UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

July 26, 2011

MEMORANDUM

Subject: Efficacy Review for Austin A-1 Ultra Disinfecting Bleach; EPA Reg. No. 1672-65; DP Barcode: D389209

From: Ibrahim Laniyan, Ph.D.
Microbiologist
Product Science Branch
Antimicrobials Division (7510P)

Thru: Tajah Blackburn, Ph.D.
Team Leader
Product Science Branch
Antimicrobials Division (7510P)

To: Wanda Henson / Thomas Luminello
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: James Austin Company
P.O. Box 827
Mars, PA 16046

Formulation from the Label:

Active Ingredient	% by wt.
Sodium Hypochlorite	6.0 %
Other Ingredients:	94.7 %
Total	100.0 %

I. BACKGROUND

The product, Austin A-1 Ultra Disinfecting Bleach (EPA Reg. No. 1672-65), is an EPA-approved disinfectant (bactericide, fungicide, tuberculocide, virucide), sanitizer, mildewcide, and deodorizer for use on hard, non-porous surfaces in household, commercial, institutional, food service, animal care, and hospital or medical environments. The applicant provided a new, complete set of efficacy studies to supplement efficacy data used to support initial product registration. 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's representative to EPA (dated April 12, 2011), twenty six studies (MRID 484527-01 through 484527-26), Statements of No Data Confidentiality Claims for all twenty six studies, and the proposed label (dated April 12, 2011).

II. USE DIRECTIONS

The product is designed for disinfecting and sanitizing hard, non-porous surfaces. The product may be used to treat hard, non-porous surfaces, including: bathtubs, countertops, equipment, floors, showers, sinks, and toilet bowls. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: ceramic tile, enamel, porcelain, and vinyl. Directions on the proposed label provide the following information regarding preparation and use of the product:

> As a disinfectant: Pre-wash surfaces and rinse. Mix ¾ cup of the product and 1 gallon of water (a 1:21.33 dilution; ~2,400 ppm available chlorine). Spray, rinse, or wipe surface. Let stand for 10 minutes. Drain and air dry.
>
> As a tuberculocide: Pre-wash surfaces and rinse. Mix 1.75 cup of the product and 1 gallon of water (a 9.1 dilution). Spray, rinse, or wipe surface. Let stand for 5 minutes. Drain and air dry.
>
> As a sanitizer on food contact surfaces: Remove gross food particles from surface. Pre-wash surface with a good detergent and rinse thoroughly with potable water. Mix ½ ounce of the product and 1 gallon of water (a 1:256 dilution; 200 ppm available chlorine). Cover surface with use solution for at least 2 minutes. Air dry.
>
> As a sanitizer on non-food contact surfaces: Pre-wash with detergent. Rinse. Mix ½ ounce of the product and 1 gallon of water (a 1:256 dilution; 200 ppm available chlorine). Cover surface with use solution for at least 2 minutes. Drain. Let air dry.

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

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

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.

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces): Sanitizing rinses may be formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, or anionic detergent-acid formulations. The effectiveness of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method. Data from the test on 1 sample from each of 3 different product lots, one of which is at least 60 days old against *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) are required. When the effectiveness of the product in hard water is made, all required data must be developed at the hard water tolerance claimed. Acceptable results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and the percentage reduction over the control. Furthermore, counts on the number controls for the product should fall between 75 and 125 x 10^6/mL for percent reductions to be considered valid. Label directions for use must state that a contact time of at least 1 minute is required for sanitization. A potable water rinse is not required (to remove the use solution for the treated surface) for products cleared for use on food contact surfaces under the Federal Food, Drug, and Cosmetic Act. Label directions must recommend a potable water rinse (to remove the use solution from the treated surface) under any other circumstances.

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces): Sanitizing rinses may be formulated with iodophors, mixed halides, or chlorine-bearing chemicals. The effectiveness of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC Available Chlorine Germicidal Equivalent Concentration Method. Data from one test on each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Salmonella enterica* (formerly *Salmonella choleraesuis*) are required. Test results must show product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. The reference standard is sodium hypochlorite.

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

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 484527-01 "AOAC Use-Dilution Method," Test Organism: *Salmonella enterica* (ATCC 10708), for Austin's A-1 Ultra Bleach, by Anne Stemper. Study conducted at ATS Labs. Study completion date – December 3, 2009. Project Number A08608.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 09267, 09309, and 09315) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01110909.UD.3 (copy provided). At least one of the product lots tested (i.e., Lot No. 09267) was at least 60 days old at the time of testing. Use solutions were prepared by adding 87.0 mL of the product and 1,853.1 mL of filter sterilized deionized water (a 1:21.3 dilution). Use solutions were not tested in the presence of a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 35-37°C at 40% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 5 minutes at 19.0-20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

