UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION
PREVENTION

MEMORANDUM

DATE: April 14, 2012

SUBJECT: Efficacy Review for PDI Sani-Cloth Bleach Wipes;
EPA Reg. No. 9480-8;
DP Barcode: D397683

FROM: Lorilyn M. Montford LM 4/19/12
Product Science Branch
Antimicrobials Division (7510P)

THRU: Dr. Tajah Blackburn, Team Leader
Product Science Branch
Antimicrobials Division (7510P) 4/19/12

TO: Jacqueline Campbell-McFarlane, PM 34/Jaclyn Carl
Regulatory Management Branch II
Antimicrobial Division (7510P)

APPLICANT: Professional Disposables International, Inc.
Two Nice-Pak Park
Orangeburg, NY 10962-1376

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Sodium Hypochlorite	0.63%
Inert Ingredients	99.37%
Total	100.00%

I BACKGROUND

The product, PDI Sani-Cloth Bleach Wipes (EPA Reg. No. 9480-8), is an EPA-approved disinfectant (bactericide, fungicide, tuberculocide, virucide) and deodorizer for use on hard, non-porous surfaces in household, commercial, institutional, animal care, and hospital or medical environments. The applicant requested to amend the registration of this product to add new claims for effectiveness as a disinfectant against additional microorganisms, including *Clostridium difficile* spores. The registration amendment also includes changes to the product formulation. The label states that the product is effective in the presence of 5% blood serum contamination. 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's representative to EPA (dated December 21, 2011), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), twelve studies (MRID 487037-02 through 487037-04 and MRID 487037-06 through 487037-14), Statements of No Data Confidentiality Claims for all twelve studies, and the proposed label. The data package also included a study assigned MRID 487037-01 (a discussion of efficacy) and a study assigned MRID 487037-05 (a custom towelette wetness test).

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: ambulance equipment, animal equipment, automatic feeders, bathroom fixtures, bathtubs, bed railings, blood glucose meters, blood pressure monitors, cabinets, cages, carts, chairs, changing tables, computers (keyboards, mice, monitors), counters, cribs, dental chairs, dental countertops, dental unit instrument trays, desks, diagnostic equipment, diaper pails, display cabinets, doorknobs, endodontic equipment, examination tables, faucets, fax machines, feed racks, filing cabinets, floors, fountains, garbage cans, grocery cart handles, gurneys, gym equipment, handles, hampers, handrails, headsets, infant incubators, infant warmers, isolettes, IV poles, laboratory equipment, lights, loupes, machine exteriors, operating room tables and lights, operatory light switches, oxygen hoods, patient monitoring equipment, patient support and delivery equipment, pens, physical therapy equipment, showers, sinks, slit lamps, spine back boards, stalls, steering wheels, stethoscopes, stretchers, stools, tables, telephones, toilet seats, trashcans, toys, troughs, ultrasound transducers and probes, urinals, vanity tops, watering appliances, and work stations. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: Formica, glass, glazed tile, metal, plastic, and stainless steel. Directions on the proposed label provide the following information regarding use of the product:

> As a disinfectant against bacteria, TB, fungi, and viruses: Use a wipe to remove gross filth or heavy soil. Unfold a clean wipe and thoroughly wet surface. Treated surface must remain visibly wet for a full 4 minutes (1 minute against bacteria and viruses; 2 minutes against *Mycobacterium bovis* BCG; 4 minutes against fungi). Use additional wipes, if needed, to assure continuous 4-minute wet contact time.
>
> As a disinfectant against *Clostridium difficile* spores: Fecal matter/waste must be

thoroughly cleaned from surfaces/objects before disinfection. Cleaning is to include vigorous wiping and/or scrubbing, until all visible soil is removed. Special attention is needed for high-touch surfaces. Surfaces in patient rooms are to be cleaned in an appropriate manner, such as from right to left, or left to right, on horizontal surfaces, and top to bottom on vertical surfaces, to minimize spreading of the spores. Restrooms are to be cleaned last. Do not reuse soiled cloths. Unfold a clean wipe and thoroughly wet surface. Treated surface must remain visibly wet for a full 4 minutes. Use additional wipes, if needed, to assure continuous 4-minute wet contact time.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Antimicrobial Products for Use on Hard Surfaces Using Pre-saturated or Impregnated Towelettes

