UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

March 9, 2010

MEMORANDUM

Subject: Efficacy Review for EPA File Symbol 70299-RO, Sanidate 5.0; DP Barcode: 372252

From: Tajah Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Emily Mitchell, Chief
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To: Marshall Swindell PM 33/ Demson Fuller
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: BioSafe Systems, LLC
22 Meadow Street
East Hartford, CT 06108

Formulation from the Label:

Active Ingredient(s)	% by wt.
Hydrogen peroxide	23.0%
Peroxyacetic Acid	5.3%
Other Ingredients	71.7%
Total	100.0%

I BACKGROUND

The product, SaniDate 5.0 (EPA File Symbol 70299-RO), is a new product. The applicant requested to register the product for use as a disinfectant (bactericide, fungicide, virucide), sanitizer, sanitizing rinse, mildewstat, and deodorizer on hard, non-porous surfaces in institutional, commercial, industrial, food processing, animal care, and hospital or medical environments. The label states that the product is effective as a disinfectant on lightly soiled surfaces. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; BioScience Laboratories, Inc., located at N. Willson Avenue in Bozeman, MT 59715; MicroBioTest, Inc., located at 105 Carpenter Drive in Sterling, VA 20164; and Presque Isle Cultures, located at 3804 West Lake Road in Erie, PA 16505.

This data package contained a letter from the applicant's representative to EPA (dated November 17, 2009), eleven studies (MRID 479153-05 through 479153-15), Statements of No Data Confidentiality Claims for all eleven studies, and the proposed label.

Note: The referenced email (included with data submission), states that the Sanidate 5.0 confirmatory efficacy data requirements are as follows: (1) Disinfectant claims: 2 product lots, 10 carriers/lot for *S. aureus*, *S. enterica*, and *P. aeruginosa*; (2) Sanitizers, Food-contact surfaces: 1 lot for both test organisms; (3) Sanitizers, Non-food contact surfaces: One lot for both test organisms; and (4) Fungicidal: No confirmatory data is required (Email from Agency to Donna Bishel, April 21, 2009).

II USE DIRECTIONS

The product is designed for disinfecting and sanitizing hard, non-porous surfaces. The product may be used to treat hard, non-porous surfaces such as appliances, barber/salon tools, bathroom fixtures, bed frames, benches, beverage dispensing equipment, bins, boxing and packing equipment, brooms, buckets, cabinets, cages, cans, carts, chairs, coolers, conveyors, corers, counter tops, crates, drains, evaporators, fan blades, filter housings, filters, floors, garbage cans/bins/pails, hooks, kennel runs, mops, pails, pans, pasteurizers, peelers, personal protective equipment, piping, platters, processing equipment, pumps, racks, rakes, scrapers, shelves, shovels, sinks, squeegees, tables, tanks, tools and equipment, trays, valves, vats, vehicles, walls, walkways, water transfer and handling systems. The proposed label indicates that the product may be used on hard, non-porous surfaces including: Formica, glass, glazed porcelain, glazed tile, linoleum, metal (e.g., aluminum, stainless steel, steel), plastic (e.g., CPVC, nylon, polyethylene, polyvinylchloride, vinyl), sealed fiberglass, and sealed wood. Directions on the proposed label provide the following information regarding preparation and use of the product:

> <u>As a disinfectant</u>: Prepare a use solution by adding 0.5 fluid ounces of product to 1 gallon of water (a 1:256 use solution). Apply use solution using a mop, cloth, sponge, brush, scrubber, or coarse spray device, or by soaking so as to wet all surfaces thoroughly. Treated surfaces must remain wet for 10 minutes. For heavily soiled surfaces, a pre-cleaning step is required.

As a sanitizer on non-food contact surfaces: Remove gross filth with a cleaner or suitable detergent. Prepare a use solution by adding 1.6 fluid ounces of the product to 5 gallons of potable water (a 1:400 use solution). Soak items using mop, wipe, coarse spray, or flood techniques. Allow contact of 1 minute. Air dry.

