*	---	---	1°=0/10 2°=0/10	1.52×10^5
		---	---	1°=0/10 2°=0/10	3.7×10^5
		---	1°=0/10 2°=0/10	---	6.1×10^5
484848-37	*Legionella pneumophila*	---	1°=0/10 2°=0/10	1°=0/10 2°=0/10	5.5×10^6
484848-38	*Escherichia coli* O157:H7	---	0/10	0/10	2.09×10^6

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Carrier Population (CFU/ carrier)
		Lot No. 10PUMA12		
4.5-Minute Exposure Time				
484848-13	*Staphylococcus aureus*	0/60	---	2.13×10^6
484848-15	*Salmonella enterica*	0/60	---	1.1×10^4
484848-17	*Pseudomonas aeruginosa*	0/60	---	6.2×10^5
		Lot No. 10PUMA13	Lot No. 10PUMA14	
484848-40	Vancomycin Resistant *Enterococcus faecalis*	0/10	0/10	1.11×10^6
484848-41	*Acinetobacter baumannii*	0/10	0/10	3.2×10^6
484848-42	*Streptococcus pneumoniae*	1°=0/10 2°=0/10	1°=0/10 2°=0/10	7.3×10^4

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 09PUMA2	Lot No. 09PUMA3	
9.5-Minute Exposure Time					
484848-18	Feline panleukopenia virus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-29	Canine parvovirus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
4.5-Minute Exposure Time					
484848-19	Herpes simplex virus type 2	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.7}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-20	Hepatitis A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.6}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-21	Respiratory syncytial virus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-25	Adenovirus type 2	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{7.6}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-26	Duck hepatitis B virus	10^{-2} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$, $10^{5.5}$, $10^{5.75}$, $10^{5.5}$, $10^{5.5}$ $TCID_{50}$/1.0 mL
		$TCID_{50}$/1.0 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
484848-27	Duck hepatitis B virus	10^{-2} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$, $10^{5.5}$, $10^{5.0}$, $10^{5.25}$, $10^{5.0}$ $TCID_{50}$/1.0 mL
		$TCID_{50}$/1.0 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
484848-28	Cytomegalovirus	$10^{-1.3}$ to $10^{-6.3}$ dilutions	Complete inactivation	Complete inactivation	$10^{5.3}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.8}$	$\leq 10^{0.8}$	

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 09PUMA2	Lot No. 09PUMA3	
484848-30	2009-H1N1 Influenza A virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.2}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-31	Influenza B virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.6}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-32	Human coronavirus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.1}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-34	Avian Influenza A (H3N2) virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.8}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-45	Parainfluenza virus type 3	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.45}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 10PUMA13	Lot No. 10PUMA14	
4.5-Minute Exposure Time					
484848-35	Varicella-Zoster virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.15}$ $TCID_{50}$/mL
		10^{-3} dilution	Cytopathic/ cytotoxic	Complete inactivation	
		10^{-4} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$10^{2.35}$	$10^{2.25}$	
		Log reduction	3.80 $\log_{10}$	3.90 $\log_{10}$	
484848-35	Rubella virus	10^{-2} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.60}$ $TCID_{50}$/mL
		$TCID_{50}$/mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
484848-43	Bovine viral diarrhea virus	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	$10^{5.5}$ and $10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	---	
484848-44	Bovine viral diarrhea virus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$, $10^{5.25}$, $10^{5.5}$, $10^{5.5}$, and $10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	Organism	Results				Dried Virus Count
			Lot No. 09PUMA 1	Lot No. 09PUMA 2	Lot No. 09PUMA 3	
4.5-Minute Exposure Time						
484848-22	Human immuno-deficiency virus type 1	10^{-1} to 10^{-7} dil	---	Complete inactivation		$10^{5.4}$ $TCID_{50}$/0.2 mL
		$TCID_{50}$/0.2 mL	---	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	Organism	Results				Dried Virus Count
			Lot No. 09PUMA 1	Lot No. 09PUMA 2	Lot No. 09PUMA 3	
484848-23	Human immuno-deficiency virus type 1 Note: 50% organic soil load	10^{-1} dilution	---	Cytotoxicity		$10^{5.6}$ $TCID_{50}$/0.2 mL
		10^{-2} to 10^{-7} dil	---	Complete inactivation		
		$TCID_{50}$/0.2 mL	---	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	---	≥ 4.1 $\log_{10}$	≥ 4.1 $\log_{10}$	
484848-24	Human immuno-deficiency virus type 1	10^{-1} to 10^{-7} dilutions	Complete inactivation			$10^{5.5}$ $TCID_{50}$/0.2 mL
		$TCID_{50}$/0.2 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484848-33	Poliovirus type 1	10^{-1} to 10^{-6} dilutions	Complete inactivation			$10^{4.6}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	Organism	Lot No.	Test Carriers	Control Carriers	Log Reduction
			(Mean $\log_{10}$ Density)		
484848-39	*Clostridium difficile* – spore form	10PUMA12	0.0	6.6	6.6
		10PUMA13	0.0	6.6	6.6
		10PUMA14	0.0	6.6	6.6

