Results:

Sample Identification: B-pHree (BF-104); Bulk Sample		
Staphylococcus aureus **(NLML B-179)**	**CFU/mL**	**Percent Reduction**
Starting Point Control (dH_2O)	10 000 000 000	
Positive Growth Control (TSB)	32 000 000 000 000	
B-pHree (BF-104)	< 10	100

Summary of Findings:

- *S. aureus* inoculated into the test product (B-pHree) was unable to grow on Tryptic Soy Agar (TSA) after 24 hours of incubation.
- All original inoculum of *S. aureus* was killed by exposure to the test product (B-pHree) after 24 hours of exposure.
- *S. aureus* (positive growth Control) exhibited extensive growth after 24 hours incubation.
- Sterility Test: No growth was detected on the uninoculated sterile distilled water.

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Laboratory Results authorized by Sean P. Abbott, Ph.D., Analytical Director

Purpose:

To test the antibacterial efficacy of a product (B-pHree) to inhibit the growth of bacteria and kill bacterial inoculum. The organism selected for this trial was *Staphylococcus aureus* (NLML B-179). The *S. aureus* stock culture was grown on Tryptic Soy agar (TSA).

Antibacterial Efficacy/Product Testing Protocol:

4. Prepare Bacterial suspensions.
   - 4.1. Swab surface of bacterial colonies from stock culture with a sterile swab and vortex in 1.00 mL sterile distilled water for the organism being challenged (*Staphylococcus aureus* NLML B-179). This master suspension will be used to prepare the test suspensions.
   - 4.2. Prepare 5 mL sterile test tube with 0.9 mL sterile distilled water as starting point Control.
   - 4.3. Prepare 5 mL sterile test tube with 0.9 mL Tryptic Soy Broth (TSB) as positive growth Control.
   - 4.4. Prepare 5 mL sterile test tube with 0.9 mL of test product (B-pHree, BF-104).
   - 4.5. Add 0.1 mL of bacterial stock from the master suspension to the previously prepared tubes of sterile distilled water (starting point control), TSB (positive growth control) and the product (challenge). This will bring the total volume to 1.0 mL. These are the primary suspensions and will be used in the serial dilutions to follow.
5. Prepare dilution series and incubate.
   - 5.1. Prepare serial dilutions and plate out the *S. aureus* starting point Control on TSA to appropriate levels after preparing the primary suspension.
   - 5.2. Incubate the remaining product test tubes and positive growth control for 24 hours at 37 ^{o} C.
   - 5.3. Prepare serial dilutions and plate out the *S. aureus* positive growth Control on TSA to appropriate levels after 24 hr incubation period.
   - 5.4. Prepare serial dilutions and plate out the product inoculated with *S. aureus* on TSA to appropriate levels after 24 hr incubation period.
   - 5.5. Incubate the plates for 24 hours at 37^{o} C
6. Count colonies and report.
   - 6.1. Visually and microscopically confirm bacterial colonies recovered are the challenge organism.
   - 6.2. Count colonies on appropriate dilution plates and calculate CFU's/mL. Report counts and percent reduction in CFU/mL from the product (challenge) versus the sterile distilled water (starting point control).

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