UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

March 28, 2011

MEMORANDUM

Subject: Efficacy Review for Blondie; EPA File Symbol 67619-EU; DP Barcode: D385825

From: Ibrahim Laniyan, Ph.D.
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3/29/11

To: Marshall Swindell / Abigail Downs
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Clorox Professional Products Company
1221 Broadway
Oakland, CA 94612

Formulation from the Label:

Active Ingredient(s)	% by wt.
Hydrogen Peroxide	1.4 %
Other ingredients	98.6 %
Total	100.0 %

I. BACKGROUND

The product, Blondie (EPA File Symbol 67619-EU), is a new product. The applicant requested to register the product for use as a ready-to-use disinfectant (bactericide, fungicide, tuberculocide, virucide) and deodorizer on hard, non-porous surfaces in household, institutional, industrial, commercial, food service, animal care, and hospital or medical environments. The label states that the product is an effective bactericide, fungicide, and virucide in the presence of organic soil (5% blood serum). Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated December 22, 2010), EPA Form 8570-4 (Confidential Statement of Formula (CSF)), CSFs for three alternate formulations, forty studies (MRID 483360-09 through 483360-48), Statements of No Data Confidentiality Claims for all forty studies, and the proposed label.

Note: EPA Form 8570-4 (Confidential Statement of Formula) contains Confidential Business Information. Data or information claimed by the applicant to be FIFRA confidential has not been included in this report.

II. USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: air vents, animal equipment, appliance exteriors, automatic feeders, bathtubs, bed frames, bed rails, cabinets, cages, carts, ceilings, cellular phones, chairs, changing tables, computer peripherals, computers, cords, counter tops, crates, cribs, desk tops, diagnostic equipment, diaper pails, dish racks, doorknobs and handles, drain boards, elevator buttons, exercise equipment, exhaust fans, faucets, feed racks, fixtures, floors, footboards, fountain exteriors, furniture, garbage bins, grocery carts, hampers, headboards, incubators, kennels, keyboards, lamps, laptops, light fixtures and switches, lockers, medical equipment and instruments, piano keys, plastic mattress covers, playpens, railings and rails, recycling bins, remote controls, salad bar sneeze guards, shelves, sports equipment, strollers, shower curtains, shower stalls, sinks, tables, telephones, toilets, tools, towel dispensers, toys, trash cans, trays, urinals, vanities, veterinary equipment, walls, and wheelchairs. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: baked enamel, finished hardwood, Formica, glass, glazed ceramic tile, glazed porcelain, glazed tile, laminated surfaces, linoleum, metal (e.g., chrome, plated steel, stainless steel), Marlite, Naugahyde, painted surfaces, plastic, Plexiglas, sealed fiberglass, sealed granite, sealed marble, synthetic marble, and vinyl. Directions on the proposed label provide the following information regarding use of the product as a disinfectant: Pre-clean heavily soiled areas. Hold container six to eight inches from surface to be treated. Spray area until it is covered with the product. Allow the product to penetrate and remain wet for the contact time listed on the label (or for 1 minute) (or for 4 minutes against TB). Wipe off with a clean cloth, mop, or sponge. Rinse food contact surfaces with potable water.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble

powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

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

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using the AOAC Germicidal Spray Products as Disinfectants Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Germicidal Spray Products as Disinfectants Method contains procedures for testing fungicidal activity. 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.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity of Disinfectants Test Method): Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. When using the existing or modified AOAC Tuberculocidal Activity Test Methods, 10 carriers for each of 2 samples, representing 2 different product lots, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). Killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then

treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

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

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. BRIEF DESCRIPTION OF THE DATA

1. MRID 483360-09 "AOAC Germicidal Spray Method," Test Organism: *Salmonella enterica* (ATCC 10708), for Blondie, F2010.0127, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – November 5, 2010. Project Number A09786.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 10ACE8, 10ACE9, and 10ACE2) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18062310.GS.3 (copy provided). At least one of the product lots tested (i.e., Lot No. 10ACE2) was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 40 minutes at 35-37°C at 40.78% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 64% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase 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 ~45.75 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.

2. MRID 483360-10 "AOAC Germicidal Spray Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Blondie, F2010.0127, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – November 5, 2010. Project Number A09785.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 10ACE8, 10ACE9, and 10ACE2) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18062310.GS.2 (copy provided). At least one of the product lots tested (i.e., Lot No. 10ACE2) was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. Testing was conducted on July 20, 2010 and July 27, 2010. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48-50 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 40 minutes at 35-37°C at 40-41% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20-23°C at 64% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°. 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.

Note: Testing conducted on July 20, 2010 against *Pseudomonas aeruginosa* at a 30-second exposure time showed growth in subcultures of 2 of the 60 carriers for Lot No. 10ACE9. Testing was repeated to test for false positives.

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

3. MRID 483360-11 "AOAC Germicidal Spray Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Blondie, F2010.0127, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – November 5, 2010. Project Number A09887.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 10ACE8, 10ACE9, and 10ACE2) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18072310.GS (copy provided). At least one of the product lots tested (i.e., Lot No. 10ACE2) was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a ~50.25 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 42% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 45 seconds at 20°C at 54% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 47 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 483360-12 "AOAC Germicidal Spray Method," Test Organism: *Acinetobacter baumannii* (ATCC 19606), for Blondie, F2010.0127, by Joshua

Luedtke. Study conducted at ATS Labs. Study completion date – September 23, 2010. Project Number A09990.

This study was conducted against *Acinetobacter baumannii* (ATCC 19606). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070210.GS.1 (copy provided). The product was received ready-to-use, as a trigger spray.Testing was conducted on August 12, 2010 and September 7, 2010. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 41% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20-23°C at 43-64% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. For testing conducted on August 12, 2010, the subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation or incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Testing conducted on August 12, 2010 against *Acinetobacter baumannii* at a 30-second exposure time showed growth in the subculture of 1 of the 10 carriers for Lot No. 10ACE9. Testing was repeated to test for false positives.