Towelette products represent a unique combination of antimicrobial chemical and applicator, pre-packaged as a unit in fixed proportions. As such, the complete product, as offered for sale, should be tested according to the directions for use to ensure the product's effectiveness in treating hard surfaces. The standard test methods available for hard surface disinfectants and sanitizers, if followed exactly, would not closely simulate the way a towelette product is used. Agency guidelines recommend that a simulated-use test be conducted by modifying the standard test methods. Agency guidelines further recommend that instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the slides after a specified holding time. Performance standards of the standard test methods must be met. These Agency standards are presented in EPA Pesticide Assessment Guidelines, Subdivision G, §9 t-2(h), Pre-saturated or impregnated towelettes; and the April 12, 2001 EPA Memorandum, Draft Interim Guidance for Non-Residual Sanitization of Hard Inanimate Food Contact Surfaces Using Pre-Saturated Towelettes.

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: 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.

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.

Supplemental Claims

An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 487037-02 "A Quantitative Hard Surface Disinfection Evaluation of One Test Formulation Versus Spores of *Clostridium difficile*" (ATCC 43598), for PDI Sani-Cloth Bleach Wipes, by Lisa Lehman. Study conducted at BioScience Laboratories, Inc. Study completion date – October 20, 2011. Laboratory Study Number 110726-204 A.

This study was conducted against spores of *Clostridium difficile* (ATCC 43598). One lot (Lot No. AE-756-168D) of the product, PDI Sani-Cloth Bleach Wipes, was tested using the

ASTM Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides, E 2197 (modified). The single product lot tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. The fluid present in the disinfectant wipes was expressed and collected in sterile containers. A spore suspension of the challenge microorganism was received from the Centre for Research on Environmental Microbiology at the University of Ottawa, Ottawa, Ontario, Canada. The product was not tested in the presence of a 5% organic soil load. Ten (10) sterile stainless steel disk carriers (1 cm diameter) were inoculated with 10 µL of the spore suspension. The carriers were dried in a vacuum desiccator containing anhydrous calcium chloride for ~21.75 hours. Each carrier was transferred, inoculated side up, to a sterile 15 mL vial, to which 50 µL of the expressed liquid was added. The carriers remained exposed to the expressed liquid for 4 minutes at 23.6-24.1°C. Following exposure, 9.95 mL of Butterfield's Phosphate Buffer solution with product neutralizers was added to each vial to neutralize. The vials were shaken vigorously for ~1 minute. Within 30 minutes, the entire liquid volume of each vial was individually filtered through a pre-wetted filter using a vacuum pump. Each vial was rinsed three times with 15 mL of 0.9% Sodium Chloride Irrigation (SCI), and the rinsate was filtered. Each filter unit was rinsed with ~40 mL of SCI, and the rinsate was filtered. Each membrane filter was placed, up side up, on the surface of a Petri plate containing Brain Heart Infusion agar modified for *Clostridium* species. Each Petri plate containing a membrane filter was incubated for 7 days at 30±2°C under anaerobic conditions. Following incubation, final plate counts were made. Controls included those for carrier count, purity, sterility, verification of neutralizer efficacy (the single product lot), and acid resistance at 2, 5, and 10 minutes. Efficacy data was generated at the lower certified limit.

2. MRID 487037-03 "A Quantitative Hard Surface Disinfection Evaluation of One Test Formulation Versus Spores of *Clostridium difficile*" (ATCC 43598), for PDI Sani-Cloth Bleach Wipes, by Lisa Lehman. Study conducted at BioScience Laboratories, Inc. Study completion date – October 20, 2011. Laboratory Study Number 110726-204 B.