2. MRID 484527-02 "AOAC Use-Dilution Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Austin's A-1 Ultra Bleach, by Anne Stemper. Study conducted at ATS Labs. Study completion date – February 16, 2010. Project Number A08857.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 09265) of the product, Austin's A-1 Ultra Bleach, was tested using ATS Laboratory Protocol No. JAC01112509.UD.1 (copy provided). The product lot tested (i.e., Lot No. 09265) was at least 60 days old at the time of testing. A use solution was prepared by adding 60.0 mL of the product and 1,278 mL of filter sterilized deionized water (a 1:21.3 dilution). The use solution was not tested in the presence of a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 48% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 21.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

3. MRID 484527-03 "AOAC Use-Dilution Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Austin's A-1 Ultra Bleach, by Anne Stemper. Study conducted at ATS Labs. Study completion date – January 29, 2010. Project Number A08858.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 09335) of the product, Austin's A-1 Ultra Bleach, was tested using ATS Laboratory Protocol No. JAC01112509.UD.2 (copy provided). A use solution was prepared by adding 60.0 mL of the product and 1,278 mL of filter sterilized deionized water (a 1:21.3 dilution). The use solution was

not tested in the presence of a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 48% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 21.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

4. MRID 484527-04 "AOAC Use-Dilution Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Austin's A-1 Ultra Bleach, by Anne Stemper. Study conducted at ATS Labs. Study completion date – January 29, 2010. Project Number A08859.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 09337) of the product, Austin's A-1 Ultra Bleach, was tested using ATS Laboratory Protocol No. JAC01112509.UD.3 (copy provided). A use solution was prepared by adding 60.0 mL of the product and 1,278 mL of filter sterilized deionized water (a 1:21.3 dilution). The use solution was not tested in the presence of a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 48% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 21.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

5. MRID 484527-05 "AOAC Use-Dilution Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Austin's A-1 Ultra Bleach, by Anne Stemper. Study conducted at ATS Labs. Study completion date – February 16, 2010. Project Number A08860.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 09265) of the product, Austin's A-1 Ultra Bleach, was tested using ATS Laboratory Protocol No. JAC01112509.UD.4 (copy provided). The product lot tested (i.e., Lot No. 09265) was at least 60 days old at the time of testing. A use solution was prepared by adding 60.0 mL of the product and 1,278 mL of filter sterilized deionized water (a 1:21.3 dilution). The use solution was not tested in the presence of a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 48% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

6. MRID 484527-06 "AOAC Use-Dilution Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Austin's A-1 Ultra Bleach, by Anne Stemper. Study

conducted at ATS Labs. Study completion date – January 29, 2010. Project Number A08861.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 09335) of the product, Austin's A-1 Ultra Bleach, was tested using ATS Laboratory Protocol No. JAC01112509.UD.5 (copy provided). A use solution was prepared by adding 60.0 mL of the product and 1,278 mL of filter sterilized deionized water (a 1:21.3 dilution). The use solution was not tested in the presence of a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 48% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

7. MRID 484527-07 "AOAC Use-Dilution Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Austin's A-1 Ultra Bleach, by Anne Stemper. Study conducted at ATS Labs. Study completion date – January 29, 2010. Project Number A08862.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 09337) of the product, Austin's A-1 Ultra Bleach, was tested using ATS Laboratory Protocol No. JAC01112509.UD.6 (copy provided). A use solution was prepared by adding 60.0 mL of the product and 1,278 mL of filter sterilized deionized water (a 1:21.3 dilution). The use solution was not tested in the presence of a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 48% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

8. MRID 484527-08 "AOAC Use-Dilution Method," Test Organism: *Escherichia coli* O157:H7 (ATCC 35150), for Austin's A-1 Ultra Bleach, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – April 1, 2010. Project Number A09160.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two lots (Lot Nos. 09280 and 09287) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01100109.UD.2 (copy provided). Use solutions were prepared by adding 16.0 mL of the product and 340.8 mL of filter sterilized deionized water (a 1:21.3 dilution). Use solutions were not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 35-37°C at 45.80% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate 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, and neutralization confirmation.

9. MRID 484527-09 "AOAC Use-Dilution Method," Test Organism: *Streptococcus pyogenes* (ATCC 12344), for Austin's A-1 Ultra Bleach, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – April 1, 2010. Project Number A09161.

This study was conducted against *Streptococcus pyogenes* (ATCC 12344). Two lots (Lot Nos. 09280 and 09287) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01100109.UD.3 (copy provided). Use solutions were prepared by adding 16.0 mL of the product and 340.8 mL of filter sterilized deionized water (a 1:21.3 dilution). Use solutions were not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 35-37°C at 62% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Brain Heart Infusion Broth at least 30 minutes after subculture of the first carrier. All subcultures were incubated for 46 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.

10. MRID 484527-10 "AOAC Use-Dilution Method," Test Organism: *Shigella dysenteriae* (ATCC 11835), for Austin's A-1 Ultra Bleach, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – April 23, 2010. Project Number A09312.