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

5. MRID 483360-13 "AOAC Germicidal Spray Method," Test Organism: Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575), for Blondie, F2010.0127, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – September 9, 2010. Project Number A10049.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070210.GS.3 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 25-30°C at 66% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 66% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 45.75 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 11 mm) confirmed antibiotic resistance of Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575) to vancomycin. See page 9 and Table 5 of the laboratory report.

6. MRID 483360-14 "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* (ATCC 11229), for Blondie, F2010.0127, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – September 13, 2010. Project Number A09978.

This study was conducted against *Escherichia coli* (ATCC 11229). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070210.GS.5 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 36.0-36.1% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 23°C at 63% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

7. MRID 483360-15 "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* O157:H7 (ATCC 35150), for Blondie, F2010.0127, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – September 13, 2010. Project Number A09979.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070210.GS.6 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 36.0-36.1% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 23°C at 63% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

8. MRID 483360-16 "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* with extended beta-lactamase resistance (ATCC BAA-196), for Blondie,

F2010.0127, by Becky Lien. Study conducted at ATS Labs. Study completion date – October 1, 2010. Project Number A09993.

This study was conducted against *Escherichia coli* with extended beta-lactamase resistance (ATCC BAA-196). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070210.GS.4 (copy provided). The product was received ready-to-use, as a trigger spray. Testing was conducted on August 12, 2010 and September 7, 2010. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 41-42% relative humidity. For each lot of product, separate carriers were sprayed until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20-23°C at 42-63% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 44 hours at 35-37°C. For testing conducted on August 12, 2010, the subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation or incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation, and antibiotic resistance.

Note: Testing conducted on August 12, 2010 against *Escherichia coli* with extended beta-lactamase resistance at a 30-second exposure time showed growth in the subculture of 1 of the 10 carriers for Lot No. 10ACE9. Testing was repeated to test for false positives.

Note: The antimicrobial susceptibility pattern of *Escherichia coli* with extended beta-lactamase resistance (presumably ATCC BAA-196) was verified on a representative culture. The laboratory performed an AB BIODISK E test Method assay. An E test strip containing Cefotaxime (CT) and Cefotaxime + Clavulanic acid (CTL) and an E test strip containing Ceftazidime (TZ) and Ceftazidime + Clavulanic acid (TZL) were used. *Escherichia coli* (ATCC 35218) served as the negative control organism. *Klebsiella pneumoniae* (ATCC 700603) served as the positive control organism. The Minimum Inhibitory Concentration (MIC) values were determined. The MIC values confirmed the antimicrobial susceptibility pattern of *Escherichia coli* with extended beta-lactamase resistance (presumably ATCC BAA-196). See page 9 and Tables 5-7 of the laboratory report.

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

9. MRID 483360-17 "AOAC Germicidal Spray Method," Test Organism: *Klebsiella pneumoniae* Carbapenem Resistant (BAA-1705), for Blondie, F2010.0127, by Anne Stemper. Study conducted at ATS Labs. Study completion date – September 16, 2010. Project Number A09992.

This study was conducted against *Klebsiella pneumoniae* Carbapenem Resistant (BAA-1705). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested ATS Laboratory Protocol No. CX18070810.GS.3 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot

were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 45% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 18-24°C at 64% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705) was verified on a representative culture. The laboratory performed a Modified Hodge Test to confirm that the test organism produces a carbapenemase and is, therefore, carbapenem resistant. *Klebsiella pneumoniae* (ATCC BAA-1705) was the positive control organism. *Klebsiella pneumoniae* (ATCC BAA-1706) was the negative control organism. By inactivating the effect of meropenem and allowing growth of *Escherichia coli* (ATCC 25922), the presence of carbapenemase was demonstrated. Thus, antibiotic resistance of *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705) to carbapenem was confirmed. See page 9 and Table 5 of the laboratory report.

10. MRID 483360-18 "AOAC Germicidal Spray Method," Test Organism: Multidrug Resistant *Klebsiella pneumoniae* (ATCC 51503), for Blondie, F2010.0127, by Anne Stemper. Study conducted at ATS Labs. Study completion date – September 30, 2010. Project Number A09991.

This study was conducted against Multidrug Resistant *Klebsiella pneumoniae* (ATCC 51503). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070810.GS.2 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 42% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 23°C at 42% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Additional antibiotic profile testing of Multidrug Resistant *Klebsiella pneumoniae* was performed using a representative culture from the day of testing. [Note that the laboratory report refers to the organism as *Klebsiella pneumoniae* ESBL.] The testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, MN. The testing was

not performed under EPA GLP regulations. The minimum inhibitory concentrations confirmed antibiotic resistance of Multidrug Resistant *Klebsiella pneumoniae* to ampicillin, ampicillin/sulbactam, cefazolin, cefepime, ceftazidime, ceftriaxone, gentamicin, piperacillin/tazo, and trimethoprim/sulfa. See page 9 and Attachment I of the laboratory report.

11. MRID 483360-19 "AOAC Germicidal Spray Method," Test Organism: *Klebsiella pneumoniae* with extended beta-lactamase resistance (ATCC 700603), for Blondie, F2010.0127, by Becky Lien. Study conducted at ATS Labs. Study completion date – September 8, 2010. Project Number A10010.