As a sanitizer on food contact surfaces: Remove gross food particulate matter and soil by a warm water flush, pre-flush, pre-scrape, or pre-soak. Thoroughly wash surfaces or equipment with a good detergent or compatible cleaning solution. Do not rinse. Prepare a use solution by adding 1.6 fluid ounces of product to 5 gallons of potable water (a 1:400 use solution). Apply by wiping, mopping, or coarse spray, or by adding to a closed system. Allow a contact time of 1 minute. Drain thoroughly. Do not rinse.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use in Hospital or Medical Environments; Confirmatory Efficacy Data Requirements

Under certain circumstances, an applicant is permitted to rely on previously submitted efficacy data to support an application or amendment for registration of a product and to submit only minimal confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formation. Confirmatory data must be developed on the applicant=s own finished product. For hospital disinfectants, 10 carriers on each of 2 samples representing 2 different product lots must be tested against *Salmonella enterica* (ATCC 10708, formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442) using either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Killing on all carriers is required.

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.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method)

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different

product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

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.

Sanitizers (For Non-Food Contact Surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces)

Sanitizing rinses may be formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, or anionic detergent-acid formulations. The effectiveness of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method. Data from the test on 1 sample from each of 3 different product lots, one of which is at least 60 days old against *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) are required. When the effectiveness of the

product in hard water is made, all required data must be developed at the hard water tolerance claimed. Acceptable results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and the percentage reduction over the control. Furthermore, counts on the number controls for the product should fall between 75 and 125 x 10^6/mL for percent reductions to be considered valid. Label directions for use must state that a contact time of at least 1 minute is required for sanitization. A potable water rinse is not required (to remove the use solution for the treated surface) for products cleared for use on food contact surfaces under the Federal Food, Drug, and Cosmetic Act. Label directions must recommend a potable water rinse (to remove the use solution from the treated surface) under any other circumstances.

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 479153-05 "An Evaluation of the Sanitizing Efficacy of One Test Formulation for Use on Non-Food Contact Surfaces," Test Organisms: *Klebsiella pneumoniae pneumoniae* (ATCC 4352) and *Staphylococcus aureus aureus* (ATCC 6538), for SaniDate 5.0, by Terri Eastman. Study conducted at BioScience Laboratories, Inc. Study completion date – July 20, 2009. Amended report date – November 11, 2009. Laboratory Study Number 090433-204.

This confirmatory study was conducted against *Staphylococcus aureus aureus* (ATCC 6538) and *Klebsiella pneumoniae pneumoniae* (ATCC 4352). One lot (Lot No. 70420083PA) of the product, SaniDate 5.0, was tested. The laboratory report referenced the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E 1153-03). The product lot tested was at least 60 days old at the time of testing. A use solution was prepared by adding 1.0 mL of the product and 399 mL of 400±20 ppm AOAC synthetic hard water (titration results not provided; a 1:400 dilution). The use solution was not tested in the presence of a 5% organic soil load. Three sterile glass carriers (1 inch x 1 inch) per microorganism were inoculated with 0.03 mL of a 48-54 hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread to within 3 mm of the edges of each carrier. The carriers were air-dried for 20-40 minutes at 30-37°C (which differs from the 40-minute drying time specified in DIS/TSS-10). Each carrier was transferred to a sterile Petri dish and treated with 5.0 mL of the use solution for 1 minute at 25±1°C. Following exposure, each carrier was placed into a sterile vessel containing 25 mL of Butterfield's Phosphate Buffer Solution with 0.1% sodium thiosulfate and Catalase. The vessels were shaken vigorously to suspend the surviving organisms. One mL and 0.1 mL aliquots were plated in duplicate on Nutrient Agar with 0.07% Lecithin and 0.5% Tween 80. All subcultures were incubated for 48-50 hours at 35±2°C prior to examination. Following incubation, the subcultures were counted manually. Controls included those for carrier population, purity, sterility, and neutralizer efficacy verification.

Note: A protocol amendment noted the inconsistencies between control carrier population recovery requirements specified by ASTM E 1153-03, DIS/TSS-10, and the EPA Pesticide Assessment Guidelines. The amendment established a minimum carrier recovery of 10^4 CFU/carrier.

2. MRID 479153-06 "A Hard Surface Disinfection Evaluation of One Test Formulation Versus Three Bacterial Species," Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538), for SaniDate 5.0, by Terri Eastman. Study conducted at BioScience Laboratories, Inc. Study completion date – July 20, 2009. Amended report date – September 11, 2009. Laboratory Study Number 090427-204.

