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# Antibacterial Efficacy Test for FORCE Additive

**Prepared for:**

ZEP Manufacturing of Canada
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**June 21, 2004**

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## 1.0 Introduction

ZEP Manufacturing of Canada (ZEP) contracted HydroQual Laboratories Ltd. to perform an antibacterial efficacy test on their product, FORCE Additive, which is intended for use in a washing product to properly clean and sanitize sports team equipment.

The study below was designed to test the efficacy of FORCE Additive at two concentrations (1 part in 380 or 1 part in 220 by volume) and at two contact times, simulating warm (30°C) water washing with agitation for 10 or 15 minutes.

The percent kill of four bacterial species representing common pathogens were tested (Gram negatives: *Escherichia coli* and *Pseudomonas aeruginosa*; Gram positives: *Staphylococcus aureus* and *Enterococcus (formerly Streptococcus) faecium*). Initial concentrations were at least 100,000 bacteria per milliliter and were tested in the presence of soluble protein and salts from the bacterial growth medium (0.5%) to simulate typical soiling from used sports equipment.

The rational for the test bacteria was as follows: *Enterococcus faecium* , which can cause gastroenteritis, can grow in the presence of 6.5% sodium chloride (i.e., sweat), pH 9.6 and at 10 to 45°C, so is a good candidate to survive in soiled sports equipment with conventional washing. *E. coli* is well known for causing gastroenteritis, but certain strains (e.g., *E. coli* O157/H7) can cause more serious hemorrhagic colitis. *Staphylococcus aureus* has been linked to MRSA (Methicillin-Resistant *Staphylococcus aureus)* outbreaks and severe infections of the hand from cuts inside soiled hockey gloves. *Pseudomonas aeruginosa* is a common opportunistic pathogen, commonly found soil and water. In swimming pools it is the cause of pink eye and swimmers ear infections.

The test was carried out under the direction of a HydroQual senior environmental microbiologist on June 18, 2004.

## 2.0 Methods

All bacteria were standard strains from the American Type Culture Collection (ATCC) as listed below:

| | |
|---|---|
| *Enterococcus faecium* | ATCC 35667 |
| *Escherichia coli* | ATCC 25922 |
| *Pseudomonas aeruginosa* | ATCC 10145 |
| *Staphylococcus aureus* | ATCC 25923 |

All bacteria were grown as pure cultures in Bacto Nutrient Broth (Difco) for 20 hours at 30°C on an orbital shaker at 150 rpm. Following growth, culture turbidity (as optical density) was measured at 660nm to estimate the culture cell density.

An Initial Test Mix of all bacterial strains was prepared by adding one to five mL of culture to a total of one litre of sterile dechlorinated tap water at 30°C to obtain initial concentration of about 100,000 to 500,000 bacteria/mL for testing.

Each bacterial type was enumerated following appropriate dilution of the Initial Test Mix in sterile dechlorinated tap water using selective growth medium as follows:

| | |
|---|---|
| *Enterococcus faecium* | IDEXX Enterolert by Most Probable Number (MPN) method (adaptation of Standard Method 9230B). Incubated at 41°C and scored after 24 and 48h. |
| *Escherichia coli* | IDEXX Colisure by MPN (Standard Method 9223B). Incubated at 35°C and scored after 24 and 48h. |
| *Pseudomonas aeruginosa* | Asparagine Broth by MPN (Standard Method 9213F). Incubated at 35°C and scored after 48 and 72h. |

| | |
|---|---|
| *Staphylococcus aureus* | Phenyl Ethyl Alcohol (PEA) Blood Agar (from Dalynn Biologicals, Calgary) for Plate Counts (adaptation of Standard Method 9213B). Plates incubated at 35°C and scored after 48 and 72h. |

Exposure tests were done in sterile 250mL Erlenmeyer flasks containing 100 mL of Initial Test Mix and either 0.26 mL (1 FORCE in 220 parts water) or 0.45 mL (1 in 330 parts) of FORCE Additive. No FORCE treatment controls were also run. Exposure was for 10 and 15 minutes at 150 rpm on an orbital shaker at 30°C.

The remaining Initial Test Mix was stored at 4°C for 24h in case further analysis was necessary to obtain countable range in the MPN (<2400 MPN/100 mL) or Plate Count (30-300 CFU/plate) methods.

After each exposure time, samples (0.1 and 0.05 mL) were plated immediately for *S. aureus* enumeration or were diluted as 10 mL Test Mix in 90 mL sterile dechlorinated tap water to quench the antibacterial action by 10-fold dilution prior to MPN enumeration for the other three bacteria types.

All MPN and plate counts were scored following 24, 48 and 72 h, as required, and final total counts were calculated based on the sample dilution factors used.