This study was conducted against *Klebsiella pneumoniae* with extended beta-lactamase resistance (ATCC 700603). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070810.GS.1 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 41% relative humidity. For each lot of product, separate carriers were sprayed until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 52% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 46 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: The antimicrobial susceptibility pattern of *Klebsiella pneumoniae* with extended beta-lactamase resistance (ATCC 700603) was verified on a representative culture. The laboratory performed an AB BIODISK E test Method assay. An E test strip containing Cefotaxime (CT) and Cefotaxime + Clavulanic acid (CTL) and an E test strip containing Ceftazidime (TZ) and Ceftazidime + Clavulanic acid (TZL) were used. *Escherichia coli* (ATCC 35218) served as the negative control organism. *Klebsiella pneumoniae* (ATCC 700603) served as the positive control organism. The Minimum Inhibitory Concentration (MIC) values were determined. The MIC values confirmed the antimicrobial susceptibility pattern of *Klebsiella pneumoniae* with extended beta-lactamase resistance (ATCC 700603). See page 9 and Tables 5-7 of the laboratory report.

12. MRID 483360-20 "AOAC Germicidal Spray Method – Quantitated," Test Organism: *Campylobacter jejuni* (ATCC 29428), for Blondie, F2010.0127, by Anne Stemper. Study conducted at ATS Labs. Study completion date – September 20, 2010. Project Number A10048.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18071610.GS (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 4 day old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 25-30°C at 66% relative

humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 50% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. The subcultures were shaken gently after addition of the carriers. The entire volume of each subculture was individually transferred to the surface of a pre-wetted filter membrane and filtered using a vacuum pump. Each filter membrane was washed, removed from the filter unit, and transferred to a plate containing tryptic soy agar with 5% sheep's blood agar. All subcultures were incubated for 2 days at 35-37°C under microaerophilic conditions. Following incubation, the plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

13. MRID 483360-21 "AOAC Germicidal Spray Method," Test Organism: *Enterobacter aerogenes* (ATCC 13048), for Blondie, F2010.0127, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – September 7, 2010. Project Number A09977.

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070210.GS.2 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 42% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 23°C at 62% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 25-30°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

14. MRID 483360-22 "AOAC Germicidal Spray Method," Test Organism: *Stenotrophomonas maltophilia* (ATCC 25596), for Blondie, F2010.0127, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – September 20, 2010. Project Number A10050.

This study was conducted against *Stenotrophomonas maltophilia* (ATCC 25596). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18071310.GS.4 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a ~52.75 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 41% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 21°C at 64% relative humidity. Following the

exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. Carriers were transferred from the primary subcultures into individual secondary subcultures containing 20 mL of tryptic soy broth at least 30 minutes following the first transfer. All subcultures were incubated for 44.5 hours at 25-30°C. The subcultures were stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

15. MRID 483360-23 "AOAC Germicidal Spray Method," Test Organism: *Streptococcus pyogenes* (ATCC 19615), for Blondie, F2010.0127, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – September 23, 2010. Project Number A10052.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18071310.GS.6 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 51.5 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 25-30°C at 69% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 50% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Brain Heart Infusion Broth with 0.01% Catalase to neutralize. All subcultures were incubated for 44 hours at 35-37°C in CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

16. MRID 483360-24 "AOAC Germicidal Spray Method," Test Organism: Methicillin Resistant *Staphylococcus aureus* – MRSA (ATCC 33592), for Blondie, F2010.0127, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – September 14, 2010. Project Number A10081.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18071310.GS.1 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 45% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 66% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls

included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 6 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) to oxacillin. See page 9 and Table 5 of the laboratory report.

17. MRID 483360-25 "AOAC Germicidal Spray Method," Test Organism: Methicillin Resistant *Staphylococcus aureus* – MRSA Hospital Acquired (ATCC 33591), for Blondie, F2010.0127, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – September 20, 2010. Project Number A10082.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* Hospital Acquired (ATCC 33591). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18071310.GS.2 (copy provided). The product was received ready-to-use, as a trigger spray. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the culture was incubated for 48-54 hours at 35-37°C. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 40% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 54% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for ~48 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* Hospital Acquired (ATCC 33591) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 6 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* Hospital Acquired (ATCC 33591) to oxacillin. See page 9 and Table 5 of the laboratory report.

18. MRID 483360-26 "AOAC Germicidal Spray Method," Test Organism: Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA (NARSA NRS384) (Genotype USA300), for Blondie, F2010.0127, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – September 17, 2010. Project Number A10083.

This study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* (NARSA NRS384) (Genotype USA300; obtained from the NARSA Contracts Administrator at Focus Technologies, Inc., Herndon, VA). Two lots (Lot Nos. 10ACE8

and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18071310.GS.3 (copy provided). The product was received ready-to-use, as a trigger spray. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the culture was incubated for 48-54 hours at 35-37°C. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 35-37°C at 42% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 45 seconds at 19°C at 55% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate and 0.01% Catalase to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus* (NARSA NRS384) (Genotype USA300) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 6 mm) confirmed antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus* (NARSA NRS384) (Genotype USA300) to oxacillin. See page 9 and Table 5 of the laboratory report.

19. MRID 483360-27 "AOAC Germicidal Spray Method," Test Organism: *Streptococcus pneumoniae* – Penicillin Resistant (ATCC 700677), for Blondie, F2010.0127, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – September 23, 2010. Project Number A10051.

This study was conducted against *Streptococcus pneumoniae* – Penicillin Resistant (ATCC 700677). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18071310.GS.5 (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 4 day old culture of the test organism. Inoculum was uniformly spread over the entire surface of each carrier. The carriers were dried for 30 minutes at 25-30°C at 60-70% (65%) relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20°C at 49% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Brain Heart Infusion with 0.01% Catalase to neutralize. All subcultures were incubated for 44 hours at 35-37°C in CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of *Streptococcus pneumoniae* – Penicillin Resistant (ATCC 700677) was verified on a representative culture. The laboratory performed an E test Method assay. An E test strip containing benzylpenicillin was used. *Streptococcus pneumoniae* (ATCC 49619)

served as the control organism. The Minimum Inhibitory Concentration (MIC) values were determined. The MIC values confirmed antibiotic resistance of *Streptococcus pneumoniae* – Penicillin Resistant (ATCC 700677). See page 9 and Table 5 of the laboratory report.