This study was conducted against spores of *Clostridium difficile* (ATCC 43598). One lot (Lot No. AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, was tested using the ASTM Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides, E 2197 (modified). The single product lot tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. The fluid present in the disinfectant wipes was expressed and collected in sterile containers. A spore suspension of the challenge microorganism was received from the Centre for Research on Environmental Microbiology at the University of Ottawa, Ottawa, Ontario, Canada. The product was not tested in the presence of a 5% organic soil load. Ten (10) sterile stainless steel disk carriers (1 cm diameter) were inoculated with 10 µL of the spore suspension. The carriers were dried in a vacuum desiccator containing anhydrous calcium chloride for ~21.75 hours. Each carrier was transferred, inoculated side up, to a sterile 15 mL vial, to which 50 µL of the expressed liquid was added. The carriers remained exposed to the expressed liquid for 4 minutes at 23.6-24.1°C. Following exposure, 9.95 mL of Butterfield's Phosphate Buffer solution with product neutralizers was added to each vial to neutralize. The vials were shaken vigorously for ~1 minute. Within 30 minutes, the entire liquid volume of each vial was individually filtered through a pre-wetted filter using a vacuum pump. Each vial was rinsed three times with 15 mL of 0.9%

Sodium Chloride Irrigation (SCI), and the rinsate was filtered. Each filter unit was rinsed with ~40 mL of SCI, and the rinsate was filtered. Each membrane filter was placed, up side up, on the surface of a Petri plate containing Brain Heart Infusion agar modified for *Clostridium* species. Each Petri plate containing a membrane filter was incubated for 7 days at 30±2°C under anaerobic conditions. Following incubation, final plate counts were made. Controls included those for carrier count, purity, sterility, verification of neutralizer efficacy (the single product lot), and acid resistance at 2, 5, and 10 minutes. Efficacy data was generated at the lower certified limit.

3. MRID 487037-04 "A Quantitative Hard Surface Disinfection Evaluation of One Test Formulation Versus Spores of *Clostridium difficile*" (ATCC 43598), for PDI Sani-Cloth Bleach Wipes, by Lisa Lehman. Study conducted at BioScience Laboratories, Inc. Study completion date – October 20, 2011. Laboratory Study Number 110726-204 C.

This study was conducted against spores of *Clostridium difficile* (ATCC 43598). One lot (Lot No. AE-756-170D) of the product, PDI Sani-Cloth Bleach Wipes, was tested using the ASTM Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides, E 2197 (modified). The single product lot tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. The fluid present in the disinfectant wipes was expressed and collected in sterile containers. A spore suspension of the challenge microorganism was received from the Centre for Research on Environmental Microbiology at the University of Ottawa, Ottawa, Ontario, Canada. The product was not tested in the presence of a 5% organic soil load. Ten (10) sterile stainless steel disk carriers (1 cm diameter) were inoculated with 10 µL of the spore suspension. The carriers were dried in a vacuum desiccator containing anhydrous calcium chloride for ~21.75 hours. Each carrier was transferred, inoculated side up, to a sterile 15 mL vial, to which 50 µL of the expressed liquid was added. The carriers remained exposed to the expressed liquid for 4 minutes at 23.6-24.1°C. Following exposure, 9.95 mL of Butterfield's Phosphate Buffer solution with product neutralizers was added to each vial to neutralize. The vials were shaken vigorously for ~1 minute. Within 30 minutes, the entire liquid volume of each vial was individually filtered through a pre-wetted filter using a vacuum pump. Each vial was rinsed three times with 15 mL of 0.9% Sodium Chloride Irrigation (SCI), and the rinsate was filtered. Each filter unit was rinsed with ~40 mL of SCI, and the rinsate was filtered. Each membrane filter was placed, up side up, on the surface of a Petri plate containing Brain Heart Infusion agar modified for *Clostridium* species. Each Petri plate containing a membrane filter was incubated for 7 days at 30±2°C under anaerobic conditions. Following incubation, final plate counts were made. Controls included those for carrier count, purity, sterility, verification of neutralizer efficacy (the single product lot), and acid resistance at 2, 5, and 10 minutes. Efficacy data was generated at the lower certified limit.