This study was conducted against *Shigella dysenteriae* (ATCC 11835). Two lots (Lot Nos. 10070 and 10081) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01030110.UD.2 (copy provided). Use solutions were prepared by adding 6.0 mL of the product and 128.0 mL of filter sterilized deionized water (a 1:21.3 dilution). Use solutions were not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 25-30°C at 62% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

11. MRID 484527-11 "AOAC Use-Dilution Method," Test Organism: Methicillin Resistant *Staphylococcus aureus* (ATCC 33592), for Austin's A-1 Ultra Bleach, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – May 13, 2010. Project Number A09313.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592). Two lots (Lot Nos. 10070 and 10081) of the product, Austin's A-1 Ultra Bleach, were

tested using ATS Laboratory Protocol No. JAC01030110.UD.1 (copy provided). Testing was conducted on April 13, 2010 and May 4, 2010. Use solutions were prepared by adding 6.0 mL of the product and 127.8 mL of filter sterilized deionized water (a 1:21.3 dilution). Use solutions were not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 40-43% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 19.0-20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Testing conducted on April 13, 2010 showed growth in 1 subculture of 10 carriers for one product lot (i.e., Lot No. 10081). Testing was repeated to test for false positives.

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 for both test dates) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33952) to oxacillin. See page 9 and Table 5 of the laboratory report.

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

12. MRID 484527-12 "Germicidal and Detergent Sanitizing Action of Disinfectants," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229), for Austin's A-1 Ultra Bleach, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – September 28, 2010. Project Number A10144.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229). Three lots (Lot Nos. 10160, 10210, and 10216) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01080510.GDST (copy provided). At least one of the product lots tested (i.e., Lot No. 10160) was at least 60 days old at the time of testing. Use solutions were prepared by adding 19.0 mL of the product and 404.7 mL of filter sterilized deionized water (a 1:21.3 dilution). The absorbance value of the *Staphylococcus aureus* culture suspension was determined (at 620 nm); no further adjustment was necessary. The absorbance value of the *Escherichia coli* culture suspension was determined (at 620 nm); sterile phosphate buffer dilution water was added to target 1 x 10^{10} CFU/mL. Use solutions were not tested in the presence of a 5% organic soil load. A 99.0-mL aliquot of each use solution was transferred to a 250-300 mL Erlenmeyer flask and placed in a water bath at 24.0°C. One-mL bacterial suspension was added to each flask. One-mL aliquots of the bacterium-product mixture were transferred to 9 mL of Letheen Broth with 0.2% sodium thiosulfate exactly 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in tryptone glucose extract agar. All plates were incubated for 45.75 hours at 35-37°C. Following incubation, the colonies were counted. Controls included those for numbers count, purity, sterility, viability, and neutralization confirmation.

13. MRID 484527-13 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), for Austin's A-1 Ultra Bleach, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – October 7, 2010. Project Number 10145.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. 10160, 10210, and 10216) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01080510.NFS (copy provided). At least one of the product lots tested (i.e., Lot No. 10160) was at least 60 days old at the time of testing. Testing was conducted on September 7, 2010 and September 21, 2010. Use solutions were prepared by adding 3.0 mL of the product and 63.9 mL of filter sterilized deionized water (a 1:21.3 dilution). Fetal bovine serum was added to each culture to achieve a 5% organic soil load. Five sterile glass carriers (1 inch x 1 inch) per product lot per microorganism were inoculated with 10.0 µL of a 48±4 hour old suspension of test organism. The inoculum was spread to within 1/8 inch of the edges of each carrier. The carriers were dried for 20 minutes at 35-37°C at 40-41% relative humidity. Each carrier was treated with 5.0 mL of the use solution for 5 minutes at 20-21°C. Following exposure, 20.0 mL of Letheen Broth with 1.0% sodium thiosulfate was added to each vessel. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. *Staphylococcus aureus* subcultures were incubated for 44 hours at 35-37°C. *Enterobacter aerogenes* subcultures were incubated for ~46.25 hours at 25-30°C. Following incubation, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation.

Note: Testing initiated on September 1, 2010 against *Staphylococcus aureus* for a 10-minute exposure time was stopped on September 2, 2010 by discarding the test subcultures. On September 2, 2010, the applicant requested the study protocol be changed to test a 5-minute exposure time. Testing was repeated for *Staphylococcus aureus* for a 5-minute exposure time on September 21, 2010.

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

14. MRID 484527-14 "Fungicidal Use-Dilution Method," Test Organism: *Trichophyton mentagrophytes* (ATCC 9533), for Austin's A-1 Ultra Bleach, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – December 3, 2009. Project Number A08511.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 09280 and 09287) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01100109.FUD.2 (copy provided). Use solutions were prepared by adding 6.0 mL of the product and 128.0 mL of filter sterilized deionized water (a 1:21.3 dilution). Use solutions were not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 10-day old suspension of test organism, at a ratio of 1 carrier per 1.0 mL suspension. The carriers were dried for 40 minutes at 35-37°C at 47% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. Carriers were transferred from

primary subculture tubes into individual secondary subculture tubes containing 10 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. 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.