MRID/ Organism	Lot No.	Average No. Surviving (CFU/ swatch)	Microbes Initially Present (mean CFU/ swatch)	"Wash" Water Test Results (CFU/ mL)	"Wash" Water Control (CFU/ mL)	% Red.
Household Laundry Operations; 9.5-Minute Exposure Time						
484848-02	Methicillin Resistant *Staphylococcus aureus*					
	09PUMA2	$<1 \times 10^1$	1.22×10^7	$<1 \times 10^1$	8.6×10^4	>99.9
	09PUMA3	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
484848-03	*Staphylococcus aureus*					
	09PUMA1	$<1 \times 10^1$	4.1×10^7	$<1 \times 10^1$	1.29×10^5	>99.9
	09PUMA2	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
	09PUMA3	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
484848-04	*Klebsiella pneumoniae*					
	09PUMA1	$<1 \times 10^1$	1.1×10^7	$<1 \times 10^1$	1.20×10^6	>99.9
	09PUMA2	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
	09PUMA3	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
484848-05	*Pseudomonas aeruginosa*					
	09PUMA1	$<1 \times 10^1$	5.4×10^6	$<1 \times 10^1$	6.5×10^5	>99.9
	09PUMA2	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
	09PUMA3	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
484848-06	*Pseudomonas aeruginosa*					
	10PUMA12	$<1 \times 10^1$	2.89×10^6	$<1 \times 10^1$	4.8×10^5	>99.9

MRID/ Organism	Lot No.	Average No. Surviving (CFU/ swatch)	Microbes Initially Present (mean CFU/ swatch)	"Wash" Water Test Results (CFU/ mL)	"Wash" Water Control (CFU/ mL)	% Red.
High Efficiency Washer Operations; 9.5-Minute Exposure Time						
484848-07	Methicillin Resistant *Staphylococcus aureus*					
	10PUMA13	2×10^3	3.0×10^7	$<1 \times 10^1$	1.17×10^6	>99.9
	10PUMA14	5×10^2		$<1 \times 10^1$		>99.9
484848-08	*Staphylococcus aureus*					
	10PUMA13	$<1 \times 10^1$	7.9×10^6	$<1 \times 10^1$	3.0×10^5	>99.9
	10PUMA14	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
484848-09	*Klebsiella pneumoniae*					
	10PUMA13	$<1 \times 10^1$	4.9×10^6	$<1 \times 10^1$	5.7×10^6	>99.9
	10PUMA14	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
484848-10	*Pseudomonas aeruginosa*					
	10PUMA13	$<1 \times 10^1$	3.2×10^6	$<1 \times 10^1$	1.80×10^6	>99.9
	10PUMA14	$<1 \times 10^1$		$<1 \times 10^1$		>99.9
484848-11	*Pseudomonas aeruginosa*					
	10PUMA12	$<1 \times 10^1$	3.4×10^6	$<1 \times 10^1$	1.83×10^6	>99.9