This confirmatory study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Two lots (Lot Nos. 80707083PA and 70420083PA) of the product, SaniDate 5.0, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 2009. Both of the product lots tested were at least 60 days old at the time of testing. Use solutions were prepared by adding 1.0 mL of the product and 255 mL of 400±20 ppm AOAC synthetic hard water (titration results not provided; a 1:256 dilution). Cultures of the challenge microorganisms were prepared in accordance with the published AOAC methods, with the following exception: the cultures were incubated for 48-54 hours at 35±2°C (which differs from the AOAC methods specification of 48-54 hours at 36±1°C). Heat-inactivated fetal bovine serum was added to each culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot per test organism were immersed for 15±2 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±2 minutes at 35±2°C (which differs from the AOAC methods specification of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 10 minutes at 25±1°C. The tubes containing the use solution were swirled after addition of the carriers. Following exposure, individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and Catalase to neutralize. [It is unknown whether tubes containing the neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods.] All subcultures were incubated for 48±2 hours at 35±2°C (which differs from the AOAC methods specification of 48±2 hours at 36±1°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.

3. MRID 479153-07 "AOAC Use-Dilution Method, Test Organism: *Bordetella bronchiseptica* (ATCC 10580)" for SaniDate 5.0, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – October 26, 2009. Project Number A08297.

This study was conducted against *Bordetella bronchiseptica* (ATCC 10580). Two lots (Lot Nos. 09141-1 and 09141-2) of the product, SaniDate 5.0, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. Use solutions were prepared by adding 1.00 mL of the product and 255.0

mL of 400 ppm AOAC synthetic hard water (titrated at 400 ppm; a 1:256 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods. 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 40% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. [It is unknown whether the tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods.] Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.05% Catalase to neutralize. [It is unknown whether tubes containing the neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods.] 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.

4. MRID 479153-08 "Use Dilution Test Methicillin-resistant *Staphylococcus aureus*" for SaniDate 5.0, by Travis R. Farley. Study conducted at MicroBioTest, Inc. Study completion date – September 30, 2009. Laboratory Project Identification Number 703-102.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33591). Two lots (Lot Nos. 09141-1 and 09141-2) of the product, SaniDate 5.0, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. Use solutions were prepared by adding 1 part of the product and 255 parts of 400 ppm ± 2.9% AOAC hard water (a 1:256 dilution). Heat-inactivated horse 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 20 carriers per tube of 20 mL broth. The carriers were dried for 25 minutes at 37±2°C. Each carrier was placed in 10 mL of the use solution for 10 minutes at 21°C. The tubes containing the use solution were swirled after addition of the carriers. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* to oxacillin. See pages 9 and 15 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed.

5. MRID 479153-09 "Use Dilution Test *Listeria monocytogenes*" for SaniDate 5.0, by Travis R. Farley. Study conducted at MicroBioTest, Inc. Study completion date – September 30, 2009. Laboratory Project Identification Number 703-101.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. 09141-1 and 09141-2) of the product, SaniDate 5.0, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. Use solutions were prepared by adding 1 part of the product and 255 parts of 400 ppm ± 2.9% AOAC hard water (a 1:256 dilution). Heat-inactivated horse 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 20 carriers per tube of 20 mL broth. The carriers were dried for 25 minutes at 37±2°C. Each carrier was placed in 10 mL of the use solution for 10 minutes at 21°C. The tubes containing the use solution were swirled after addition of the carriers. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC methods specification of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed.

6. MRID 479153-10 "Virucidal Efficacy Test Using Human Influenza Virus (H1N1)" for SaniDate 5.0, by Zheng Chen. Study conducted at MicroBioTest, Inc. Study completion date – October 16, 2009. Laboratory Project Identification Number 703-104.