4. MRID 487037-05 "Custom Towelette Wetness Test" for PDI Sani-Cloth Bleach Wipes, by Ashley Rex. Study conducted at Antimicrobial Test Laboratories. Study completion date – November 3, 2011. Protocol Number P1086.

This study was conducted to demonstrate the wetness of the surfaces after wiping.

Three lots (Lot Nos. AE-756-168D, AE-756-169D, and AE-756-170D) were tested. All lots were ≥0 days old at the time of testing. Three carriers were tested per lot. The carriers consistent of 12" x 12" Formica tiles. The test carriers were wiped with a damp cloth and allowed to air dry for at least 3 hours, to ensure that only completely dry carriers were used in the study. The back of each carrier was labeled with a unique identifier. Temperature and humidity of the testing area were recorded immediately before testing occurred and immediately after completion of all testing. Three towelettes were evaluated per lot. To begin the study, laboratory staff initiated the video recording of the study. The first test carrier was weighted prior to treatment using a calibrated laboratory balance with an accuracy of 0.01 g. The test carrier was placed on a flat lab bench. Each towelette contained in a single packet was examined for uniform wetness. The wipe was unfolded and then refolded in half twice. The test carrier was wiped in a side to side motion, each stroke slightly overlapping the last; until the entire test carrier was completely covered (6 total strokes with the wipe were used on each 12" x 12" carrier). Immediately after the entire test surface area was treated, the carrier was placed on the balance and the initial wet weight of the test carrier was recorded. The timer was started. The test carrier was allowed to sit undisturbed for the 5 minute contact time. Upon completion of the contact time, the final wet eight of the test carrier was recorded. Immediately after weighing, a single sheet of unfolded tissue paper was wiped across the test surface to assist in visualization of wetness for the laboratory technician and video camera due to the clean, colorless nature of the test substance. Visual wetness of the tissue paper was defined as wetness. The paper wetness was observed and recorded.

5. MRID 487037-06 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Escherichia coli* (ATCC 11229)" for PDI Sani-Cloth Bleach Wipes, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – October 12, 2011. Project Number A11900.

This study was conducted against *Escherichia coli* (ATCC 11229). Two lots (Lot Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 2009 (modified for towelette products). The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inch x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over a ~1 inch x 1 inch area of each carrier. The carriers were dried for 30 minutes at 35-37°C at 50% relative humidity (which differs from the AOAC method specification of 30-40 minutes at 37°C). Each carrier was wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat 10 carriers. A different area of towelette was used to wipe each carrier. The carriers were allowed to remain wet for 1 minute at 22°C at 63% relative humidity. Following the exposure period, individual carriers were transferred to 40 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method.] All subcultures were incubated for 48±2 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 (both product lots). Efficacy data was generated at the lower certified limit.

6. MRID 487037-07 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Escherichia coli* - NDM-1 positive (CDC 1001728)" for PDI Sani-Cloth Bleach Wipes, by Anne Stemper. Study conducted at ATS Labs. Study completion date – October 14, 2011. Project Number A11868.

This study was conducted against *Escherichia coli* - NDM-1 positive (CDC 1001728). Two lots (Lot Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 2009 (modified for towelette products). The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inch x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over a ~1 inch x 1 inch area of each carrier. The carriers were dried for 30 minutes at 35-37°C at 50% relative humidity. Each carrier was wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat 10 carriers. A different area of towelette was used to wipe each carrier. The carriers were allowed to remain wet for 1 minute at 22.91°C at 45.60% relative humidity. Following the exposure period, individual carriers were transferred to 40 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method.[All subcultures were incubated for 48±2 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 (both product lots), and antibiotic resistance. Efficacy data was generated at the lower certified limit.

