



# The Use of *Escherichia coli* as an Alternate Test Organism in the ASTM E1174 Health Care Personnel Hand Wash Method

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## ABSTRACT

Health Care Personnel Hand Wash methods are used as surrogate end-point tests to determine the clinical effectiveness of antimicrobial cleansing products. The method proposed in the 1994 FDA tentative monograph is ASTM E-1174-87, which indicates the use of *Serratia marcescens* as the marker organism. The newest version of E1174-00 (2001) includes the use of *E. coli* as an alternative marker organism. The purpose of this study was to compare the results obtained in this method using both *E. coli* and *S. marcescens* as marker organisms. The efficacy of formulas containing triclosan, (2,4-dichloro-2'-hydroxy diphenyl ether), and 4% chlorhexidine gluconate (CHG) were compared. Activity was measured following both single and multiple 30 second washes. Against *E. coli*, the triclosan containing formula achieved a 3.78 log<sub>10</sub> reduction after a single wash and 4.04 log<sub>10</sub> following 10 consecutive washes compared to 3.78 log<sub>10</sub> and 4.06 log<sub>10</sub> following 10 consecutive washes against *S. marcescens*. The results for the CHG containing formula was a 2.86 log<sub>10</sub> and 4.22 log<sub>10</sub> reduction against *E. coli* and 2.54 log<sub>10</sub> and 3.78 log<sub>10</sub> against *S. marcescens*. These results show that there was no difference in end-point against either marker organism for the triclosan containing hand wash. The CHG containing product showed no statistical difference between test organisms after a single wash and only a 0.44 log<sub>10</sub> increase against *E. coli* following the tenth wash. The results of surrogate end-point tests can only be used as indicators of clinical efficacy and therefore the results obtained in this study supports the use of *E. coli* as alternative marker organism for use in the E1174 hand wash method.

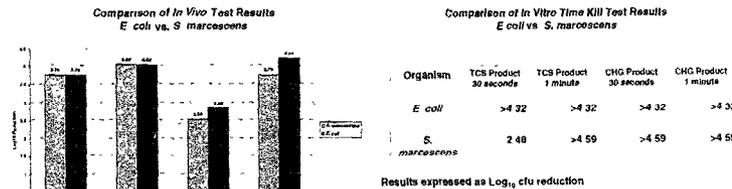
## INTRODUCTION and BACKGROUND

The ability to accurately and consistently assess the antimicrobial effectiveness of products used for hand antisepsis is critical when the products are used as part of an infection control program, or to prevent transmission of microorganisms. The ASTM E1174 Health Care Personnel Hand Wash Test is a surrogate end-point test that has been used for many years to evaluate the potential effectiveness of antimicrobial wash products. *Serratia marcescens* has always been used as the surrogate test organism, primarily due to the ability to visually differentiate it from other contaminating organisms, and its ability to survive on the skin. The use of other organisms of clinical significance, such as *Escherichia coli*, as surrogates is desirable both from a test flexibility standpoint, as well as by providing additional information on the potential efficacy of the test product. In order to maintain the validity of results obtained from such tests it is important to ensure that any changes, or modifications do not adversely affect the data, and that test results continue to accurately reflect an indication of product efficacy under use conditions. The potential effect of using *E. coli* in this test was explored by comparing the results obtained from two Health Care Personnel Hand Wash products. Results from *in vitro* time kill testing of the two products was also compared.

## METHODS

The E1174-00 procedure is an *in vivo* test designed to simulate routine hand washing conducted for the purpose of reducing the level of hand contamination under conditions of frequent use. Broth cultures of either *Serratia marcescens* ATCC 14756, or *Escherichia coli* ATCC 11229 were used as an artificial contaminant, or marker bacteria on the hands. Effectiveness was measured by comparing the number of marker bacteria surviving on the hands following hand washing with test formulations, to the baseline number of organisms recovered from contaminated, unwashed hands. A similar comparison was made following the 10<sup>th</sup> wash of a multiple wash procedure. The hands were contaminated with approximately 10<sup>8</sup> cfu/mL of either *S. marcescens* or *E. coli* prior to each wash. Organisms were recovered from the hands using a sampling fluid (0.075M phosphate buffer with 0.1% Triton X-100) containing chemical neutralizers (0.3% lecithin and 1.0% polysorbate 80). Aliquots were removed and serial dilutions prepared for enumeration. *S. marcescens* plates were incubated at 25°C to induce pigmentation for ease of identification. *E. coli* plates containing MUG (4-methylumbelliferyl-β-D gluconide) were incubated at 35°C and counted under long-wave UV light. The time kill test is an *in vitro* method designed to provide basic data on the rate-of-kill of antimicrobial formulations or ingredients against selected microorganisms. In this test 1 mL aliquots of *E. coli* or *S. marcescens* containing approximately 10<sup>8</sup> cfu/mL were combined with 99 mL of product use dilution. Following the selected time points, aliquots were removed, immediately neutralized in D/E Neutralizing broth, and serial dilutions prepared for enumeration. The test formulations consisted of a marketed hand wash (Dial Complete® Antimicrobial Foaming Hand Wash) containing activated triclosan (TCS) and a chlorhexidine gluconate (CHG) hand wash (Hibiclen®).