15. MRID 484527-15 "Fungicidal Use-Dilution Method," Test Organism: *Aspergillus niger* (ATCC 16404), for Austin's A-1 Ultra Bleach, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – April 5, 2010. Project Number A09159.

This study was conducted against *Aspergillus niger* (ATCC 16404). Two lots (Lot Nos. 09280 and 09287) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01100109.FUD.1 (copy provided). Use solutions were prepared by adding 16.0 mL of the product and 340.8 mL of filter sterilized deionized water (a 1:21.3 dilution). Use solutions were not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in an 8-day old suspension of test organism, at a ratio of 1 carrier per 1 mL suspension. The carriers were dried for 40 minutes at 35-37°C at 58.35% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.2% sodium thiosulfate to neutralize. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Sabouraud Dextrose Broth with 0.7% 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.

16. MRID 484527-16 "AOAC Tuberculocidal Activity of Disinfectants," Test Organism: *Mycobacterium bovis* BCG, for Austin's A-1 Ultra Bleach, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – December 20, 2010. Project Number A10134.

This study was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation, Durham, NC). Three lots (Lot Nos. 10160, 10210, and 10216) of the product, Austin's A-1 Ultra Bleach, were tested using ATS Laboratory Protocol No. JAC01080510.TB (copy provided). Use solutions were prepared by adding 6.0 mL of the product and 127.8 mL of filter sterilized deionized water (a 1:21.3 dilution). Fetal bovine serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) porcelain penicylinder carriers per product lot were immersed for 15 minutes in a 25-day old suspension of the test organism, at a ratio of 1 carrier per 1.0 mL suspension. The carriers were dried for 30 minutes at 35-37°C at 31.96% humidity. Each carrier was placed in 10.0 mL of the use solution for 10 minutes at 19.0°C. Following exposure, the carriers were transferred to individual tubes of 10 mL of Letheen Broth with 0.2% sodium thiosulfate. The carriers were transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. All tubes used for secondary transfers were incubated for 31, 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 carrier population, purity, sterility, viability, and neutralization confirmation.

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

17. MRID 484527-17 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rotavirus" for Austin's A-1 Ultra Bleach, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – December 22, 2009. Project Number A08654.

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; maintained in-house) as the host system. Two lots (Lot Nos. 09280 and 09287) of the product, Austin's A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01100109.ROT (copy provided). Use solutions were prepared by adding 4.0 mL of the product and 85.6 mL of filter sterilized deionized water (a 1:21.4 dilution). The stock virus culture was adjusted to contain 1% 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 exposed to 2.00 mL of the use solution for 5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in serum-free Minimum Essential Medium with 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 0.5 µg/mL trypsin, and 2.0 mM L-glutamine. 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. 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.

18. MRID 484527-18 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A (H3N2) virus (Avian Reassortant)" for Austin's A-1 Ultra Bleach, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – December 22, 2009. Project Number A08655.

This study was conducted against Avian influenza A (H3N2) virus, Avian Reassortant (Strain A/Washington/897/80 X A/Mallard/New York/6750/78; ATCC VR-2072), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09280 and 09287) of the product, Austin's A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01100109.AFLU (copy provided). Use solutions were prepared by adding 4.0 mL of the product and 85.6 mL of filter sterilized deionized water (a 1:21.4 dilution). The stock virus culture was adjusted to contain 1% 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 exposed to 2.00 mL of the use solution for 5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL

amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

19. MRID 484527-19 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rhinovirus type 37" for Austin's A-1 Ultra Bleach, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – December 21, 2009. Project Number A08656.

This study was conducted against Rhinovirus type 37 (Strain 151-1; ATCC VR-1147), using MRC-5 cells (human embryonic lung cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 09280 and 09287) of the product, Austin's A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01100109.R37 (copy provided). Use solutions were prepared by adding 4.0 mL of the product and 85.6 mL of filter sterilized deionized water (a 1:21.4 dilution). The stock virus culture was adjusted to contain 1% 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 exposed to 2.00 mL of the use solution for 5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. 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.

20. MRID 484527-20 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Hepatitis A virus" for Austin's A-1 Ultra Bleach, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 13, 2010. Project Number A09365.

This study was conducted against Hepatitis A virus (Strain HM-175; obtained from AppTec Laboratory Services, Camden, NJ), using FRhK-4 cells (fetal Rhesus monkey kidney cells; ATCC CRL-1688; propagated in-house) as the host system. Two lots (Lot Nos. 10070 and 10081) of the product, Austin's A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01030110.HAV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 21.33 mL of filter sterilized deionized water (a 1:21.33 dilution). The stock virus culture contained 1% 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 exposed to 2.00 mL of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 10% heat-

inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, and 2.0 mM L-glutamine. FRhK-4 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The inoculum was allowed to adsorb for 90 minutes at 36-38°C in a humidified atmosphere of 5-7% CO_2. Following adsorption, the cultures were re-fed. The cultures were then incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 15 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

21. MRID 484527-21 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Adenovirus type 2" for Austin's A-1 Ultra Bleach, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 11, 2010. Project Number A09400.

