

Evaluation of Methods for Enumerating Enterococci in Beach Sand

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Research Project

The background of the slide is a solid blue color. In the lower right quadrant, there are several concentric, light blue circular ripples, resembling water droplets or waves, which add a decorative element to the presentation.

Background

- Recent International interest in beach sand as a source or reservoir of fecal bacteria
- Many published protocols for enumerating bacteria in sand
- No standard method
 - Prevents assessment of problem
 - Limits comparability of data between beaches
- Goal: Identify a standard method for measuring enterococci in beach sand

Approach

- Bring together leading researchers in field
- Process a common set of samples



Participants

Jenny Jay – UCLA

Richard Whitman – USGS

Helena Solo-Gabriel - U of Miami

Ali Boehm - Stanford

Tom Edge- Environment Canada

Marty Getrich - OCPHL

Details

- 21 permutations of 4 treatments
- Four replicates processed by each method
 - Duplicates by each of two researchers
- Three sand samples
 - One sample processed each day
- Analyze for enterococci using EPA Method 1600



Treatments

➤ Hand Shaking

➤ Mechanical Shaking

➤ Sonication

➤ Blending



Variables

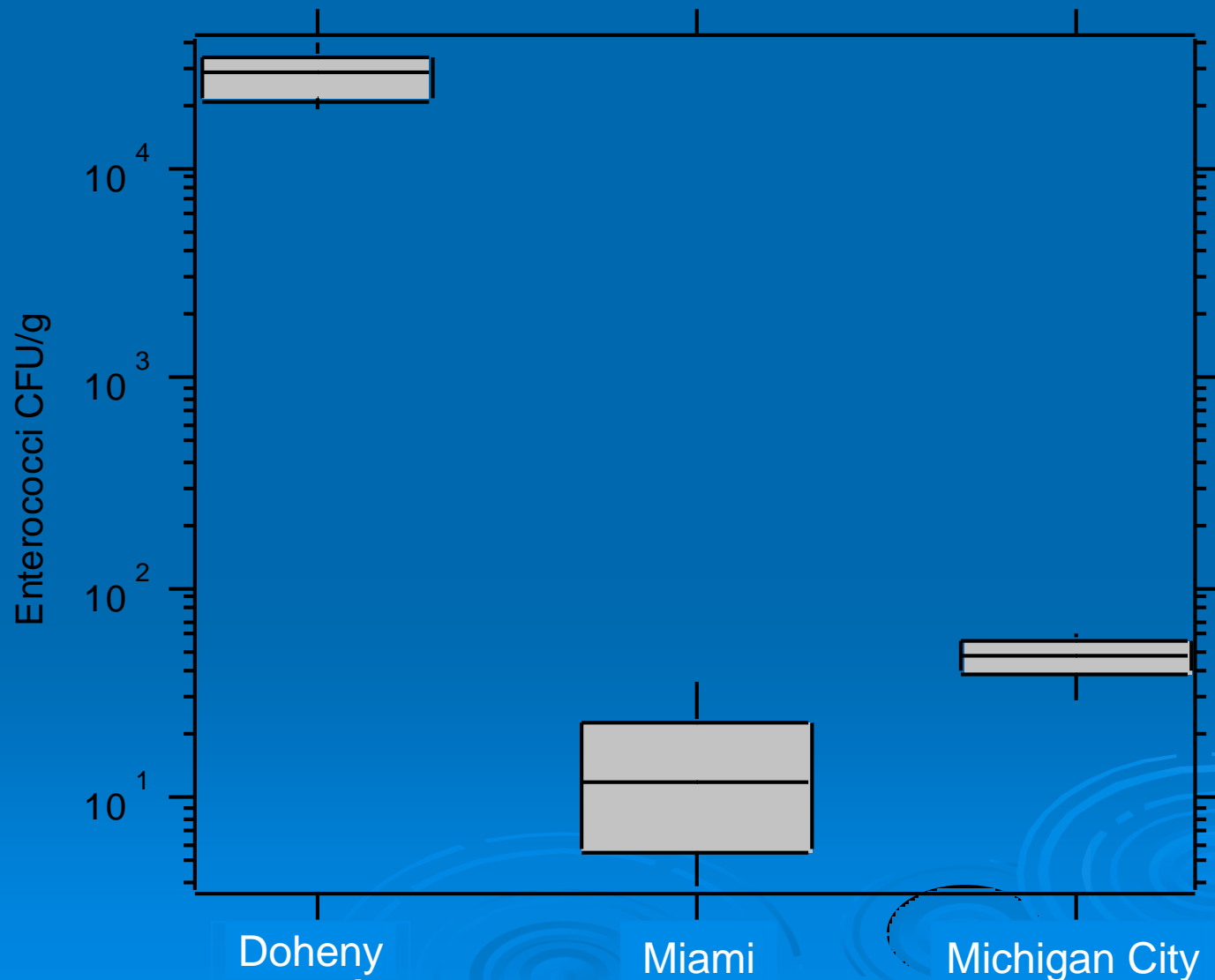
- Mass of sand
- Duration of shaking
- Settling time
- Number of rinses
- Eluant (PBS or DI water)
- Surfactant (Tween 80 or metaphosphate)
- Eluant collection (decant, pipette, vacuum)

Sand Sources

- Doheny State Beach, Dana Point, CA
- Hobie Cat Beach, Miami, FL
- Washington Park Beach, Michigan City, IN



Enterococcus Concentration



Results

- Blending produced lower numbers than sonication or shaking
- Lower eluant to sand ratio led to reduced recovery
 - Mechanical shaking produced lower numbers than hand shaking
- No effects noted for shaking time, sonication, number of rinses, or settling time

Bottom Line

- Hand-shaking performed as well or better than more complex treatments
 - Simple
 - Inexpensive
 - Consistent


Method Recommended by Experts


- 10g sand
- 60ml PBS
- 2 min. hand shake
- Settle 30s
- Decant eluant
- 40ml PBS rinse
- Settle 30s
- Decant eluant

What Next?

- Laboratory Intercalibration Exercise
 - Conducted November 2008
- Test method on more challenging sands
 - High silt
 - High organic content

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Part A: Step 1

Method for Detecting and Enumerating Enterococci Bacteria in Beach Sand

Standard Operating Procedure

developed cooperatively by:

Southern California Coastal Water Research Project
University of California, Los Angeles
United States Geological Survey
Stanford University
University of Miami
Environment Canada
Orange County Public Health Laboratory



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