Note: Antibiotic sensitivity testing of *Escherichia coli* - NDM-1 positive (CDC 1001728) was performed on a representative culture from the day of testing. The testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, MN. The testing was not performed under EPA GLP regulations. An E Test Bioassay confirmed antibiotic resistance of *Escherichia coli* - NDM-1 positive (CDC 1001728) to ertapenem. The minimum inhibitory concentrations confirmed antibiotic resistance of *Escherichia coli* - NDM-1 positive (CDC 1001728) to amikacin, ampicillin, ampicillin/sulbactam, cefazolin, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, levofloxacin, tobramycin, trimethoprim/sulfa, and meropenem. See page 9 and Attachment I of the laboratory report.

7. MRID 487037-08 "Pre-Saturated Towelettes for Hard Surface Disinfection - Quantitated, Test Organism: *Bordetella pertussis* (ATCC 12743)" for PDI Sani-Cloth Bleach Wipes, by Becky Lien. Study conducted at ATS Labs. Study completion date – October 11, 2011. Project Number A11889.

This study was conducted against *Bordetella pertussis* (ATCC 12743). Two lots (Lot Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000 (modified for towelette products). The

product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was not prepared in accordance with the published AOAC method, with deviations for the test organism. Deviations included: (1) the test culture was initiated by inoculating agar plates with the test organism; (2) daily transfers were not made; (3) the culture was incubated for 4-7 days at 35-37°C (which differs from the AOAC method specification of 48 hours for all bacterial cultures except *Pseudomonas aeruginosa*); and (4) following incubation, the culture was suspended in sterile diluent to approximately match a 4.0 McFarland turbidity standard. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inch x 1 inch) per product lot were inoculated with 10.0 µL of a 4-7 day old suspension of test organism. Inoculum was uniformly spread over a ~1 inch x 1 inch area of each carrier. The carriers were dried for 30-34 minutes at 25-30°C at 60-66% relative humidity. Each carrier was wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat 10 carriers. A different area of towelette was used to wipe each carrier. The carriers were allowed to remain wet for 1 minute at 20.84-21°C at 50.52-65% relative humidity. Following the exposure period, individual carriers were transferred to 40 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. Tubes containing neutralizer were gently shaken. The entire volume of the subculture broths was individually filtered through a pre-wetted filter using a vacuum pump. Each filter membrane was then washed, removed from the filter unit, and placed on a tryptic soy agar with 5% sheep's blood plate. All subcultures were incubated for 3-7 days 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 (both product lots). Efficacy data was generated at the lower certified limit.

8. MRID 487037-09 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527)" for PDI Sani-Cloth Bleach Wipes, by Anne Stemper. Study conducted at ATS Labs. Study completion date – October 12, 2011. Project Number A11869.

This study was conducted against *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527). Two lots (Lot Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 2009 (modified for towelette products). The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inch x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over a ~1 inch x 1 inch area of each carrier. The carriers were dried for 30 minutes at 35-37°C at 40% relative humidity. Each carrier was wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat 10 carriers. A different area of towelette was used to wipe each carrier. The carriers were allowed to remain wet for 1 minute at 21.49°C at 50.76% relative humidity. Following the exposure period, individual carriers were transferred to 40 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method.[All subcultures were incubated for 48±2 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 (both product lots), and antibiotic resistance. Efficacy data was generated at the lower certified limit.

Note: Antibiotic sensitivity testing of *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527) was performed on a representative culture from the day of testing. The testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, MN. The testing was not performed under EPA GLP regulations. An E Test Bioassay confirmed antibiotic resistance of *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527) to ertapenem. The minimum inhibitory concentrations confirmed antibiotic resistance of *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527) to amikacin, ampicillin, ampicillin/sulbactam, cefazolin, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, levofloxacin, tobramycin, trimethoprim/sulfa, and meropenem. See page 9 and Attachment I of the laboratory report.