This study was conducted against Human influenza (H1N1) virus (Strain A/PR/8/34; obtained from Charles River Laboratories), using MDCK cells (ATCC CCL-34) as the host system. Two lots (Lot Nos. 09141-1 and 09141-2) of the product, SaniDate 5.0, were tested according to a MicroBioTest protocol titled "Virucidal Efficacy Test Using Human Influenza Virus (H1N1)," dated September 10, 2009 (copy provided). Use solutions were prepared by adding 1 part of the product and 255 parts of 400 ppm ± 2.9% AOAC hard water (a 1:256 dilution). The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum uniformly over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 10 minutes at 20°C. Following exposure, the plates were neutralized with 2.0 mL of Minimal Essential Medium supplemented with 10% fetal bovine serum, 1% Polysorbate 80, 5% $NaHCO_3$, 5% Hepes, and 0.1% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were diluted serially in Minimum Essential Medium with 1.0 µg/mL Trypsin. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-6 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/ sterility, virus stock titer, plate

recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

7. MRID 479153-11 "Virucidal Efficacy Test Using Avian Influenza Virus (H9N2)" for SaniDate 5.0, by Zheng Chen. Study conducted at MicroBioTest, Inc. Study completion date – October 16, 2009. Laboratory Project Identification Number 703-105.

This study was conducted against Avian influenza (H9N2) virus (Strain Turkey/Wis/66; obtained from Charles River Laboratories), using MDCK cells (ATCC CCL-34) as the host system. Two lots (Lot Nos. 09141-1 and 09141-2) of the product, SaniDate 5.0, were tested according to a MicroBioTest protocol titled "Virucidal Efficacy Test Using Avian Influenza Virus (H9N2)," dated September 10, 2009 (copy provided). Use solutions were prepared by adding 1 part of the product and 255 parts of 400 ppm ± 2.9% AOAC hard water (a 1:256 dilution). The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum uniformly over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 10 minutes at 20°C. Following exposure, the plates were neutralized with 2.0 mL of Minimal Essential Medium supplemented with 10% fetal bovine serum, 1% Polysorbate 80, 5% $NaHCO_3$, 5% Hepes, and 0.1% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were diluted serially in Minimum Essential Medium with 1.0 µg/mL Trypsin. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-6 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/ sterility, virus stock titer, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

8. MRID 479153-12 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Canine Distemper virus" for SaniDate 5.0, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 3, 2009. Project Number A08388.

This study was conducted against Canine distemper virus (Strain Lederle; ATCC VR-128), using Vero cells (ATCC CCL-81; propagated in-house) as the host system. Two lots (Lot Nos. 09141-1 and 09141-2) of the product, SaniDate 5.0, were tested according to ATS Labs Protocol No. BSS01082809.CDIS (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 255 mL of 400 ppm AOAC synthetic hard water (titrated at 396 ppm; a 1:256 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 undersides of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20°C

at 40% relative humidity. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 10 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 supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. 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 titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

9. MRID 479153-13 "Use Dilution Test *Aspergillus fumigatus*" for SaniDate 5.0, by Travis R. Farley. Study conducted at MicroBioTest, Inc. Study completion date – October 29, 2009. Laboratory Project Identification Number 703-103.

This study was conducted against *Aspergillus fumigatus* (ATCC 36607). Two lots (Lot Nos. 09141-1 and 09141-2) of the product, SaniDate 5.0, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. Use solutions were prepared by adding 1 part of the product and 255 parts of 400 ppm ± 2.9% AOAC hard water (a 1:256 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the suspension was filtered through sterile glass wool (which differs from the AOAC method specification to filter through sterile absorbent cotton). Heat-inactivated horse 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 10-15 day old suspension of test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 30-40 minutes at 37±2°C (which differs from the AOAC methods specification of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 10 minutes at 20°C. The tubes containing the use solution were swirled after addition of the carriers. Following exposure, individual carriers were transferred to NeoPeptone Glucose Broth with 7% Polysorbate 80, 1% Lecithin, and 0.3% thioglycollic acid to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. All subcultures were incubated for up to 10 days at 25-30°C (which differs from the AOAC method specification of 7 days at 25-30°C (for *Trichophyton mentagrophytes*)). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for pre-test inoculum counts, inoculum counts, carrier counts, sterility, viability, neutralizer effectiveness, fungistasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed.