This study was conducted against Adenovirus type 2 (Strain Adenoid 6; ATCC VR-846), using A-549 cells (human lung carcinoma cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10070 and 10081) of the product, Austin's A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01030110.ADV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 21.3 mL of filter sterilized deionized water (a 1:21.3 dilution). The stock virus culture was adjusted to contain 1% 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 exposed to 2.00 mL of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, and 10 mM HEPES. A-549 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

22. MRID 484527-22 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus" for A-1 Ultra Bleach, by Shanen Conway. Study conducted at ATS Labs. Study completion date – July 14, 2010. Project Number A09670.

This study was conducted against Influenza A 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. 10118 and 10131) of the product, A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01052410.FLUA (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 21.3 mL of filter sterilized deionized water (a 1:21.3 dilution). The stock virus culture contained 1% 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 exposed to 2.00 mL of the use solution for

10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium 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.

23. MRID 484527-23 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes simplex virus type 2" for A-1 Ultra Bleach, by Shanen Conway. Study conducted at ATS Labs. Study completion date – July 19, 2010. Project Number A09684.

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. 10118 and 10131) of the product, A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01052110.HSV2 (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 21.3 mL of filter sterilized deionized water (a 1:21.3 dilution). The stock virus culture contained 1% 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 exposed to 2.00 mL of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium 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.

24. MRID 484527-24 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Respiratory syncytial virus" for A-1 Ultra Bleach, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – July 20, 2010. Project Number A09697.

This study was conducted against Respiratory syncytial virus (Strain Long; ATCC VR-26), using Hep-2 cells (human larynx carcinoma cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 10118 and 10131) of the product, A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01052110.RSV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 21.3 mL of filter sterilized deionized water (a 1:21.3 dilution). The stock virus culture was adjusted to contain 1% 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 exposed to 2.00 mL of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, 10 µg/mL vancomycin, 2 mM L-glutamine, and 10 mM HEPES. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

25. MRID 484527-25 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Cytomegalovirus" for A-1 Ultra Bleach, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 1, 2010. Project Number A09789.

This study was conducted against Cytomegalovirus (Strain AD-169; ATCC VR-538), using MRC-5 cells (human embryonic lung fibroblasts; ATCC CCL-171; propagated in-house) as the host system. Two lots (Lot Nos. 10118 and 10131) of the product, A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01052110.CMV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 21.3 mL of filter sterilized deionized water (a 1:21.3 dilution). The stock virus culture was adjusted to contain 1% 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 exposed to 2.00 mL of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium 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 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 27 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

26. MRID 484527-26 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Immunodeficiency virus type 1" for Austin's A-1 Ultra Bleach, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – October 7, 2010. Project Number A10155.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using MT-2 cells (human T-cell leukemia cells; obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; maintained in-house) as the host system. Two lots (Lot Nos. 10210 and 10216) of the product, Austin's A-1 Ultra Bleach, were tested according to ATS Labs Protocol No. JAC01080510.HIV (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 21.3 mL of filter sterilized deionized water (a 1:21.3 dilution). The stock

virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 21.0°C. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 10 minutes at 21.0°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, 50 µg/mL gentamicin, and 2.0 mM L-glutamine. 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 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.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ carrier)
5-Minute Exposure Time					
		Lot No. 09267	**Lot No. 09309**	**Lot No. 09315**	
484527-01	*Salmonella enterica* Test Date: 11/17/2009	0/60	0/60	0/60	7.9×10^6
		Lot No. 09280	**Lot No. 09287**		
484527-14	*Trichophyton mentagrophytes* Test Date: 11/04/2009	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	---	1.27×10^6
10-Minute Exposure Time					
484527-02	*Staphylococcus aureus* Test Date: 1/12/2010	0/60	---	---	9.9×10^6
		Lot No. 09265	**Lot No. 09335**	**Lot No. 09337**	
484527-03	*Staphylococcus aureus* Test Date: 1/12/2010	---	1/60	---	7.5×10^6
484527-04	*Staphylococcus aureus* Test Date: 1/12/2010	---	---	1/60	6.7×10^6
484527-05	*Pseudomonas aeruginosa* Test Date: 1/12/2010	1/60	---	---	5.2×10^5
484527-06	*Pseudomonas aeruginosa* Test Date: 1/12/2010	---	1/60	---	6.6×10^6
484527-07	*Pseudomonas aeruginosa* Test Date: 1/12/2010	---	---	0/60	6.8×10^5
		Lot No.	**Lot No.**	---	