20. MRID 483360-28 "AOAC Tuberculocidal Activity of Disinfectant Spray Products," Test Organism: *Mycobacterium bovis* BCG, for Blondie, F2010.0127, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – October 22, 2010. Project Number A09776.

This study was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation, Durham, NC). Three lots (Lot Nos. 10ACE8, 10ACE9, and 10ACE2) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18062310.TB.3 (copy provided). At least one of the product lots tested (i.e., Lot No. 10ACE2) was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 10.0 µL of a 24 day old suspension of the test organism. The carriers were dried for 30 minutes at 35-37°C at 42% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 4 minutes at 22.3°C at 50.9% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to individual tubes of 20 mL of Letheen Broth with 0.1 % sodium thiosulfate and 0.01% Catalase. The carriers were transferred to individual tubes containing 20 mL of Middlebrook 7H9 Broth. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Modified Proskauer-Beck Medium and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. All tubes used for secondary transfers were incubated for 30, 60, and 90 days at 35-37°C under aerobic conditions. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for initial suspension population, carrier population, purity, sterility, viability, and neutralization confirmation.

21. MRID 483360-29 "AOAC Germicidal Spray Method," Test Organism: *Trichophyton mentagrophytes* (ATCC 9533), for Blondie, F2010.0127, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – November 5, 2010. Project Number A09775.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Three lots (Lot Nos. 10ACE8, 10ACE9, and 10ACE2) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18062310.FGS (copy provided). At least one of the product lots tested (i.e., Lot No. 10ACE2) was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the conidial suspension to yield a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) were inoculated with 0.01 mL of a 10 day old suspension of test organism. The inoculum was uniformly spread over the surface of each carrier. The carriers were dried for 40 minutes at 35-37°C at 44% relative humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 22.7°C at 46.9% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Sabouraud Dextrose Broth with 0.01% Catalase to neutralize. Carriers were transferred from the primary subcultures into individual secondary subcultures containing 20 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5%

Tween 80 at least 30 minutes following the first transfer. All subcultures were incubated for 10 days 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, and neutralization confirmation.

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

22. MRID 483360-30 "AOAC Germicidal Spray Method, Test Organism: *Candida albicans* (ATCC 10231)" for Blondie, F2010.0127, by Becky Lien. Study conducted at ATS Labs. Study completion date – September 8, 2010. Project Number A10029.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested using ATS Laboratory Protocol No. CX18070210.FGS (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the conidial suspension to yield a 5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of a 2 day old suspension of test organism. The inoculum was uniformly spread over the surface of each carrier. The carriers were dried for 30 minutes at 25-30°C at 62% humidity. For each lot of product, separate carriers were sprayed (3 pumps) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 3 minutes at 20°C at 60% relative humidity. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. Carriers were transferred from the primary subcultures into individual secondary subcultures containing 20 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 at least 30 minutes following the first transfer. All subcultures were incubated for 44 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, and neutralization confirmation.

23. MRID 483360-31 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Cytomegalovirus, Strain AD-169, ATCC VR-538" for Blondie, F2010.0127, by Shanen Conway. Study conducted at ATS Labs. Study completion date – November 18, 2010. Project Number A09976.

This study was conducted against Cytomegalovirus (Strain AD-169; ATCC VR-538), using MRC-5 cells (human embryonic lung fibroblasts; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.CMV.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. For each lot of product, separate dried virus films were sprayed (4 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, a 2.00 mL aliquot of test medium was added, and the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 10% (v/v)

heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. To reduce the cytotoxicity of the test substance, the $10^{-1.3}$ dilutions were passed through individual Sephadex columns following titration. 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. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

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

Note: The laboratory reported a failed study set up on August 11, 2010. In that study, a recoverable virus titer of at least 10^4 was not achieved. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on October 19, 2010. See page 8 and Attachment I of the laboratory report.

24. MRID 483360-32 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 8, 2010. Project Number A09889.

This study, under the direction of Study Director Mary J. Miller, was conducted against Duck hepatitis B virus (Strain 7/31/07; obtained from HepadnaVirus Testing Inc., Palo Alto, CA), using primary duck hepatocytes (cultures prepared by Valley Research Institute personnel using hatchling ducks received from Metzer Farms) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18071310.DHBV.2 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 30 minutes at 20.0°C at 40% relative humidity. Two replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. Carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, each plate 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 using Leibovitz L-15 medium with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with 1.0 mL of selected dilutions. Test medium was added to sufficiently cover the monolayer during the adsorption period. The cultures were incubated overnight at 36-38°C in a humidified atmosphere of 5-7% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for 9 days at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were re-fed, as necessary. On the final day of incubation, the cultures were scored microscopically for cytotoxicity. An indirect immunofluorescence assay was then performed. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

25. MRID 483360-33 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus" for Blondie, F2010.0127, by Kelleen Gutzman. Study conducted at ATS Labs. Study completion date – November 11, 2010. Amended report date – November 19, 2010. Project Number A09888.

This confirmatory study, under the direction of Study Director Kelleen Gutzmann, was conducted against Duck hepatitis B virus (Strain 7/31/07; obtained from HepadnaVirus Testing Inc., Palo Alto, CA), using primary duck hepatocytes (cultures prepared by Valley Research Institute personnel using hatchling ducks received from Metzer Farms) as the host system. One lot (Lot No. 10ACE8) of the product, Blondie, F2010.0127, was tested according to ATS Labs Protocol No. CX18071310.DHBV.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 30 minutes at 20.0°C at 50% relative humidity. Two replicates were tested. For the single product lot, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. Carriers were allowed to remain wet for 30 seconds at 20.0°C. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially using Leibovitz L-15 medium with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with 1.0 mL of selected dilutions. Test medium was added to sufficiently cover the monolayer during the adsorption period. The cultures were incubated overnight at 36-38°C in a humidified atmosphere of 5-7% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for 9 days at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were re-fed, as necessary. On the final day of incubation, the cultures were scored microscopically for cytotoxicity. An indirect immunofluorescence assay was then performed. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: The initial laboratory report was amended to correct information regarding the cytotoxicity control.