10. MRID 479153-14 "AOAC Use Dilution Testing of SaniDate 5.0 in the Presence of 5% Serum and 400 ppm Hard Water," Test Organisms: *Streptococcus uberis* (ATCC 19436), *Enterobacter aerogenes* (ATCC 13048), *Bacteroides melaninogenica* (ATCC 25845), *Streptococcus agalactiae* (ATCC 13813), *Streptococcus dysgalactiae* (ATCC 43078), *Klebsiella pneumoniae* (ATCC 13883), and *Fusobacterium necrophorum* (ATCC 25286), by Richard A. Gammon. Study conducted at Presque Isle Cultures. Study completion date – July 21, 2009. Laboratory Project #: Biosafe 09-01.

This study was conducted against *Streptococcus uberis* (ATCC 19436), *Enterobacter aerogenes* (ATCC 13048), *Bacteroides melaninogenica* (ATCC 25845), *Streptococcus agalactiae* (ATCC 13813), *Streptococcus dysgalactiae* (ATCC 43078), *Klebsiella pneumoniae* (ATCC 13883), and *Fusobacterium necrophorum* (ATCC 25286). Three lots (Lot Nos. 80707083PA, 71213082PA, and 80707082PA) of the product, SaniDate 5.0, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis (edition not specified). Use solutions (1:256 dilution) were prepared by adding the product and 400 ppm AOAC hard water (titration results not provided). Bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot per test organism were immersed for 15±2 minutes in a 48-54 hour old suspension of test organism. The carriers were dried for 40±2 minutes at 36±1°C. Each carrier was placed in 10 mL of the use solution for 10 minutes at ambient temperature. The tubes containing the use solution were swirled after addition of the carriers. Following exposure, individual carriers were transferred to subculture medium. [It is unknown whether tubes containing the neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods.] Carriers were transferred from primary subculture tubes into individual secondary subculture tubes after 30 minutes following the first transfer. All subcultures were incubated for 48±2 hours at 36±1°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.

11. MRID 479153-15 "Germicidal and Detergent Sanitizing Action of Disinfectants," Test Organism: *Escherichia coli* O157:H7 (ATCC 43888), for SaniDate 5.0, by Becky Lien. Study conducted at ATS Labs. Study completion date – July 3, 2008. Project Number A06399.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 43888). Two lots (Lot Nos. 71213082PA and 71213081PA) of the product, SaniDate 5.0, were tested. The laboratory report referenced the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Use solutions were prepared by adding 1.00 mL of the product and 399.0 mL of 400 ppm AOAC synthetic hard water (titrated at 408 ppm; a 1:400 dilution). A culture of the challenge microorganism was <u>not</u> prepared in accordance with the published AOAC method. Deviations included: (1) synthetic broth was used as the culture medium to prepare an initial suspension; (2) multiple tubes of synthetic broth were inoculated and incubated for 48±4 hours at 35-37°C; (3) the harvested growth was centrifuged following collection; and (4) the resulting pellet was re-suspended in 1/50th the original volume using sterile phosphate buffered dilution water. The absorbance value of the culture suspension was measured (at 620 nm) and the

culture was adjusted to approximately 2.6. The use solution was not tested in the presence of a 5% organic soil load. A 99.0-mL aliquot of each use solution was transferred to a 250-300 mL Erlenmeyer flask and placed in a water bath at 26.0°C. One-mL bacterial suspension was added to each flask. One-mL aliquots of the bacterium-product mixture were transferred to 9 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.05% Catalase exactly 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in tryptic soy agar with 5% sheep's blood (which differs from the AOAC method specification of tryptone glucose extract agar). All plates were incubated for 46 hours at 35-37°C (which differs from the AOAC method specification of 48 hours at 35°C). The plates were stored for 2 days at 2-8°C prior to reading. Following incubation and storage, the colonies were counted. Controls included those for numbers count, purity, sterility, viability, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were reviewed.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Count (CFU/ carrier)
		Lot No. 80707083PA	**Lot No. 70420083PA**	---	
479153-06	*Staphylococcus aureus*	0/10	0/10	---	7.44×10^5
479153-06	*Salmonella enterica*	0/10	0/10	---	2.68×10^4
479153-06	*Pseudomonas aeruginosa*	0/10	0/10	---	4.68×10^5
		Lot No. 09141-1	**Lot No. 09141-2**	---	
479153-07	*Bordetella bronchiseptica*	0/10	0/10	---	3.7×10^6
479153-08	Methicillin Resistant *Staphylococcus aureus*	0/10	0/10		1.9×10^6
479153-09	*Listeria monocytogenes*	0/10	0/10	---	5.9×10^5
479153-13	*Aspergillus fumigatus*	0/10	0/10	---	4.0×10^5
		Lot No. 80707083 PA	**Lot No. 71213082 PA**	**Lot No. 80707082 PA**	
479153-14	*Streptococcus uberis*	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	8.3×10^5
	Enterobacter aerogenes	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1.41×10^6