9. MRID 487037-10 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Burkholderia cepacia* (ATCC 25416)" for PDI Sani-Cloth Bleach Wipes, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – October 12, 2011. Project Number A11871.

This study was conducted against *Burkholderia cepacia* (ATCC 25416). Two lots (Lot Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 2009 (modified for towelette products). The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inch x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over a ~1 inch x 1 inch area of each carrier. The carriers were dried for 30 minutes at 35-37°C at 40% relative humidity. Each carrier was wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat 10 carriers. A different area of towelette was used to wipe each carrier. The carriers were allowed to remain wet for 1 minute at 20°C at 67% relative humidity. Following the exposure period, individual carriers were transferred to 40 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method.[All subcultures were incubated for 48±2 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 (both product lots). Efficacy data was generated at the lower certified limit.

10. MRID 487037-11 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Serratia marcescens* (ATCC 14756)" for PDI Sani-Cloth Bleach Wipes, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – October 12, 2011. Project Number A11872.

This study was conducted against *Serratia marcescens* (ATCC 14756). Two lots (Lot

Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 2009 (modified for towelette products). The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inch x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over a ~1 inch x 1 inch area of each carrier. The carriers were dried for 35 minutes at 35-37°C at 42-43% relative humidity. Each carrier was wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat 10 carriers. A different area of towelette was used to wipe each carrier. The carriers were allowed to remain wet for 1 minute at 20°C at 67% relative humidity. Following the exposure period, individual carriers were transferred to 40 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method.] All subcultures were incubated for 48±2 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 (both product lots). Efficacy data was generated at the lower certified limit.

11. MRID 487037-12 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Enterobacter cloacae* NDM-1 positive (CDC 1000654)" for PDI Sani-Cloth Bleach Wipes, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – October 13, 2011. Project Number A11870.

This study was conducted against *Enterobacter cloacae* NDM-1 positive (CDC 1000654). Two lots (Lot Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 2009 (modified for towelette products). The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inch x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over a ~1 inch x 1 inch area of each carrier. The carriers were dried for 30 minutes at 35-37°C at 40-41% relative humidity. Each carrier was wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat 10 carriers. A different area of towelette was used to wipe each carrier. The carriers were allowed to remain wet for 1 minute at 22.3°C at 52.9% relative humidity. Following the exposure period, individual carriers were transferred to 40 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method.] All subcultures were incubated for 48±2 hours at 25-30°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 (both product lots), and antibiotic resistance. Efficacy data was generated at the lower certified limit.

Note: Antibiotic sensitivity testing of *Enterobacter cloacae* NDM-1 positive (CDC 1000654) was performed on a representative culture from the day of testing. The testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, MN. The testing was not performed under EPA GLP regulations. An E Test Bioassay confirmed antibiotic resistance of *Enterobacter cloacae* NDM-1 positive (CDC 1000654) to ertapenem. The minimum inhibitory concentrations confirmed antibiotic resistance of *Enterobacter cloacae* NDM-1 positive (CDC 1000654) to amikacin, ampicillin, ampicillin/sulbactam, cefazolin, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, levofloxacin, tobramycin, trimethoprim/sulfa, and meropenem. See page 9 and Attachment I of the laboratory report.

12. MRID 487037-13 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, Virus: Cytomegalovirus" for PDI Sani-Cloth Bleach Wipes, by Shanen Conway. Study conducted at ATS Labs. Study completion date – October 18, 2011. Project Number A11901.

This study was conducted against Cytomegalovirus (Strain AD-169; ATCC VR-538), using MRC-5 cells (human embryonic lung fibroblast cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested according to ATS Labs Protocol No. NPP01080811.CMV (copy provided). The product was received ready-to-use, as a pre-saturated towelette. 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 a defined area (~8 x 8 cm) on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, individual carriers were wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat one carrier. The carriers were allowed to remain wet for 1 minute at 20.0°C. Following exposure, 4.00 mL of test medium was added to each Petri dish and each Petri dish was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures 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 product 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 28 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 (both product lots). Viral and cytotoxicity titers were calculated by the method of Spearman Karber. Efficacy data was generated at the lower certified limit.