26. MRID 483360-34 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 15, 2010. Project Number A10028.

This study, under the direction of Study Director Mary J. Miller, was conducted against Bovine viral diarrhea virus (Strain Oregon C24v-genotype 1; obtained from National Veterinary Services Laboratories, Ames IA), using bovine turbinate cells (BT cells; ATCC CRL-1390; propagated in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Protocol No. CX18071310.BVD.2 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained 5% horse serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity.

Two replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. Carriers were allowed to remain wet for 30 seconds at 21.0°C. Following exposure, each plate 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 using Minimum Essential Medium with 5% (v/v) non-heat inactivated horse serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. BT cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., rounded, dark, with a granular appearance), cytotoxicity, and viability. On the final day of incubation, infectious virus was assayed by a direct immunofluorescence assay to verify the CPE reading. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

27. MRID 483360-35 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus" for Blondie, F2010.0127, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – September 17, 2010. Project Number A10027.

This confirmatory study, under the direction of Study Director Kelleen Gutzmann, was conducted against Bovine viral diarrhea virus (Strain Oregon C24v-genotype 1; obtained from National Veterinary Services Laboratories, Ames, IA), using bovine turbinate cells (BT cells; ATCC CRL-1390; propagated in-house) as the host system. One lot (Lot No. 10ACE8) of the product, Blondie, F2010.0127, was tested according to ATS Protocol No. CX18071310.BVD.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained 5% horse serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0ºC at 50% relative humidity. Two replicates were tested. For the single product lot, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. Carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, each plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns. A 0.2 mL aliquot of the test virus was resuspended in 2.00 mL of the test substance, which equals a 1:10 dilution. Ten-fold serial dilutions were prepared, using Minimum Essential Medium with 5% (v/v) non-heat inactivated horse serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. BT cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., rounded, dark, with a granular appearance), cytotoxicity, and viability. On the final day of incubation, infectious virus was assayed by a direct immunofluorescence assay to verify the CPE reading. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

28. MRID 483360-36 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes simplex virus type 1, Strain F(1), ATCC VR-

733" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 10, 2010. Project Number A09939.

This study was conducted against Herpes simplex virus type 1 (Strain F(1); ATCC VR-733), using RK cells (rabbit kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.HSV1.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

29. MRID 483360-37 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes simplex virus type 2, Strain G, ATCC VR-734" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 10, 2010. Project Number A09974.

This study was conducted against Herpes simplex virus type 2 (Strain G; ATCC VR-734), using RK cells (rabbit kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.HSV2.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count,

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

30. MRID 483360-38 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human immunodeficiency virus type 1, Strain HTLV-III_B" for Blondie, F2010.0127, by Shanen Conway. Study conducted at ATS Labs. Study completion date – September 29, 2010. Project Number A10012.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using MT-2 cells (human T-cell leukemia cells; obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; maintained in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.HIV.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 22.0°C. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 22.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in RPMI-1640 with 15% (v/v) heat inactivated fetal bovine serum, 2.0 mM L-glutamine, and 50 µg/mL gentamicin. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 9 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

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

31. MRID 483360-39 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A (H5N1) virus, Strain VNH5N1-PR8/CDC-RG CDC #2006719965" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 8, 2010. Project Number A09886.

This study was conducted against Avian influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG; CDC #2006719965), 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. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18071310.AFLU.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 21.5°C at 45.6% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30

seconds at 21.5°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

32. MRID 483360-40 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A (H3N2) virus, Strain Hong Kong, ATCC VR-544" for Blondie, F2010.0127, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – September 3, 2010. Project Number A09975.

This study was conducted against Influenza A (H3N2) virus (Strain Hong Kong; ATCC VR-544), 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. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.FLUA.3 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 21.5°C at 45.6% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

33. MRID 483360-41 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: 2009-H1N1 Influenza A virus (Novel H1N1), Strain A/Mexico/4108/2009, CDC #2009712192" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 10, 2010. Project Number A09949.

This study was conducted against 2009-H1N1 Influenza A virus (Novel H1N1) (Strain A/Mexico/4108/2009; CDC #2009712192), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.FLUA.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to

contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 22.0°C at 40.7% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 22.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: An unforeseen circumstance reported in the study was reviewed and found to be acceptable.

34. MRID 483360-42 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza B virus, Strain B/Hong Kong/5/72, ATCC VR-823" for Blondie, F2010.0127, by Shanen Conway. Study conducted at ATS Labs. Study completion date – November 8, 2010. Project Number A09994.

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. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.FLUB.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

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

35. MRID 483360-43 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Human Norovirus" for Blondie, F2010.0127, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – September 20, 2010. Project Number A10080.

This study, under the direction of Study Director Kelleen Gutzmann, was conducted against Feline calicivirus (Strain F-9; ATCC VR-782), using CRFK cells (Crandel Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18071310.FCAL.2 (copy provided). The product was received ready-to-use, as a trigger spray. 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 pre-marked bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. Two replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. Carriers were allowed to remain wet for 50 seconds at 20.0°C. Following exposure, each plate 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 using Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

36. MRID 483360-44 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Human Norovirus – Confirmatory Assay" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 21, 2010. Project Number A10079.