VI. CONCLUSION

1. The submitted efficacy data **support** the use of 1:32 dilution of the product, Puma, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 100 ppm hard water and a 5% organic soil load for a 4.5-minute contact time:

Staphylococcus aureus	MRID 484848-12 and -13
Salmonella enterica	MRID 484848-14 and -15
Pseudomonas aeruginosa	MRID 484848-16 and -17

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. [Note that repeat testing was conducted on one product lot against *Pseudomonas aeruginosa* to evaluate for false positives.] In testing against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa*, at least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2. The submitted efficacy data **support** the use of a 1:32 dilution of the product, Puma, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 100 ppm hard water and a 5% organic soil load for a 4.5-minute contact time:

Streptococcus pyogenes	MRID 484848-36
Legionella pneumophila	MRID 484848-37
Escherichia coli O157:H7	MRID 484848-38

Vancomycin Resistant *Enterococcus faecalis*	MRID 484848-40
Acinetobacter baumannii	MRID 484848-41
Streptococcus pneumoniae	MRID 484848-42

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

3. The submitted efficacy data **support** the use of a 1:32 dilution of the product, Puma, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 100 ppm hard water and a 5% organic soil load (a 5% organic soil load in addition to 100% duck serum against Duck hepatitis B virus) for a 4.5-minute contact time (a 9.5-minute contact time against Feline panleukopenia virus and Canine parvovirus):

Feline panleukopenia virus	MRID 484848-18
Herpes simplex virus type 2	MRID 484848-19
Hepatitis A virus	MRID 484848-20
Respiratory syncytial virus	MRID 484848-21
Human immunodeficiency virus type 1	MRID 484848-22 and -24
Adenovirus type 2	MRID 484848-25
Duck hepatitis B virus	MRID 484848-26 and -27
Cytomegalovirus	MRID 484848-28
Canine parvovirus	MRID 484848-29
2009-H1N1 Influenza A virus	MRID 484848-30
Influenza B virus	MRID 484848-31
Human coronavirus	MRID 484848-32
Poliovirus type 1	MRID 484848-33
Avian Influenza A (H3N2) virus	MRID 484848-34
Varicella-Zoster virus	MRID 484848-35
Rubella virus	MRID 484848-35
Bovine viral diarrhea virus	MRID 484848-43 and -44
Parainfluenza virus type 3	MRID 484848-45

Recoverable virus titers of at least 10^4 were achieved. In studies against Varicella-Zoster virus, cytotoxicity and/or cytopathic/cytotoxic effects were observed in the 10^{-2} and 10^{-3} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against all other viruses, cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

In studies against Duck hepatitis B virus, the initial and confirmatory studies were performed at the same laboratory, but under the direction of different study directors. Both the initial and confirmatory studies tested five replicates per product lot.

In studies against Bovine viral diarrhea virus, the initial and confirmatory studies were performed at the same laboratory, but under the direction of different study directors. The initial study tested five replicates per product lot; the confirmatory study tested two replicates per product lot. The confirmatory study tested one product lot, not the standard two product lots.

4. The submitted efficacy data (MRID 484848-23) **support** the use of a 1:32 dilution of the product, Puma, as a disinfectant with virucidal activity against Human immunodeficiency virus type 1 on hard, non-porous surfaces in the presence of 100 ppm hard water and a 50% organic soil load for a 4.5-minute contact time. A recoverable virus titer of at least 10^4 was achieved. Cytotoxicity was observed in the 10^{-1} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

5. The submitted efficacy data (MRID 484848-39) **support** the use of a **1:9** dilution of the product, Puma, as a disinfectant against spores of *Clostridium difficile* on pre-cleaned, hard, non-porous surfaces in the presence of 100 ppm hard water for a 4-minute contact time. A >6-log reduction in viable spores was reported by the laboratory. At least one of the product lots tested was at least 60 days old at the time of testing. Carrier counts met the acceptance criterion of $>10^6$ spores/carrier. Neutralizer efficacy verification testing demonstrated that the neutralizer was effective in neutralizing the antimicrobial activity of the product. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Test spores showed resistance to acid for >10 minutes.