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ carrier)
		09280	**09287**		
484527-08	*Escherichia coli* O157:H7 Test Date: 3/15/2010	0/10	0/10	---	3.1×10^6
484527-09	*Streptococcus pyogenes* Test Date: 3/15/2010	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	---	1.6×10^4
484527-15	*Aspergillus niger* Test Date: 3/15/2010	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	---	4.5×10^5
		Lot No. 10070	**Lot No. 10081**	---	
484527-10	*Shigella dysenteriae* Test Date: 4/14/2010	0/10	0/10	---	5.5×10^5
484527-11	Methicillin Resistant *Staphylococcus aureus* Test Date: 4/13/2010 Test Date: 5/04/2010	0/10 ---	1/10 0/10	---	5.3×10^6 1.90×10^6

MRID Number	Organism	Media	No. Exhibiting Growth/ Total No. Tested		
			Lot No. 10160, 90 Days	**Lot No. 10210, 90 Days**	**Lot No. 10216, 90 Days**
10-Minute Exposure Time					
484527-16	*Mycobacterium bovis* BCG Carrier Population: 5.4×10^5 CFU/carrier	Modified Proskauer-Beck Medium[1]	0/10	0/10	0/10
		Middlebrook 7H9 Broth	0/10	1/10[2]	0/10
		Kirchner's Medium[1]	0/10	0/10	0/10

[1]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 for all three product lots in these two media.

[2]Neutralization confirmation testing failed to show growth of *Mycobacterium bovis* BCG in Middlebrook 7H9 Broth for one product lot (i.e., Lot No. 10210). This media type did show growth in the test subcultures, indicating test substance neutralization.

MRID Number	Organism	Results			Dried Virus Count
5-Minute Exposure Time					
			Lot No. 09280	**Lot No. 09287**	
484527-17	Rotavirus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484527-18	Avian influenza A (H3N2) virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	Organism	Results			Dried Virus Count
484527-19	Rhinovirus type 37	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
10-Minute Exposure Time					
			Lot No. 10070	**Lot No. 10081**	
484527-20	Hepatitis A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484527-21	Adenovirus type 2	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
			Lot No. 10118	**Lot No. 10131**	
484527-22	Influenza A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484527-23	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	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484527-24	Respiratory syncytial virus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
484527-25	Cytomegalovirus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
			Lot No. 10210	**Lot No. 10216**	
484527-26	Human immunodeficiency virus type 1	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.25}$ $TCID_{50}$/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	≥ 3.75 $\log_{10}$	≥ 3.75 $\log_{10}$	

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/carrier)		
30-Second Exposure Time					
484527-12	*Staphylococcus aureus*	**10160**	$<1 \times 10^1$	1.21×10^8	>99.999
		10210	$<1 \times 10^1$	1.21×10^8	>99.999
		10216	$<1 \times 10^1$	1.21×10^8	>99.999
	Escherichia coli	**10160**	$<1 \times 10^1$	1.11×10^8	>99.999
		10210	$<1 \times 10^1$	1.11×10^8	>99.999
		10216	$<1 \times 10^1$	1.11×10^8	>99.999

MRID Number	Organism	Lot No.	Total No. Surviving	Parallel Control	Percent Reduction
			(CFU/carrier)		
5-Minute Exposure Time					
484527-13	*Staphylococcus aureus*	**10160**	$<3.02 \times 10^1$	1.32×10^6	>99.9
		10210	$<9.55 \times 10^1$	1.32×10^6	>99.9
		10216	$<3.02 \times 10^1$	1.32×10^6	>99.9
	Enterobacter aerogenes	**10160**	$<3.02 \times 10^1$	3.24×10^6	>99.9
		10210	$<3.02 \times 10^1$	3.24×10^6	>99.9
		10216	$<3.02 \times 10^1$	3.24×10^6	>99.9

VI. CONCLUSIONS

1. The submitted efficacy data **support** the use of 1:22.3 dilution of the product, Austin's A-1 Ultra Bleach, as a disinfectant with bactericidal activity against the following microorganisms on pre-cleaned, hard, non-porous surfaces for the contact times listed:

Salmonella enterica	5 minutes	MRID 484527-01
Staphylococcus aureus	10 minutes	MRID 484527-02, -03, -04
Pseudomonas aeruginosa	10 minutes	MRID 484527-05, -06, -07
Escherichia coli O157:H7	10 minutes	MRID 484527-08
Streptococcus pyogenes	10 minutes	MRID 484527-09
Shigella dysenteriae	10 minutes	MRID 484527-10
Methicillin Resistant *Staphylococcus aureus*	10 minutes	MRID 484527-11

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

2. The submitted efficacy data **support** the use of 1:22.3 dilution of the product, Austin's A-1 Ultra Bleach, as a disinfectant with fungicidal activity against the following microorganisms on pre-cleaned hard, non-porous surfaces for the contact times listed:

Trichophyton mentagrophytes	5 minutes	MRID 484527-14
Aspergillus niger	10 minutes	MRID 484527-15

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

3. The submitted efficacy data (MRID 484527-16) **do not support** the use of a 1:22.3 dilution of the product, Austin's A-1 Ultra Bleach, 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 **10-minute** contact time. Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots (i.e., two product lots). **Growth was observed in one subculture for a third product lot tested**. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