This confirmatory study, under the direction of Study Director Mary J. Miller, was conducted against Feline calicivirus (Strain F-9; ATCC VR-782), using CRFK cells (Crandel-Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. One lot (Lot No. 10ACE8) of the product, Blondie, F2010.0127, was tested according to ATS Labs Protocol No. CX18071310.FCAL.1 (copy provided). The product was received ready-to-use, as a trigger spray. 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 pre-marked bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. Two replicates were tested. For the single product lot, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. Carriers were allowed to remain wet for 50 seconds at 20.0°C. Following exposure, each plate 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 using Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells

in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

37. MRID 483360-45 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rhinovirus type 37, Strain 151-1, ATCC VR-1147" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 9, 2010. Project Number A09913.

This study was conducted against Rhinovirus type 37 (Strain 151-1; ATCC VR-1147), using MRC-5 cells (human embryonic lung fibroblasts; ATCC CCL-171; propagated in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.R37.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 15.0°C at 50% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 50 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 10% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

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

38. MRID 483360-46 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rotavirus, Strain WA" for Blondie, F2010.0127, by Shanen Conway. Study conducted at ATS Labs. Study completion date – November 11, 2010. Project Number A09914.

This study was conducted against Rotavirus (Strain WA; obtained from the University of Ottawa, Ontario, Canada), using MA-104 cells (Rhesus monkey kidney cells; obtained from Diagnostic Hybrids, Inc., Athens, OH; propagated in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.ROT.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. For each lot of product, separate dried virus

films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 50 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 0.5 µg/mL trypsin, 2.0 mM L-glutamine, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MA-104 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The inoculum was allowed to adsorb for 60 minutes at 36-38°C in a humidified atmosphere of 5-7% CO_2. Following adsorption, the cultures were re-fed and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

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

39. MRID 483360-47 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Respiratory syncytial virus, Strain Long, ATCC VR-26" for Blondie, F2010.0127, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 11, 2010. Project Number A09915.

This study was conducted against Respiratory syncytial virus (Strain Long; ATCC VR-26), using Hep-2 cells (human larynx carcinoma; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.RSV.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 2% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, 10 µg/mL vancomycin, 2 mM L-glutamine, and 10 mM HEPES. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

40. MRID 483360-48 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human coronavirus, Strain 229E, ATCC VR-740" for Blondie, F2010.0127, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – September 10, 2010. Project Number A09912.

This study was conducted against Human coronavirus (Strain 229E; ATCC VR-740), using WI-38 cells (human embryonic lung fibroblasts; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10ACE8 and 10ACE9) of the product, Blondie, F2010.0127, were tested according to ATS Labs Protocol No. CX18070810.COR.1 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were sprayed (3 sprays) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 2% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. WI-38 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ carrier)
		Lot No. 10ACE8	Lot No. 10ACE9	Lot No. 10ACE2	
30-Second Exposure Time					
483360-09	*Salmonella enterica* Test Date: 7/20/2010	0/60	0/60	0/60	1.1×10^4
483360-10	*Pseudomonas aeruginosa* Test Date: 7/20/2010 Test Date: 7/27/2010	1/60 ---	2/60 0/60	0/60 ---	1.93×10^6 1.20×10^6
483360-12	*Acinetobacter baumannii* Test Date: 8/12/2010 Test Date: 9/07/2010	0/10 ---	1/10 0/10	--- ---	1.23×10^6 9.3×10^5
483360-13	Vancomycin Resistant *Enterococcus faecalis* Test Date: 8/23/2010	0/10	0/10	---	5.1×10^5
483360-14	*Escherichia coli* Test Date: 8/11/2010	0/10	0/10	---	3.3×10^6
483360-15	*Escherichia coli* O157:H7 Test Date: 8/11/2010	0/10	0/10	---	1.35×10^6
483360-16	*Escherichia coli* with extended beta-lactamase resistance Test Date: 8/12/2010	0/10	1/10	---	4.6×10^5

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ carrier)
		Lot No. 10ACE8	Lot No. 10ACE9	Lot No. 10ACE2	
	Test Date: 9/07/2010	---	0/10	---	1.00×10^6
483360-17	*Klebsiella pneumoniae* Carbapenem Resistant Test Date: 8/12/2010	0/10	0/10	---	7.2×10^5
483360-18	Multidrug Resistant *Klebsiella pneumoniae* Test Date: 8/12/2010	0/10	0/10	---	6.6×10^5
483360-19	*Klebsiella pneumoniae* with extended beta-lactamase resistance Test Date: 8/16/2010	0/10	0/10	---	3.8×10^4
483360-20	*Campylobacter jejuni* Test Date: 8/24/2010	0/10	0/10	---	4.0×10^5
483360-21	*Enterobacter aerogenes* Test Date: 8/12/2010	0/10	0/10	---	1.8×10^7
483360-22	*Stenotrophomonas maltophilia* Test Date: 8/20/2010	1°=0/10 2°=0/10	1°=0/10 2°=0/10	---	5.7×10^6
483360-23	*Streptococcus pyogenes* Test Date: 8/24/2010	0/10	0/10	---	4.0×10^5
483360-24	Methicillin Resistant *Staphylococcus aureus* Test Date: 8/26/2010	0/10	0/10	---	3.6×10^6
483360-25	Methicillin Resistant *Staphylococcus aureus* Hospital Acquired Test Date: 8/26/2010	0/10	0/10	---	1.67×10^6
483360-27	*Streptococcus pneumoniae* – Penicillin Resistant Test Date: 8/24/2010	0/10	0/10	---	4.1×10^4
45-Second Exposure Time					
483360-11	*Staphylococcus aureus* Test Date: 7/28/2010	0/60	1/60	0/60	3.9×10^6
483360-26	Community Acquired Methicillin Resistant *Staphylococcus aureus* (NARSA NRS384) (Genotype USA300) Test Date: 8/26/2010	0/10	0/10	---	4.0×10^6
3-Minute Exposure Time					
483360-29	*Trichophyton mentagrophytes* Test Date: 7/19/2010	1°=0/10 2°=0/10	1°=0/10 2°=0/10	1°=0/10 2°=0/10	6.8×10^5
483360-30	*Candida albicans* Test Date: 8/18/2010	1°=0/10 2°=0/10	1°=0/10 2°=0/10	--- ---	3.1×10^6