13. MRID 487037-14 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, Virus: Influenza B virus, Strain B/HongKong/5/72, ATCC VR-823" for PDI Sani-Cloth Bleach Wipes, by Shanen Conway. Study conducted at ATS Labs. Study completion date – October 11, 2011. Project Number A11902.

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. AE-756-168D and AE-756-169D) of the product, PDI Sani-Cloth Bleach Wipes, were tested according to ATS Labs Protocol No. NPP01080811.FLUB (copy provided). The product was received ready-to-use, as a pre-saturated towelette. 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 a defined area (~8 x 8 cm) on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 40% relative humidity. For each lot of product, individual carriers were wiped with a saturated towelette with two wipes back and forth for a total of four passes. One towelette was used to treat one carrier. The carriers were allowed to remain wet for 1 minute at 21.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish and each Petri dish was 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 (both product lots). Viral and cytotoxicity titers were calculated by the method of Spearman Karber. Efficacy data was generated at the lower certified limit.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Carrier Population (Average log_{10})
		Lot No. AE-756-168D	Lot No. AE-756-169D	
1-Minute Exposure Time				
487037-06	*Escherichia coli*	0/10	0/10	6.58
487037-07	*Escherichia coli* - NDM-1 positive	0/10	0/10	6.81
487037-08	*Bordetella pertussis* Test Date: 9/2/2011	0/10	---	3.7×10^6 (CFU/carrier)
	Bordetella pertussis Test Date: 9/12/2011	---	0/10	7.6×10^5 (CFU/carrier)
487037-09	*Klebsiella pneumoniae* - NDM-1 positive	0/10	0/10	6.01
487037-10	*Burkholderia cepacia*	0/10	0/10	6.16
487037-11	*Serratia marcescens*	0/10	0/10	7.54
487037-12	*Enterobacter cloacae* NDM-1 positive	0/10	0/10	7.03

MRID Number	Organism	Results			Dried Virus Count
			Lot No. AE-756-168D	Lot No. AE-756-169D	
1-Minute Exposure Time					
487037-13	Cytomegalovirus	$10^{-1.3}$ to $10^{-6.3}$ dilutions	Complete inactivation	Complete inactivation	$10^{4.80}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.80}$	$\leq 10^{0.80}$	
487037-14	Influenza B virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.50}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	

MRID Number	Organism	Lot No.	Mean Log_{10} Density Surviving	Mean Log_{10} Density of Numbers Control	Log Reduction
			(CFU/ carrier)		
4-Minute Exposure Time					
487037-02	*Clostridium difficile* spores	AE-756-168D	0.0	6.9	6.9
487037-03	*Clostridium difficile* spores	AE-756-169D	0.3	6.9	6.6
487037-04	*Clostridium difficile* spores	AE-756-170D	0.7	6.9	6.2

487037-05, Study Conducted August 24

Lot No.	Carrier#	Initial Carrier Weight Dry (g)	Initial Carrier Weight Wet (g)	Final Carrier Weight (g)	Remaining Test Substance Weight (g)	Wetness Confirmed
AE-756-168D	1	106.72	107.68	107.24	0.52	Yes
	2	107.13	108.29	107.87	0.74	Yes
	3	107.87	108.53	108.11	0.24	Yes
AE-756-169D	1	109.33	110.76	110.08	0.75	Yes
	2	82.73	83.43	83.05	0.32	Yes
	3	82.70	83.99	83.47	0.77	Yes
AE-756-170D	1	112.98	114.00	113.58	0.60	Yes
	2	108.01	108.82	108.43	0.42	Yes
	3	106.53	107.20	106.75	0.22	Yes