4. The submitted efficacy data **support** the use of a 1:22.3-1:22.4 dilution of the product, Austin's A-1 Ultra Bleach (or A-1 Ultra Bleach), as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 1% organic soil load (a 5% organic soil load against Human immunodeficiency virus type 1) for the contact times listed:

Rotavirus	5 minutes	MRID 484527-17
Avian influenza A (H3N2) virus	5 minutes	MRID 484527-18
Rhinovirus type 37	5 minutes	MRID 484527-19
Hepatitis A virus	10 minutes	MRID 484527-20
Adenovirus type 2	10 minutes	MRID 484527-21
Influenza A virus	10 minutes	MRID 484527-22
Herpes simplex virus type 2	10 minutes	MRID 484527-23
Respiratory syncytial virus	10 minutes	MRID 484527-24
Cytomegalovirus	10 minutes	MRID 484527-25
Human immunodeficiency virus type 1	10 minutes	MRID 484527-26

Recoverable virus titers of at least 10^4 were achieved. In studies against Human immunodeficiency virus type 1, 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.

5. The submitted efficacy data **support** the use of a 1:22.3 dilution of the product, Austin's A-1 Ultra Bleach, as a sanitizing rinse against the following microorganisms on pre-cleaned, hard, non-porous, food contact surfaces for a 30-second contact time:

Staphylococcus aureus	MRID 484527-12
Escherichia coli	MRID 484527-12

Bacterial reductions of at least 99.999 percent over the parallel control were observed within 30 seconds. In studies against *Staphylococcus aureus* and *Escherichia coli*, at least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

6. The submitted efficacy data **support** the use of a 1:22.3 dilution of the product, Austin's A-1 Ultra Bleach, as a sanitizer against the following microorganisms on hard, non-porous, non-food contact surfaces in the presence of a 5% organic soil load for a 5-minute contact time:

Staphylococcus aureus	MRID 484527-13
Enterobacter aerogenes	MRID 484527-13

Bacterial reductions of at least 99.9 percent over the parallel control were observed within 5 minutes. In testing against both organisms, at least one of the product lots tested was at least 60 days old at the time of testing. The inoculum counts demonstrated an average of at least 7.5 x 10^5 surviving organisms, which is the criterion set forth in ASTM 1153. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Purity controls were reported as pure. Sterility controls did not show growth.

VII. LABEL

1. The registrant must make the following changes to the dilution factors:

- When **¾ cup** of the product **is added to 1 gallon** of water, it is one part of product and 21.33 parts of water; making it a **1:22.3 dilution**; ~2,400 ppm available chlorine.

- When **1.75 cup** of the product **are added to 1 gallon** of water, it is one part of product and 9.1 parts of water; making it a **10.1 dilution**.

2. The proposed label [see page 5 of the proposed label] claims that a **1:22.3** dilution (~2,400 ppm available chlorine) of the product, Austin A-1 Ultra Disinfecting Bleach, is an effective disinfectant against the following microorganisms on **pre-cleaned**, hard, non-porous surfaces for a 10-minute contact time:

Pseudomonas aeruginosa
Salmonella enterica (demonstrated to be effective with a 5-minute contact time)
Staphylococcus aureus

Escherichia coli O157:H7
Methicillin Resistant *Staphylococcus aureus*
Shigella dysenteriae
Streptococcus pyogenes
Aspergillus niger
Trichophyton mentagrophytes (demonstrated to be effective with a 5-minute contact time)

Adenovirus type 2
Avian influenza A virus (demonstrated to be effective with a 5-minute contact time)
Cytomegalovirus
Hepatitis A virus
Herpes simplex virus type 2
H1N1 Influenza A virus (Strain A/PR/8/34; ATCC VR-1469)
Influenza A virus
Influenza A2 virus
Respiratory syncytial virus
Rhinovirus type 37 (demonstrated to be effective with a 5-minute contact time)
Rotavirus (demonstrated to be effective with a 5-minute contact time)
Human immunodeficiency virus type 1 (demonstrated to be effective in the presence of a 5% organic soil load)

Data provided in the data package support these claims.

Directions on page 11 of the proposed label state that a 600 ppm use solution of the product (i.e., 13 ounces of the product per 10 gallons of water) is an effective disinfectant on pre-cleaned, hard, non-porous surfaces for a 10-minute contact time. New efficacy data were not provided to support this claim.

Directions on page 12 of the proposed label state that a 2,700 ppm use solution of the product is an effective bactericide/fungicide/virucide on hard, non-porous surfaces in the presence of a light to moderate organic soil load for a 5-minute contact time. New efficacy data were not provided to support this claim.

Directions on page 20 of the proposed label state that the product is an effective disinfectant against Human immunodeficiency virus type 1 on pre-cleaned, hard, non-porous surfaces for a 1-minute contact time. New efficacy data were not provided to support this claim.