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 10ACE8	Lot No. 10ACE9	
30-Second Exposure Time					
483360-31	Cytomegalovirus	$10^{-1.3}$ dilution	Cytotoxicity	Cytotoxicity	$10^{4.8}$ $TCID_{50}$/0.1 mL
		$10^{-2.3}$ to $10^{-6.3}$ dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.8}$	$\leq 10^{1.8}$	
		Log reduction	≥ 3.0 $\log_{10}$	≥ 3.0 $\log_{10}$	
483360-32	Duck hepatitis B virus	10^{-2} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$ and $10^{5.75}$ $TCID_{50}$/1.0 mL
		$TCID_{50}$/1.0 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
483360-33	Duck hepatitis B virus	10^{-2} to 10^{-4} dilutions	Complete inactivation	---	$10^{5.75}$ and $10^{5.5}$ $TCID_{50}$/1.0 mL
		$TCID_{50}$/1.0 mL	$\leq 10^{1.5}$	---	
483360-34	Bovine viral diarrhea virus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$ and $10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-35	Bovine viral diarrhea virus	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	$10^{5.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	---	
483360-36	Herpes simplex virus type 1	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-37	Herpes simplex virus type 2	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-38	Human immunodeficiency virus type 1	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.0}$ $TCID_{50}$/0.2 mL
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.2 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 3.5 $\log_{10}$	≥ 3.5 $\log_{10}$	
483360-39	Avian influenza A (H5N1) virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-40	Influenza A (H3N2) virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-41	2009-H1N1 Influenza A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-42	Influenza B virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-47	Respiratory syncytial virus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{4.75}$ $TCID_{50}$/0.1 mL
		10^{-2} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 10ACE8	Lot No. 10ACE9	
		Log reduction	≥3.25 $\log_{10}$	≥3.25 $\log_{10}$	
483360-48	Human coronavirus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.0}$ $TCID_{50}$/0.1 mL
		10^{-2} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥3.5 $\log_{10}$	≥3.5 $\log_{10}$	
50-Second Exposure Time					
483360-43	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ and $10^{6.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-44	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	$10^{6.5}$ and $10^{6.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	---	
483360-45	Rhinovirus type 37	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
483360-46	Rotavirus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{4.5}$ $TCID_{50}$/0.1 mL
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥3.0 $\log_{10}$	≥3.0 $\log_{10}$	

MRID Number	Organism	Media	No. Exhibiting Growth/ Total No. Tested		
			Lot No. 10ACE8	Lot No. 10ACE9	Lot No. 10ACE2
			90 Days		
4-Minute Exposure Time					
483360-28	*Mycobacterium bovis* BCG Carrier Population: 5.3×10^5 CFU/carrier	Modified Proskauer-Beck Medium[1, 2]	0/10	0/10	0/10
		Middlebrook 7H9 Broth	0/10	0/10	0/10
		Kirchner's Medium[2]	0/10	0/10	0/10

[1]Modified Proskauer-Beck Medium results could not be used to support product efficacy because viability testing failed to show growth of *Mycobacterium bovis* BCG.
[2]Modified Proskauer-Beck Medium and Kirchner's Medium results could not be used to support product efficacy because neutralization confirmation testing failed to show growth of *Mycobacterium bovis* BCG in all three product lots.

VI. CONCLUSIONS

1. The submitted efficacy data support the use of the product, Blondie, F2010.0127, 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 the indicated contact times:

30-Second Contact Time

Salmonella enterica	MRID 483360-09
Pseudomonas aeruginosa	MRID 483360-10
Acinetobacter baumannii	MRID 483360-12
Vancomycin Resistant *Enterococcus faecalis*	MRID 483360-13
Escherichia coli	MRID 483360-14
Escherichia coli O157:H7	MRID 483360-15
Escherichia coli with extended beta-lactamase resistance	MRID 483360-16
Klebsiella pneumoniae Carbapenem Resistant	MRID 483360-17
Klebsiella pneumoniae	MRID 483360-18
Klebsiella pneumoniae with extended beta-lactamase resistance	MRID 483360-19
Campylobacter jejuni	MRID 483360-20
Enterobacter aerogenes	MRID 483360-21
Stenotrophomonas maltophilia	MRID 483360-22
Streptococcus pyogenes	MRID 483360-23
Methicillin Resistant *Staphylococcus aureus*	MRID 483360-24
Methicillin Resistant *Staphylococcus aureus* ~~Hospital Acquired~~	MRID 483360-25
Streptococcus pneumoniae – Penicillin Resistant	MRID 483360-27

45-Second Contact Time

Staphylococcus aureus	MRID 483360-11
Community Acquired Methicillin Resistant *Staphylococcus aureus* (NARSA NRS384) (Genotype USA300	MRID 483360-26

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. In testing against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella enterica*, at least one of the product lots tested was at least 60 days old at the time of testing. [Note that repeat testing was conducted on one product lot against *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli* with extended beta-lactamase resistance to evaluate for false positives.] Neutralization confirmation testing (for all organisms except for *Campylobacter jejuni)* showed positive growth of the microorganisms. Neutralization confirmation testing for *Campylobacter jejuni* met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. The proposed claim for Multi-Drug Resistant *Klebsiella pneumoniae* is unacceptable. The end-user is unaware of the drugs for which resistance has been demonstrated. Currently, the Agency is not accepting claims for Hospital Acquired Methicillin Resistant *Staphylococcus aureus*. 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 the product, Blondie, F2010.0127, as a disinfectant with fungicidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 3 minutes:

Trichophyton mentagrophytes	MRID 483360-29
Candida albicans	MRID 483360-30

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

3. The submitted efficacy data **support** the use of the product, Blondie, F2010.0127, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load (a 100% organic soil load for Duck hepatitis B virus) for the indicated contact times:

30-Second Contact Time

Cytomegalovirus	MRID 483360-31
Duck hepatitis B virus	MRID 483360-32 and -33
Bovine viral diarrhea virus	MRID 483360-34 and -35
Herpes simplex virus type 1	MRID 483360-36
Herpes simplex virus type 2	MRID 483360-37
Human immunodeficiency virus type 1	MRID 483360-38
Avian influenza A (H5N1) virus	MRID 483360-39
Influenza A (H3N2) virus	MRID 483360-40
2009-H1N1 Influenza A virus	MRID 483360-41
Influenza B virus	MRID 483360-42
Respiratory syncytial virus	MRID 483360-47
Human coronavirus	MRID 483360-48

50-Second Contact Time

Feline calicivirus	MRID 483360-43 and -44
Rhinovirus type 37	MRID 483360-45
Rotavirus	MRID 483360-46

Recoverable virus titers of at least 10^4 were achieved. In studies against Cytomegalovirus, cytotoxicity was observed in the $10^{-1.3}$ dilutions. In studies against Human immunodeficiency virus type 1, Rotavirus, Respiratory syncytial virus, and Human coronavirus, cytotoxicity was observed in the 10^{-1} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against all other viruses, cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested. In studies against Duck hepatitis B virus and Bovine viral diarrhea virus, the initial and confirmatory studies were performed at the same laboratory but under the direction of different study directors. Both the initial and confirmatory studies tested two replicates per product lot. The confirmatory study tested one product lot, not the standard two product lots.

4. The submitted efficacy data (MRID 483360-28) **do not support** the use of the product, Blondie, F2010.0127, as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on hard, non-porous surfaces in the presence of a 5% organic soil load for a 4-minute contact time. Product efficacy was successfully demonstrated in one medium, Middlebrook 7H9 Broth. **Product efficacy was not successfully demonstrated in the other two media**. Although completing killing was observed in these two media, the viability and neutralization confirmation controls for Modified Proskauer-Beck Medium failed to show growth; and the neutralization confirmation control for Kirchner's Medium failed to show growth. Without

acceptable neutralization confirmation test results, product efficacy in the 2 additional media cannot be confirmed.

Neutralization confirmation testing showed positive growth of the microorganism in Middlebrook 7H9 Broth. Viability controls conducted with Middlebrook 7H9 Broth and Kirchner's Medium were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

VII. LABEL

1. The product Blondie is a spray only product and cannot be used in immersion application. **The applicant must delete all immersion applications from the proposed label.**

2. The proposed label claims that the product, Blondie, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 5% blood serum for a 30-second contact time:

Salmonella enterica
Pseudomonas aeruginosa

Acinetobacter baumannii
Campylobacter jejuni
Carbapenem Resistant *Klebsiella pneumoniae*
Enterobacter aerogenes
Escherichia coli
Escherichia coli O157:H7
Extended spectrum beta-lactamase producing *Escherichia coli*
Extended spectrum beta-lactamase producing *Klebsiella pneumoniae*
~~Multidrug Resistant~~ *Klebsiella pneumoniae*
Penicillin-Resistant *Streptococcus pneumoniae*
Stenotrophomonas maltophilia
Streptococcus pyogenes
Vancomycin Resistant *Enterococcus faecalis*

Avian influenza A (H5N1) virus
Bovine viral diarrhea virus
Cytomegalovirus
Duck hepatitis B virus (surrogate for Human hepatitis B virus)
Hepatitis B virus
Hepatitis C virus
Herpes simplex virus type 1
Herpes simplex virus type 2
Human coronavirus
Human immunodeficiency virus type 1
Influenza A (H3N2) virus
2009-H1N1 Influenza A virus
Influenza B virus
Respiratory syncytial virus

These claims are acceptable as they are supported by the submitted data. The proposed claim for Multi-Drug Resistant *Klebsiella pneumoniae* is unacceptable. The end-user is unaware of the drugs for which resistance has been demonstrated.

3. The proposed label claims that the product, Blondie, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 5% blood serum for a 1-minute contact time:

Community Acquired Methicillin Resistant *Staphylococcus aureus*
Methicillin Resistant *Staphylococcus aureus* (ATCC 33592)
Methicillin Resistant *Staphylococcus aureus* (ATCC 33591)
Staphylococcus aureus

Feline calicivirus
Norovirus
Rhinovirus type 37
Rotavirus

These claims are acceptable as they are supported by the submitted data. Currently, the Agency is not accepting claims for Hospital Acquired Methicillin Resistant *Staphylococcus aureus.*

4. The proposed label claims that the product, Blondie, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 5% blood serum for a 3-minute contact time:

Candida albicans
Trichophyton mentagrophytes

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

5. The proposed label claims that the product, Blondie, is an effective tuberculocide on hard, non-porous surfaces for a 4-minute contact time. As noted in the "Conclusions" section of this report, product efficacy was demonstrated in only one of the media. **References to *Mycobacterium bovis* BCG must be deleted from the proposed label**.

6. The following revisions to the proposed label are recommended:

- On page 8 of the proposed label, **delete** the following claim as pre-cleaning is required for heavily soiled surfaces: "**Precleaning is not required**."

- On page 8 of the proposed label, change "***Enterococcus faecalis***" to read "**Vancomycin Resistant *Enterococcus faecalis***."

- On page 15 of the proposed label, change "**enamel**" to read "**baked enamel**." Enamel is a porous surface.

- On page 16 of the proposed label, change "**plexiglass**" to read "**Plexiglas**."