6. The submitted efficacy data support the use of a 1:583 dilution of the product, Puma, to sanitize laundry during household laundry operations against the following microorganisms in the presence of 100 ppm hard water and a 5% organic soil load for a 9.5 minute contact time:

Methicillin Resistant *Staphylococcus aureus*	MRID 484848-02
Staphylococcus aureus	MRID 484848-03
Klebsiella pneumoniae	MRID 484848-04
Pseudomonas aeruginosa	MRID 484848-05 and -06

A 99.9% reduction in population was observed for both the fabric swatches and the "wash" water. Three fabric swatches per product lot were tested. At least one of the product lots tested against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* was at least 60 days old at the time of testing. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

7. The submitted efficacy data **support** the use of a 1:583 dilution of the product, Puma, to sanitize laundry during high efficiency washer operations against the following microorganisms in the presence of 100 ppm hard water and a 5% organic soil load for a 9.5 minute contact time:

Methicillin Resistant *Staphylococcus aureus*	MRID 484848-07
Staphylococcus aureus	MRID 484848-08
Klebsiella pneumoniae	MRID 484848-09
Pseudomonas aeruginosa	MRID 484848-10 and -11

A 99.9% reduction in population was observed for both the fabric swatches and the "wash" water. Three fabric swatches per product lot were tested. At least one of the product lots tested against *Pseudomonas aeruginosa* was at least 60 days old at the time of testing. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

VII. LABEL

1. The proposed label claims that a 1:32 dilution of the product, Puma, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 5-minute contact time (10-minute contact time against *Pseudomonas aeruginosa*, Feline panleukopenia virus, and Canine parvovirus):

Pseudomonas aeruginosa
Salmonella enterica
Staphylococcus aureus

Acinetobacter baumannii
Escherichia coli O157:H7
Legionella pneumophila
Streptococcus pneumoniae
Streptococcus pyogenes
Vancomycin Resistant *Enterococcus faecalis*

Adenovirus type 2
Avian Influenza A (H3N2) virus
Canine parvovirus
Cytomegalovirus
Feline panleukopenia virus
Hepatitis A virus
Hepatitis B virus (as Duck hepatitis B virus)
Hepatitis C virus (as Bovine viral diarrhea virus)
Herpes simplex virus type 2
Human coronavirus
Human immunodeficiency virus type 1
2009-H1N1 Influenza A virus
Influenza B virus
Parainfluenza virus type 3
Poliovirus type 1
Respiratory syncytial virus
Rubella virus
Varicella-Zoster virus

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that a **1:10** dilution of the product, Puma, is an effective disinfectant against *Clostridium difficile* spores on pre-cleaned, hard, non-porous surfaces for a 10-minute contact time. **This claim is not acceptable**. Efficacy testing was conducted using a **1:9 dilution** of the product, which is more concentrated than a 1:10 dilution of the product. **The proposed label must be revised to specify the use of a 1:9 dilution of the product to disinfect against *Clostridium difficile* spores.**

3. The proposed label claims that adding a ½ cup of the product, Puma, to a standard washing machine (i.e., a 1:585 dilution) will sanitize laundry against the following microorganisms:

Staphylococcus aureus
Klebsiella pneumoniae

Pseudomonas aeruginosa
Methicillin Resistant *Staphylococcus aureus*

These claims are acceptable as they are supported by the submitted data.

4. The proposed label claims that adding a ½ cup of the product, Puma, to a high efficiency washing machine (i.e., a 1:585 dilution) will sanitize laundry against the following microorganisms:

Staphylococcus aureus
Klebsiella pneumoniae
Pseudomonas aeruginosa
Methicillin Resistant *Staphylococcus aureus*

These claims are acceptable as they are supported by the submitted data.