3. The proposed label [see pages 4 and 7 of the proposed label] claims that a 1.75 cup of the product per gallon of water (1:10.1dilution), Austin A-1 Ultra Disinfecting Bleach, is an effective tuberculocide on pre-cleaned, hard, non-porous surfaces for a 5-minute contact time. **This claim is not acceptable. Registrant may keep a 10-minute contact time for the same 1.75 cup of the product per gallon of water**. [Note: The efficacy study provided in the data package tested a 1:22.3 dilution (~2,400 ppm available chlorine) of the product in the presence of a 5% organic soil load for a 10-minute contact time.]

Directions on page 20 of the proposed label state that a 10,000 ppm use solution of the product (i.e., 22 ounces of the product per 1 gallon of water) is an effective tuberculocide on hard, non-porous surfaces in the presence of a light to moderate organic soil load for a 5-minute contact time. New efficacy data were not provided to support this claim.

4. The proposed label [see page 5 of the proposed label] claims that a 1:256 dilution (200 ppm available chlorine) of the product, Austin A-1 Ultra Disinfecting Bleach, is an effective sanitizing rinse (no bacteria identified) on pre-cleaned, hard, non-porous, **food contact surfaces** for a 2-minute contact time. This claim **is not acceptable** as it is not supported by the submitted data. The submitted data demonstrated efficacy at a 1:21.3 dilution (~2,400 ppm available chlorine), which is a more concentrated use solution than the label-specified use solution. Repeat testing at the appropriate use-dilution is required, or the label can be amended to indicate the more concentrated use-dilution.

Directions on page 10 of the proposed label state that a 100 ppm use solution of the product (i.e., 2 ounces of the product per 10 gallons of water) is an effective sanitizer on pre-cleaned, hard, non-porous, food contact surfaces for a 2-minute contact time. New efficacy data were not provided to support this claim.

5. The proposed label [see pages 6 and 11 of the proposed label] claims that a 1:256 dilution (200 ppm available chlorine) of the product, Austin A-1 Ultra Disinfecting Bleach, is an effective sanitizer (no bacteria identified) on pre-cleaned, hard, non-porous, **non-food contact surfaces** for a 2-minute contact time. This claim **is not acceptable** as it is not supported by the submitted data. The submitted data demonstrated efficacy at a 1:22.3 dilution (~2,400 ppm available chlorine), which is a more concentrated use solution than the label-specified use solution. Repeat testing at the appropriate use-dilution is required, or the label can be amended to

indicate the more concentrated use-dilution (for a 5-minute contact time in the presence of light to moderate organic soil loads).

6. New studies demonstrating efficacy as a disinfectant were not provided for the following microorganisms, a number of which were not identified on the last accepted label:

Canine parvovirus
Feline parvovirus
Herpes simplex virus type 1 (ATCC VR-733)
Human coronavirus
Rubella virus (ATCC VR-315)
Varicella zoster virus (Strain G; ATCC VR-586)

Clostridium difficile spores

Campylobacter jejuni (ATCC 29428)
Enterobacter aerogenes (ATCC 13048)
Klebsiella pneumoniae (ATCC 4352)
Legionella pneumophila (ATCC 33153)
Listeria monocytogenes (ATCC 19111)
Candida albicans (ATCC 10231)

The applicant needs to identify MRIDs for studies previously provided to the Agency, or copies of laboratory studies demonstrating efficacy of the product against these microorganisms.

7. The registrant must make the following revisions to the proposed label:

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

- Under the "Environmental Hazards" section on the proposed label, change "public waters" to read "**other waters**."

- Under the "Storage and Disposal" section on the proposed label, change "Do not re-use empty container and place in trash collection." to read "**Do not re-use empty container. Offer for recycling, if available, or place in trash collection**."

- On pages 4 and 7 of the proposed label, change "*Escherichia coli* 0157:H7" to read "***Escherichia coli* O157:H7**."

- On page 5 of the proposed label, change "enamel" to read "**baked enamel**." Enamel is a porous surface.

- On page 5 of the proposed label, change "porcelain" to read "**glazed porcelain**." Porcelain is a porous surface.

- On page 6 of the proposed label under the instructions for disinfecting bathtubs, showers, and kitchen sinks, **change the contact time to 10 minutes**.

- On page 8 of the proposed label, change “ceramic tile” to read “**glazed ceramic tile**.” Ceramic tile is a porous surface.

- On page 12 of the proposed label, change “Cytomegalorvirus” to read “**Cytomegalovirus**.”

- On page 12 of the proposed label, change “Rhinovirus type 17” to read “**Rhinovirus type 37**.”

- ATCC numbers identified on the proposed label do not necessarily match ATCC numbers identified in the efficacy studies included in this data package (e.g., *Shigella dysenteriae*, *Streptococcus pyogenes*, Rotavirus, *Aspergillus niger*).

- On the proposed label, cold virus must be qualified in the absence of acceptable data against all three qualifying viruses (Coronavirus, Respiratory syncytial virus (RSV), and Rhinovirus).