487037-05, Study Conducted August 26

Lot No.	Carrier#	Initial Carrier Weight Dry (g)	Initial Carrier Weight Wet (g)	Final Carrier Weight (g)	Remaining Test Substance Weight (g)	Wetness Confirmed
AE-756-168D	1	113.03	114.05	113.64	0.61	Yes
	2	108.07	108.98	108.58	0.51	Yes
	3	106.56	107.49	107.04	0.48	Yes
AE-756-169D	1	108.22	109.03	108.64	0.42	Yes
	2	109.36	110.10	109.67	0.31	Yes
	3	82.74	83.58	83.16	0.42	Yes
AE-756-170D	1	82.68	83.61	83.25	0.57	Yes
	2	106.77	107.82	107.40	0.63	Yes
	3	107.15	108.08	107.66	0.51	Yes

VI CONCLUSIONS

1. The submitted efficacy data (MRID 487037-02 through 487037-04) support the use of the towelette, product, PDI Sani-Cloth Bleach Wipes, as a disinfectant against spores of *Clostridium difficile* on pre-cleaned, hard, non-porous surfaces 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 testing demonstrated that the neutralizer was effective in neutralizing the antimicrobial activity of the product. Purity controls were reported as pure. Sterility controls did not show growth of *Clostridium difficile* spores. Test spores showed resistance to acid for ≥10 minutes.

2. The submitted efficacy data support the use of the towelette product, PDI Sani-Cloth Bleach Wipes, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 1-minute contact time:

Escherichia coli	MRID 487037-06
Escherichia coli - NDM-1 positive	MRID 487037-07
Bordetella pertussis	MRID 487037-08
Klebsiella pneumoniae - NDM-1 positive	MRID 487037-09
Burkholderia cepacia	MRID 487037-10
Serratia marcescens	MRID 487037-11
Enterobacter cloacae NDM-1 positive	MRID 487037-12

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. Neutralization confirmation for *Bordetella pertussis* testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. 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 the towelette product, PDI Sani-Cloth Bleach Wipes, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 1-minute contact time:

Cytomegalovirus	MRID 487037-13
Influenza B virus	MRID 487037-14

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

VII RECOMMENDATIONS

1. The proposed label claims that the towelette product, PDI Sani-Cloth Bleach Wipes, is an effective disinfectant against *Clostridium difficile* spores on pre-cleaned, hard, non-porous surfaces for a 4-minute contact time. This claim is acceptable as it is supported by the submitted data.

2. The proposed label claims that the towelette product, PDI Sani-Cloth Bleach Wipes, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 5% blood serum contamination for a 1-minute contact time:

Escherichia coli
Escherichia coli - NDM-1 positive
Bordetella pertussis
Klebsiella pneumoniae - NDM-1 positive
Burkholderia cepacia
Serratia marcescens
Enterobacter cloacae NDM-1 positive
Cytomegalovirus
Influenza B virus

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

3. The proposed label claims that the towelette product, PDI Sani-Cloth Bleach Wipes, is an effective disinfectant against *Legionella pneumophilia* (ATCC 33153) on hard, non-porous surfaces in the presence of 5% blood serum contamination for a 1-minute contact time. This claim is acceptable.

4. The following claims are unacceptable and must be modified or removed from the proposed label:

- On page 9 of the proposed label, remove the reference to CDC.
- On page 11 of the proposed label, qualify the different enveloped and non-enveloped viruses.
- On page 11 of the proposed label, remove the claim "Kills major forms of microbes" as this claim is too ambiguous.

- On page 11 of the proposed label, remove the claims "terminal cleaner or terminal disinfectant" as it may be misinterpreted as a terminal sterilant/high level disinfectant.

- On page 12 of the proposed label, remove the claim "To fight *Clostridium difficile* spores outbreaks" and "To reduce transmission of *Clostridium difficile* spore". To support exposure reduction claims, the Agency requires documentation.