## 3.0 Results and Discussion

To prepare the Initial Test Mix, 1 mL of *E. coli, P. aeruginosa,* and *S. aureus* was added. Because of its lower initial turbidity, 5 mL of *Ent. faecium* was added to ensure a sufficient initial cell count. As all strains were grown in nutrient broth, this meant that a total of 8 mL of nutrient broth was added with the cultures to the Initial Test Mix. Since, Bacto Nutrient Broth contains 0.3% beef extract and 0.5% peptone (a protein digest), the culture dilution would result in less than 0.8% of these concentrations of residual protein and salts remaining from the nutrient broth. These were present to serve as simulated

contaminants from dirt and sweat from used sports equipment that could potentially compete with FORCE for its antibacterial action.

The test results are presented in Table 1. All four bacterial types tested showed at least 99.99% kill efficacy of >100,000 cells/mL after 10 minute exposure at 30°C at the lower FORCE Additive concentration of 0.45 mL/L or 1 part FORCE in 380 parts water.

All no FORCE exposure controls showed counts in excess of the maximum detection limit for undiluted samples of 3,000 CFU/mL for *S. aureus* and 1/10 diluted samples of 242 MPN/mL of the other three bacteria following 15 minutes at 30°C. These results confirm that the antibacterial activity observed was caused by FORCE Additive exposure and not just the conditions of the test. Based on general microbiological experience, no significant loss of these bacteria in the Test Mix would be expected at 30°C for the short test duration (10-15 minutes) without the FORCE Additive exposure.

## 4.0 Conclusions

These results confirm that FORCE Additive is highly effective antibacterial agent for its intended use in washing of soiled sporting equipment and uniforms.

Under the lowest exposure conditions of this test (i.e., 1 part FORCE to 380 parts water at 30°C for 10 minutes), the antibacterial efficacy of FORCE additive, expressed as percent kill efficiency can be stated:

| | | |
|---|---|---|
| *Enterococcus faecium* | ATCC 35667 | 99.9976% of 208,000 MPN/mL |
| *Escherichia coli* | ATCC 25922 | 99.9979% of 242,000 MPN/mL |
| *Pseudomonas aeruginosa* | ATCC 10145 | 99.9997% of 180,000 MPN/mL |
| *Staphylococcus aureus* | ATCC 25923 | 99.9999% of 427,000 CFU/mL |

## 5.0 References:

Clesceri, L.S., Greenberg, A.E., and Eaton, A.D. (eds.). 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. APHA/AWWA/WEF, Washington, DC.

## 6.0 Closure:

HydroQual is certified by the Canadian Association of Environmental Analytical Laboratories (CAEAL) and accredited by the Standards Council of Canada (SCC). We comply with American, Canadian, and European standards for laboratory practice and the requirements of ISO/IEC Guide 25.

This test was done under the direction of Dr. J. Jeffrey Wilson, Ph.D., P.Biol. at HydroQual Laboratories.

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June 22, 2004.

## 6.0 Tables

## Table 1. FORCE Additive Antibacteria Efficacy Test Summary

| Bacterial Strain | ATCC No. | Gram Reaction | Count Units | Avg. Test Count/mL | Test Mix Count Dilution | Initial Test Mix Count | Post Treatment Count Dilution | A-10 Count | A-15 Count | B-10 Count | B-15 Count | A-10 Percent Kill |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 25923 | Positive | CFU/mL | 427 | 1000 | 427,000 | 1 | 0 | 0 | 0 | 0 | 99.9999 |
| *Escherichia coli* | 25922 | Negative | MPN/mL | 24.2 | 10000 | 242,000 | 10 | 0 | 0 | 0 | 0 | 99.9979 |
| *Enterococcus faecium* | 35667 | Positive | MPN/mL | 20.8 | 10000 | 208,000 | 10 | 0 | 0 | 0 | 0 | 99.9976 |
| *Psuedomonas aeruginosa* | 10145 | Negative | MPN/mL | 18.0 | 10000 | 180,000 | 10 | 0 | 0 | 0 | 0 | 99.9997 |

**Test Conditions**

All exposures done at 30°C and 150 RPM circular agitation. Initial mix test culture also contained 0.5% Nutrient Broth to simulate competing organics (dirt).

A-10 = FORCE Additive Concentration 2.6 mL/L or 1 part to 380 parts water, exposure time 10 minutes at 30°C and 150 rpm agitation

A-15 = FORCE Additive Concentration 2.6 mL/L or 1 part to 380 parts water, exposure time 15 minutes at 30°C and 150 rpm agitation

B-10 = FORCE Additive Concentration 4.5 mL/L or 1 part to 220 parts water, exposure time 10 minutes at 30°C and 150 rpm agitation

B-15 = FORCE Additive Concentration 4.5 mL/L or 1 part to 220 parts water, exposure time 15 minutes at 30°C and 150 rpm agitation

No FORCE Exposure Controls, C-10 and C- 15, same as above but without FORCE Additive

All No FORCE Exposure Control counts were above undiluted maximum detection limit of 242 MPN/mL or 3,000 CFU/mL for *S. aureus* plate counts.

For Percent Kill calculations where no growth or zero count was obtained, 0.5 MPN or CFU/mL was used (1/2 detection limit) to generate a value other than 100%.