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John Boyd, General Manager
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Dear Mr. Boyd:

The following is a narrative describing the microbial challenge study performed by MICRO-CHEM Laboratories using your Polarzone Cryotherapy Spa System.

Hypothesis:

The “triple-hurdle sanitation system” (automatic Chlorination, Ozone Injection, and a 254 nm pass-through C band Ultra-Violet antimicrobial light) employed in your Polarzone Cryotherapy Spa will be able to maintain sanitary water-quality under conditions simulating very heavy contamination of the water with the microorganisms shed by the human beings using it.

To test this hypothesis we devised the following study. We inoculated the water in a Polarzone Cryotherapy Spa with four different microorganisms known to be commonly shed by human beings. We purposely chose two microorganisms which are difficult to treat with antibiotic therapy and are of major concern within the medical community.

We employed the following bacteria/fungi in this study:

1. *Staphylococcus aureus* (MRSA): ATCC: BAA-39, a Methicillin Resistant Staph (MRSA)
2. *Enterococcus faecium* (VRE): ATCC: 700221, Vancomycin Resistant Enterococcus (VRE)
3. *Escherichia coli* (*E. coli*) ATCC: 8739, a fecal microorganism (E-coli.)
4. *Trichophyton mentagrophytes* (yeast): ATCC: 9533, an (Athletes Foot Fungus)

The 350 gallons of water in the Polarzone Cryotherapy Spa was inoculated with approximately 200-300 CFU/ml (Colony Forming Units/ml) of **each** of the four above-mentioned microorganisms. We inoculated with 2 ml of a McFarland Standard 0.5 concentration of each microorganism. The cold tub contains ~1,325,000 ml of water (3,785 X 350). 2 ml of a McFarland Standard 0.5 contains ~300,000,000 (300 million) bacteria. Thus, each milliliter of inoculated water initially contained a total of ~900 CFU (225 X 4) from the four microorganisms.

We took samples of cold spa water before inoculation; three minutes after inoculation (with thorough rapid agitation) called “Time Zero” sample; and at ten minute intervals for the next hour resulting in 6 more samples. Microbial results from that data are listed on the accompanying laboratory report.

The temperature of the inoculated water in the Spa remained precisely at 49 F throughout the study.

The chemical make up of the water before inoculation was:

1. Free chlorine: 5 ppm.
2. Alkalinity: 100 ppm
3. pH: 7.4
4. Ca (Hardness): 300

The chemical make up of the water approximately 1 hour later, after the completion of the sampling phase of our study, was the same for all tested chemical attributes:

5. Free chlorine: 5 ppm.
6. Alkalinity: 100 ppm
7. pH: 7.4
8. Ca (Hardness): 300

Results & Discussion:

The positive control samples for the four microorganisms in this study, as tested at MICRO-CHEM, all grew well on the laboratory media we used for the spa-water analysis. The amount of nutrient originally available, temperature of incubation, and the elapsed time of incubation were designed to assure that the microorganisms would still be in log-phase growth at the time of their inoculation into the Spa water.

When we inoculated these test microorganisms into the Polarzone Cryotherapy Spa system, in less than three minutes (the mixing time used to circulate the organisms into the spa water) the chemical (Ozone and Chlorine), and Ultra-Violet antimicrobial light immediately *inactivated* all of the inoculated microorganisms. As the attached laboratory report indicates, no microorganisms remained viable in the spa water when the Time Zero sample (and subsequent samples) were taken.

This analytical result supports our original hypothesis above, and also supports the reasonable presumption that individual(s), having a large microbial population on their bodies could enter the water in a Polarzone Cryotherapy Spa and the chemical (Ozone and Chlorine) plus Ultra-Violet light antimicrobial hurdles would be sufficient to render the water safe for further use after a very short (>3 minutes) elapsed time. The Polarzone Cryotherapy “triple hurdle sanitation system” is able to kill high concentrations of MRSA and VRE organisms, which are of concern in some infectious disease.

We did not test the efficacy of the Cryotherapy Spa sanitizing system under conditions of very dirty or turbid water. Any soil or turbidity present in the spa water would adversely affect both Chemical and UV Light sanitation capabilities. Also, we did not test the spa water under conditions where depleted concentrations of sanitizing chemicals were present.

These two caveats highlight the responsibility of spa owners and operators to maintain clean spa water and to maintain the required (as tested) sanitizing chemical concentration in the spa water during all use.

Sincerely,

Matthew V. Andrews, President
MICRO-CHEM Laboratories, Inc.