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Antimicrobial Efficacy Test for FORCE Additive

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Antimicrobial Efficacy Test for FORCE Additive

1.0 Introduction

Zep Manufacturing of Canada (Zep) and Esporta Wash Systems Inc. (Esporta) contracted HydroQual Laboratories Ltd. to perform an antimicrobial efficacy test on their product, FORCE Additive, which is intended for use as a washing product to properly clean and sanitize sports team equipment. The test was designed using sports equipment and real washing conditions.

The study below was designed to test the efficacy of FORCE Additive at a concentration of 1 part FORCE to 380 parts water during the washing procedure developed by Zep and Esporta.

The percent kill values of two microbial species representing common skin pathogens (bacterial: *Staphylococcus aureus*; fungal: *Trichophyton rubrum*) were tested.
S. aureus can be found normally on the skin of healthy individuals, but can cause disease when the skin is frequently injured (such as from chaffing during sports games). Diseases resulting from *S. aureus* infection include impetigo, boils, and folliculitis.
T. rubrum is the most common causative agent of dermatophytosis and is able to infect nails, skin, and hair.

The test was carried out between December 13 and 29, 2004.

2.0 Methods

Two sets of sports equipment were obtained and each was contaminated with either bacteria or fungi. The equipment was tested for initial microbial levels, washed, and then tested for final microbial levels, providing the percent efficiency of the equipment washing procedure both on the surface and within the foam of the equipment.

Two cultures were obtained from the American Type Culture Collection (ATCC) for this study:

Staphylococcus aureus	ATCC 25923
Trichophyton rubrum	ATCC 28188

Bacteria were grown in Tryptic Soy Broth for 20 hours at 30°C on an orbital shaker at 150rpm. This culture was used to spike dechlorinated tap water, which was then used to contaminate the sports equipment. The spiked tap water had approximately 100,000 MPN/mL.

Fungi were grown on Sabouraud Dextrose Agar for 3-5 days at 35ºC. Following growth, colonies (~20 1cm colonies) were homogenized in 50mL of sterile tap water (modified from Granade and Artis, 1980). The resulting homogenate was concentrated by centrifugation and re-suspended in 25mL of sterile tap water. This preparation was used to spike dechlorinated tap water, which was then used to contaminate a second set of sports equipment. Spiked tap water contained approximately 35 MPN/mL. It was difficult to achieve a higher fungal inoculum using this method. Bacterial and fungal inocula were enumerated using a Most Probable Number method and suitable selective media.

Staphylococcus aureus was enumerated in Tryptic Soy Broth with 8% sodium chloride and confirmed on Baird-Parker Egg Yolk Tellurite Agar. MPNs were scored after 2 days and confirmations after 24 hours (Figure 1A).

Trichophyton rubrum was enumerated in Tryptic Soy Broth supplemented with penicillin-streptomycin and confirmed by morphology on Sabouraud Dextrose Agar. MPNs were scored after 5 days and confirmations after 5 days (Figure 1B).

The following children's sports equipment was supplied by Esporta:

- Mascot head*

- Football helmet*
- Football shoulder pads*
- Baseball helmet*
- Hockey helmet*
- Hockey gloves
- Hockey shoulder pads*
- Hockey shin pads
- Hockey elbow pads
- Hockey pants

Some equipment (marked with *) was cut in half for logistical purposes.

Exposure of the equipment to the microbes was conducted in pails containing 10L of dechlorinated tap water. Pails were prepared and allowed to stand and equilibrate to room temperature (22ºC) prior to adding the microbes. Two pails were used for bacterial contamination and two for fungal. Each piece of equipment was manually submerged into the spiked water and massaged for approximately 2 minutes to allow penetration of the water into the foam materials (Figure 2). Equipment was then wrung out by hand and placed in a biohazard bag for storage. Bags were stored at 22ºC for 24 hours.

Initial (pre-wash) samples were taken after 24 hours. Both surface (swab) and foam samples were taken to assess the level of contamination achieved in each piece of equipment. Surface sampling involved swabbing an area of approximately 24cm^2 using a rolling motion with a sterile swab (Figure 3A). The tip of the swab was then cut off into 10mL of sterile tap water using sterile scissors. Foam sampling involved cutting a piece of foam 2.5cm x 2.5cm from the equipment using sterile scissors (Figure 3B). The foam was placed into 10mL of sterile tap water.

Both swabs and foam samples were extracted by compressing the material ten times using a sterile homogenizer pestle (Figure 4A). The water was then sampled and tested for either *S. aureus* or *T. rubrum*. Duplicate samples were taken, but were analyzed as composites to reduce the number of enumeration plates required.