## RESULTS



## CONCLUSIONS

- *Escherichia coli* can be used as an alternative marker organism when assessing the antimicrobial activity of TCS or CHG containing products
- Additional testing should be done to confirm the applicability when assessing other active ingredients
- This modification to the 2000 ASTM method does not adversely affect the ability to compare data from older versions of the method

## DISCUSSION

The 1994 Tentative Final Monograph (TFM) for Over-the-Counter Health Care Antiseptic Drug Products requires the demonstration of antimicrobial effectiveness via *in vivo* test methods. The test that is referenced in the TFM for antiseptic hand washes is ASTM E1174. The assessment of effectiveness in this test is based on the use of *Serratia marcescens* as the surrogate or marker organism. The ease with which *S. marcescens* can be differentiated by its stable red pigment, and the belief that it was a safe, representative non-pathogenic organism led to its use. *S. marcescens* has been identified as the source of a significant number of nosocomial infections and while this validates its relevance as a surrogate test organism, testing laboratories have seen an increase in test subject adverse reactions linked to *Serratia*. The ability to use alternate, relevant test organisms, while maintaining the integrity and safety of the test, and the ability to compare historical data is an important aspect in the utility of E1174. *Escherichia coli* has also been identified as responsible for a significant number of nosocomial infections (Weinstein, 1998). Its use as a surrogate test organism is well documented in Europe, where it is the standard test organism for the evaluation of hygienic hand rubs (CEN, 1997). It is logical to assume, therefore, that *E. coli* could be used as a suitable surrogate organism in the E1174 handwash method. The two products chosen to test that assumption contain the two most common active ingredients found in health care personnel hand washes, triclosan and chlorhexidine gluconate. Both test products had previously been shown to meet the criteria set forth by the FDA for antiseptic handwashes in the 1994 TFM. The test results obtained in this study confirm that *E. coli* can be used as an alternative to *S. marcescens* in the E1174 method. Additional testing, using products containing different active ingredients should be undertaken, as well as research into the use of additional relevant marker organisms.

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# ANTIMICROBIAL EFFICACY OF ACTIVATED 2,4-TRICHLORO-2'-HYDROXY DIPHENYL ETHER (TRICLOSAN) IN SURFACTANT BASED FORMULATIONS

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## ABSTRACT

The antimicrobial activity of triclosan (2,4-trichloro-2'-hydroxy diphenyl ether) in surfactant based formulations can vary greatly as measured by standard in vivo or in vitro tests, and may be more dependent on formulation saturation, than its total concentration. Due to its sparse water solubility, and the high levels of surfactant present in most hand washes, increased micellar solubilization of triclosan is observed. Prior research indicates that a key factor to improving the antibacterial efficacy of triclosan is increasing the percent saturation of triclosan, rather than increasing its overall concentration. We discovered that it was possible to enhance the activity of triclosan in a handwash product through this mechanism, resulting in a significant improvement in antimicrobial activity as measured by standard tests. This study examined the efficacy of "activated" triclosan formulas, compared to a formula containing 4% chlorhexidine gluconate, as well as to traditional triclosan containing hand soaps. In vivo antimicrobial efficacy was measured using the ASTM E-1174 (Healthcare Personnel Handwash) method with *Serratia marcescens* as the test organism. Activity was measured following both a single as well as multiple 30 second washes. The activated triclosan formula achieved a 3.47 log<sub>10</sub> reduction after a single wash and 3.58 log<sub>10</sub> following ten consecutive washes compared to a 2.54 log<sub>10</sub> and 3.78 log<sub>10</sub> reduction for 4% chlorhexidine gluconate. The activated triclosan formula also demonstrated a greater than 1 log<sub>10</sub> improvement in efficacy compared to traditional formulas with comparable concentrations of triclosan. Similar improvements in activity, as great as a 2 to 3 log<sub>10</sub>, as measured by an in vitro time-kill method were seen against several Gram negative and Gram positive bacteria. These results show that triclosan activity can be significantly enhanced by increasing its saturation in surfactant based formulas rather than on increasing total concentration.

## INTRODUCTION

Antimicrobial efficacy of surfactant based wash products can vary greatly as measured by standard in-vivo or in-vitro tests. Prior research (Taylor et al.) indicates that a key factor to improving the antibacterial efficacy of triclosan is increasing the percent saturation of triclosan (>25%) in the aqueous phase of the formula, rather than increasing its overall concentration.

These studies examined whether decreasing the micellar solubilization of triclosan (activating TCS) in a hand wash formula would significantly increase antimicrobial efficacy, as compared to traditional surfactant based formulas.