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Count (CFU/ carrier)
	Bacteroides melaninogenica	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	8.4×10^5
	Streptococcus agalactiae	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	7.7×10^5
	Streptococcus dysgalactiae	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	5.1×10^4
	Klebsiella pneumoniae	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1.32×10^5
	Fusobacterium necrophorum	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1.0×10^6

MRID Number	Organism	Results			Dried Virus or Plate Recovery Control
			Lot No. 09141-1	Lot No. 09141-2	
479153-10	Human influenza (H1N1) virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.00}$ $TCID_{50}$/mL
		$TCID_{50}$/mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
479153-11	Avian influenza (H9N2) virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{7.00}$ $TCID_{50}$/mL
		$TCID_{50}$/mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
479153-12	Canine distemper	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	Organism	Lot No.	Total No. Surviving	Parallel Control	Percent Reduction
			(CFU/carrier)		
479153-05	*Staphylococcus aureus aureus*	7042008 3PA	2.50×10^1	7.28×10^6	99.99
	Klebsiella pneumoniae pneumoniae	7042008 3PA	2.50×10^1	9.06×10^4	99.97

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/mL)		
479153-15	*Escherichia coli* O157:H7	71213082PA 71213081PA	$<1 \times 10^1$ $<1 \times 10^1$	8.0×10^7 8.0×10^7	>99.999 >99.999

VI CONCLUSIONS

1. The submitted confirmatory efficacy data support the use of a 1:256 dilution of the product, SaniDate 5.0, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a 10-minute contact time:

Staphylococcus aureus	MRID 479153-06
Salmonella enterica enteric	MRID 479153-06
Pseudomonas aeruginosa	MRID 479153-06

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

2. The submitted efficacy data support the use of a 1:256 dilution of the product, SaniDate 5.0, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a 10-minute contact time:

Bordetella bronchiseptica	MRID 479153-07
Methicillin Resistant *Staphylococcus aureus*	MRID 479153-08
Listeria monocytogenes	MRID 479153-09
Aspergillus fumigatus	MRID 479153-13
Streptococcus uberis	MRID 479153-14
Enterobacter aerogenes	MRID 479153-14
Bacteroides melaninogenica	MRID 479153-14
Streptococcus agalactiae	MRID 479153-14
Streptococcus dysgalactiae	MRID 479153-14
Klebsiella pneumoniae	MRID 479153-14
Fusobacterium necrophorum	MRID 479153-14

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralizer effectiveness/ neutralization confirmation testing showed positive growth of the microorganisms. When reported, purity controls were reported as pure. Viability controls were positive for growth. When reported, sterility controls did not show growth.

3. The submitted efficacy data support the use of a 1:256 dilution of the product, SaniDate 5.0, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a 10-minute contact time:

Human influenza (H1N1) virus	MRID 479153-10
Avian influenza (H9N2) virus	MRID 479153-11
Canine distemper virus	MRID 479153-12

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

4. The submitted confirmatory efficacy data support the use of a 1:400 dilution of the product, SaniDate 5.0, as a sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, non-food contact surfaces in the presence of 400 ppm hard water for a 1-minute contact time:

Staphylococcus aureus aureus	MRID 479153-05
Klebsiella pneumoniae pneumoniae	MRID 479153-05

Bacterial reductions of at least 99.9 percent over the parallel control were observed within 1 minute. At least one of the product lots tested was at least 60 days old at the time of testing. The control carrier population recovery count for *Staphylococcus aureus aureus* (i.e., 9.37×10^6 CFU/carrier) demonstrated an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. The control carrier population recovery count for *Klebsiella pneumoniae pneumoniae* (i.e., 6.40×10^5 CFU/carrier) did not demonstrate an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. Neutralizer efficacy verification showed growth of no more than 0.25 $\log_{10}$ less than the average control population. Purity controls were reported as pure. Sterility controls did not show growth.