Washing of the equipment was done in two batches to prevent cross-contamination between equipment contaminated with bacteria and that contaminated with fungi. The machine used for washing was an Esporta washing machine (model ES-3250, serial no. 0022, made in 2003) located at the Zep Manufacturing testing facility in Edmonton, Alberta (Figure 5A). Hockey and football equipment was loaded into separate folding racks, while the mascot head and baseball helmet were placed in a rigid rack (with the helmet inside a mesh bag) (Figure 5B). All equipment was loaded into the machine according to the manufacturer's instructions.

The washing procedure was as follows:

1. rotation
2. water in (270L,~43ºC)
3. addition of chemical 1
4. addition of chemical 2
5. run (40 minutes)
6. drain
7. water in
8. run (rinse)
9. drain
10. extract (removes excess water)
11. water in (270L)
12. addition of chemical 3 (Force Additive)
13. run (10 minutes)
14. drain
15. water in
16. addition of chemical 4 (fragrance)
17. run (5 minutes)
18. drain
19. extract (removes excess water)
20. rotation
21. heating/drying

The heating/drying cycle is normally 45-55 minutes. However, this was deemed unnecessary for the purpose of this test. As such, the heating/drying cycle was reduced to 10 minutes.

Following washing, all equipment was removed from the machine and placed in clean biohazard bags for transport back to the lab. Bags were stored overnight at 4ºC. Following storage, samples were obtained and analyzed as previously described.

3.0 Results and Discussion

The test results are presented in section 7.0. The percentage kill values are presented for both target and non-target organisms. Target organisms were *S. aureus* and *T. rubrum*, while non-target organisms include any microbes already present in the used equipment that were able to grow in the media used for enumeration of the target organisms. For samples with values below the detection limit, half of the detection limit was used to calculate the percentage kill value.

Results indicate that the initial contamination levels for both bacteria and fungi were variable among the different types of sports equipment. This was a result of different materials used in the fabrication of the equipment. Some of the foams were observed to be tighter, making penetration of the contamination mixture more difficult. In addition, the surface contamination levels were lower due to the nature of the material used. In some equipment, nylon fabrics were present and did not readily absorb the contamination mixture.

Many of the percent kill values for *S. aureus* exceeded 99.9% (Table 1). Those that did not exceed this value did not have sufficient contamination in the initial samples to demonstrate reduction in microbial numbers of several orders of magnitude. The confirmed numbers for *S. aureus* in the washed equipment were below the detection

limit of the method used (1MPN/10cm^2 for surface samples and 3MPN/10cm^2 for foam samples).

For the equipment contaminated with *T. rubrum*, the percent kill values were lower than those with *S. aureus* (Table 2). This was, again, due to low initial contamination levels in the equipment. Because of the growth habit of fungi, it was difficult to achieve a uniform inoculum and difficult to effectively contaminate the equipment, particularly that made with the tighter foams. If the non-target kill values are examined, these indicate a percent kill of 99.9% in most cases. The primary non-target organism was identified as *Rhodotorula*, a yeast commonly found in a variety of environments and rarely found as a causative agent of opportunistic mycoses (de Hoog *et al*, 2000).

4.0 Conclusions and Recommendations

These results confirm that FORCE Additive was an effective antimicrobial agent for washing of soiled sports equipment contaminated with *S. aureus* and *T. rubrum* under the conditions of this study.

Additional study could be done to increase the initial contamination levels (particularly for the *T. rubrum* study) to demonstrate the desirable 99.9% kill in all pieces of equipment. Due to the filamentous growth nature of fungi, it is difficult to achieve uniform inocula for contaminating the equipment. In addition, the fungal fragments in the inocula are much larger compared to bacterial cells, making it more difficult to achieve contamination within the equipment. The additional study would optimize the contamination procedure.

5.0 References

de Hoog, G.S, J. Guarro, J. Gene, and M.J. Figueras. 2000. *Atlas of Clinical Fungi*, 2nd edition. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Granade, T.C. and W.M. Artis. 1980. Antimycotic susceptibility testing of dermatophytes in microcultures with a standardized fragmented mycelial inoculum. *Antimicrobial Agents and Chemotherapy* 17(4): 725-729.

6.0 Closure

HydroQual is certified by the Canadian Association of Environmental Analytical Laboratories (CAEAL). 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 Susan Rowsell, M.E.Des., M.Sc. at HydroQual Laboratories Ltd.