A formula containing "activated" triclosan was tested, and compared to a formula containing 4% chlorhexidine gluconate (CHG) and a traditional triclosan containing formula. Antimicrobial efficacy was measured using both standard in-vivo and in-vitro tests.

## In-vivo Activity

Studies were performed using the current revision of ASTM E-1174-00, Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations. The revision provides procedures to assure adequate rapid neutralization of the antimicrobial in the handwash formulation. A neutralizer was incorporated at both sampling points.

Activated TCS hand wash formula vs a non-activated TCS formula and a 4% CHG formula were evaluated.

A broth culture of *Serratia marcescens* ATCC 14756 was grown at 25° C for 24-h. Subject's hands (n = 30) were contaminated with three 1.5 mL aliquots of *S. marcescens* (~ 10<sup>8</sup> cfu/mL).

A series of 10 contaminations followed by 30 second washes were performed with sampling taking place after the 1st and 10<sup>th</sup> wash.

Serial dilutions of sampling fluid were enumerated on Trypticase Soy Agar (spread plate method) and incubated at 25 C for 48 hours. Standard plate counting procedures were used to count colony forming units. Bacteria counts recovered were converted to into log<sub>10</sub> counts.

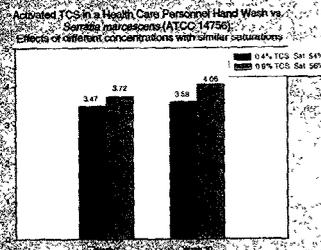
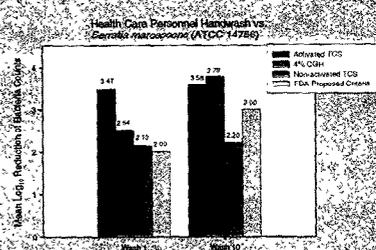
## In-vitro Activity

An in-vitro time kill test was performed to assess the rapid germicidal activity of activated TCS vs a non-activated TCS hand wash. A range of representative Gram negative and positive organisms were evaluated. The method included procedures to confirm neutralization of the antimicrobial agents under the test conditions.

Concentrations of test products were prepared based on the products use instructions. The dilution of test material was brought into contact with a known population of test organisms (~10<sup>8</sup> cfu/mL). 1 mL aliquots were sampled at 30 and 60 seconds. The 1 mL aliquot was placed into 9 mL of neutralizing broth and the number of surviving organisms were enumerated using standard plate count procedures.

Each time point was tested in triplicate. Colony counts were converted into log counts. Starting counts for each organism were calculated and used to compute difference between initial counts and surviving organisms at each time point.

## RESULTS



Activated TCS vs. Non-Activated TCS in an In-Vitro Time Kill Test (Average Log<sub>10</sub> Reduction at 30 Seconds)

Organism	Activated TCS	Non-Activated TCS
<i>S. marcescens</i> (ATCC 14756)	2.37	0.24
<i>E. coli</i> (ATCC 11229)	>4.32	0.10
<i>K. pneumoniae</i> (ATCC 10031)	>4.27	0.14
<i>S. sonnei</i> (ATCC 11060)	>4.36	0.19
<i>B. cepacia</i> (ATCC 25416)	>4.53	0.29
<i>P. aeruginosa</i> (ATCC 9027)	>4.21	0.74
<i>S. choleraesuis</i> (typhi ATCC 6539)	>4.59	0.59
<i>L. faecalis</i> VRI (ATCC 51299)	4.39	0.18
<i>S. aureus</i> (ATCC 6538)	>4.59	0.34
<i>S. aureus</i> MRSA (ATCC 33592)	4.62	0.14

Activated triclosan was significantly better than the non-activated TCS hand wash and 4% CHG hand wash at reducing *S. marcescens* following a single wash (p<.001).

Activated triclosan and 4% CHG were significantly better than non-activated TCS after 10 washes (p<.001). There was no significant difference in reduction between the activated TCS and 4% CHG product.

Two activated TCS formulas with different concentrations of TCS but similar saturation percents showed similar superior efficacy in the HCCHW test.

Activated TCS showed significant in-vitro efficacy vs. the non-activated TCS formula against all 10 organisms within 30 seconds.

## CONCLUSION

By increasing the water phase saturation of TCS in a hand wash formula, antimicrobial efficacy is significantly enhanced as was demonstrated in both in-vivo and in-vitro tests compared to a traditional surfactant hand wash formula containing similar concentrations of TCS.

Activated TCS showed rapid in-vitro germ kill against both Gram negative and positive organisms.

Compared to the non-activated TCS liquid hand wash, activated TCS formula was superior at reducing the test organisms by greater than 3 logs at 30 seconds.

The activated TCS formula and 4% CHG both met the proposed FDA monograph criteria of a 2 log<sub>10</sub> reduction after a single wash and a 3 log<sub>10</sub> reduction after the 10<sup>th</sup> wash for a Health Care Personnel Handwash Product.

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