5. The submitted efficacy data (MRID 479153-15) support the use of a 1:400 dilution of the product, SaniDate 5.0, as a sanitizing rinse against *Escherichia coli* O157:H7 on pre-cleaned, hard, non-porous, food contact surfaces in the presence of 400 ppm hard water for a 30-second contact time. Bacterial reductions of at least 99.999 percent over the parallel control were observed within 30 seconds. Neutralization confirmation 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.

VII RECOMMENDATIONS

1. The proposed label claims that a 1:256 dilution of the product, SaniDate 5.0, is an effective disinfectant against the following microorganisms on lightly soiled, hard, non-porous surfaces for a 10-minute contact time:

Pseudomonas aeruginosa
Salmonella enterica
Staphylococcus aureus

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

2. The proposed label claims that a 1:256 dilution of the product, SaniDate 5.0, is an effective disinfectant against the following microorganisms on lightly soiled, hard, non-porous surfaces for a 10-minute contact time:

Bordetella bronchiseptica
Methicillin Resistant *Staphylococcus aureus*

Listeria monocytogenes
Aspergillus fumigatus
Streptococcus uberis
Enterobacter aerogenes
Bacteroides melaninogenica
Streptococcus agalactiae
Streptococcus dysgalactiae
Klebsiella pneumoniae
Fusobacterium necrophorum
Human influenza virus (H1N1)
Avian influenza virus (H9N2)
Canine distemper virus

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

3. The proposed label claims that a 1:256 dilution of the product, SaniDate 5.0, is an effective disinfectant against the following microorganisms on lightly soiled, hard, non-porous surfaces for a 10-minute contact time:

Escherichia coli
Saccharomyces cerevisiae
Lactobacillus malefermentans
Pediococcus damnosus
Trichophyton mentagrophytes

Data were not provided to support these claims. References to these microorganisms must be deleted from the proposed label.

4. The proposed label claims that a 1:400 dilution of the product, SaniDate 5.0, is an effective sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, non-food contact surfaces for a 1-minute contact time:

Staphylococcus aureus
Klebsiella pneumoniae

These claims are acceptable as they are supported by confirmatory data. Only one product lot was tested.

5. The proposed label claims that a 1:400 dilution of the product, SaniDate 5.0, is an effective sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, food contact surfaces for a 1-minute contact time:

Escherichia coli
Staphylococcus aureus
Escherichia coli O157:H7
Pediococcus damnosus
Lactobacillus malefermentans
Saccharomyces cerevisiae

These claims are not yet acceptable, as confirmatory data for *Escherichia coli* and *Staphylococcus aureus* have not been provided. References to use of the product as a sanitizer on food contact surfaces must be deleted from the proposed label.

6. Deodorizing directions are required on the proposed label.

7. ATCC designation numbers are required in one of the following locations:

   - on the data matrix;
   - on the master label (as optional text) with the listing of the organisms claimed; or
   - as the final page of the master label (as optional text).

8. The following revisions to the proposed label must be made:

   - On page 8 of the proposed label under the "Sanitization of Non-Food Contact Surfaces" section, include a pre-wash step for all applications. Efficacy testing was not performed in the presence of an organic soil load. Not only does gross filth need to be removed, any filth must be removed (i.e., surfaces must be pre-washed).

   - On page 10 of the proposed label under the "Sanitization of Food Storage Areas" section, include a pre-wash step. Efficacy testing for *Escherichia coli* O157:H7 was not performed in the presence of an organic soil load.

   - On page 11 of the proposed label, under the "Surface Disinfection" section, include the following (or a similar) statement: "For heavily soiled surfaces, a pre-cleaning step is required."

9. The following revisions to the proposed label are required:

   - Under the "Precautionary Statements" section of the proposed label, change "before eating, drinking, or using tobacco" to read "before eating, drinking, chewing gum, using tobacco, or using the toilet."

   - On page 9 of the proposed label, change "cutting boards" to read "non-wooden cutting boards."

   - On page 12 of the proposed label, change "*Streptococcus agalacticiae*" to read "*Streptococcus agalactiae*."