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7.0 Tables

Table 1 – Levels of *Staphylococcus aureus* in sports equipment

		Unwashed Equipment		Washed Equipment		% Kill	
		Non-target	Target	Non-target	Target	Non-target	Target
Hockey Helmet	Surface	55	3.3	0.5	0.5	99.0909	84.8485
	Foam	5600	370	784	1.5	86.0000	99.5946
Hockey Shoulder Pads	Surface	14	0.5	0.5	0.5	96.4286	0.0000
	Foam	56000	350	1.5	1.5	99.9973	99.5714
Hockey Pants	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	780	780	12	1.5	98.4615	99.8077
Hockey Shin Pads	Surface	33	33	0.5	0.5	98.4848	98.4848
	Foam	5300	5300	1.5	1.5	99.9717	99.9717
Hockey Gloves	Surface	290	290	0.5	0.5	99.8276	99.8276
	Foam	5000	5000	3.2	1.5	99.9360	99.9360
Hockey Elbow Pads	Surface	33	33	0.5	0.5	98.4848	98.4848
	Foam	7400	7400	1.5	1.5	99.9797	99.9797
Baseball Helmet	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	53	37	1.5	1.5	97.1698	95.9459
Football Helmet	Surface	1.9	1.9	0.5	0.5	73.6842	73.6842
	Foam	130	22	1.5	1.5	98.8462	93.1818
Football Shoulder Pads	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	1.5	1.5	1.5	1.5	0.0000	0.0000
Mascot Costume Head	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	120	72	1.5	1.5	98.7500	97.9167

Table 2 – Levels of *Trichophyton rubrum* in sports equipment

		Unwashed Equipment		Washed Equipment		% Kill	
		Non-target	Target	Non-target	Target	Non-target	Target
Hockey Helmet	Surface	190	0.5	0.5	0.5	99.7368	0.0000
	Foam	1000000	1.5	110	1.5	99.9890	0.0000
Hockey Shoulder Pads	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	2720	780	1.5	1.5	99.9449	99.8077
Hockey Pants	Surface	71	0.5	0.5	0.5	99.2958	0.0000
	Foam	4960	3.2	1.5	1.5	99.9698	53.1250
Hockey Shin Pads	Surface	9.2	0.5	0.5	0.5	94.5652	0.0000
	Foam	350	1.5	1.5	1.5	99.5714	0.0000
Hockey Gloves	Surface	3.3	0.5	0.5	0.5	84.8485	0.0000
	Foam	180	7.2	1.5	1.5	99.1667	79.1667
Hockey Elbow Pads	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	130	7.2	1.5	1.5	98.8462	79.1667
Baseball Helmet	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	74	2.9	1.5	1.5	97.9730	48.2759
Football Helmet	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	78	3.2	1.5	1.5	98.0769	53.1250
Football Shoulder Pads	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	7.2	1.5	1.5	1.5	79.1667	0.0000
Mascot Costume Head	Surface	0.5	0.5	0.5	0.5	0.0000	0.0000
	Foam	27	1.9	1.5	1.5	94.4444	21.0526

8.0 Figures

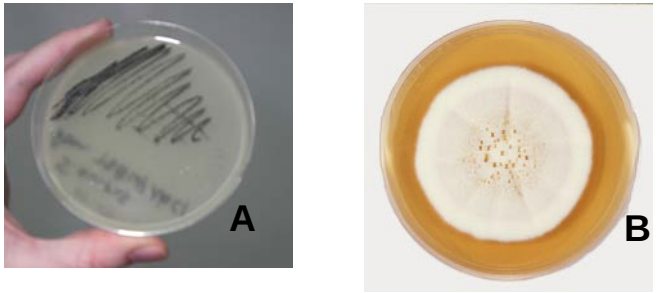

Figure 1 – *Staphylococcus aureus* (A) and *Trichophyton rubrum* (B)

Figure 2 - Contamination of equipment (A, hockey elbow pad; B, mascot costume head)

Figure 3 - Obtaining surface (A) and foam (B) samples

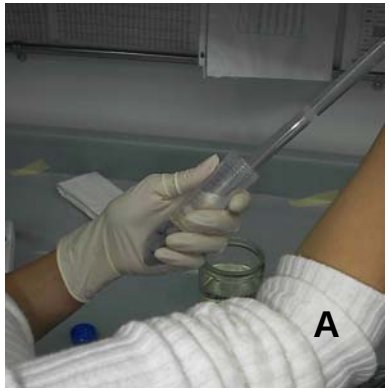

Figure 4 - Compressing foam sample (A) and sample tubes (B)

Figure 5 - Esporta equipment washing machine (A) and folding rack (B)