A Comparison of Commercially Available Bacteria Test Kits

Joe Grenier
Ray Denkewicz, Jr.
Zodiac North American Pool Care Sector

Three types of commercially available bacteria test paddles were evaluated on the basis of precision and accuracy of results. Bodies of water tested included residential swimming pools, spas and laboratory test beakers. All types of paddles tested tended to underestimate the actual numbers of bacteria present. An inverse relationship was found between the accuracy and precision of the test paddles that were evaluated. Also, it was found that some of the test paddles were very sensitive to poor sampling technique while others were more forgiving. Further study of a wider variety of bacteria test kits is warranted.

Introduction

Recent outbreaks of pathogenic bacteria in recreational waters have focused public attention on the health effects of improperly sanitized recreational bathing and drinking water. Concurrently there has been an increase in the number of home bacteria test kits on the market. These tests purportedly allow consumers to test the safety of drinking and bathing water by estimating the number of bacteria in a given volume of water. A few different types are available, ranging from those that simply indicate the presence or absence of a particular microbe to those that claim to give semi–quantitative results. The presence/absence tests consist of a dry media to which a water sample is added. The color of the resulting solution indicates the presence or absence of the microbe of interest. The semi–quantitative testers consist of a filter grid resting on, or embedded with bacterial growth media, or simply the media itself, in the form of a paddle which is immersed into a water sample for a proscribed period of time. The current study investigates the accuracy and precision of data obtained by four different semi–quantitative paddle testers.

Proceedings of the 3rd Annual Chemistry Symposium
National Spa and Pool Institute – October 1998
Pages 14–17

Materials and Methods

The paddle testers fell into two major groups, each with two media types for different sampling needs.

1. ***Type A:** A filter grid overlaying an absorbent pad impregnated with growth media.*
   - The sample is taken in the test paddle's plastic cover, the paddle inserted and the entire apparatus laid on it's side for 30 seconds. This allows a measured volume of water (1 mL according to the manufacturer) to be absorbed into the pad, filtering any organisms onto the overlying filter grid.
     - ***a.** Low Nutrient Medium*
       - Low nutrient medium for recovery of organisms which are "stressed" due to low nutrient conditions.
     - ***b.** High Nutrient Medium*
       - High nutrient medium for non–selective growth of aerobic bacteria. Tends to give lower counts than the low nutrient agar.
2. ***Type B:** Semi–solid (agar) growth media adhering directly to the paddle.*
   - This type of paddle tester is simply dipped briefly into the body of water to be sampled and any excess water allowed to drip off the agar surface.
     - **a.** *High Nutrient Medium*
       - High nutrient medium for non–selective growth of total aerobic bacteria. Contains an indicator that stains bacterial colonies red.
     - **b.** *Neutralizing Medium*

- Contains neutralizing agents for a number of disinfectants including chlorine and quaternary ammonium based disinfectants.

Three types of bodies of water were tested:

1. A spa inoculated with laboratory maintained bacteria.
2. Beakers inoculated with laboratory maintained bacteria.
3. A swimming pool undergoing minimal use.

Spa

A 350 gallon Coleman model 411 spa was inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa* to various levels. After five minutes of circulation samples were taken. No sanitizer was present.

Beakers

Four liter beakers containing 3 liters of balanced water (pH = 7.6; total alkalinity = 100 ppm; Ca hardness = 250 ppm) were inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa* to various levels. After five minutes of stirring, samples were taken. No sanitizer was present.

Swimming Pool

These tests were done to investigate the effect of bad sampling technique on paddle test results. One set of samples was taken adhering strictly to the directions provided with the test paddle. A second set of samples was taken less rigorously, allowing the sampler's fingers to contact the sample water. At the time of sampling, 1.0 ppm free chlorine was present in the pool water.

Conventional bacterial analysis was done using Standard Method 9215 B (Heterotrophic Plate count – pour plate method using R2A agar). Statistical analysis of the data consisted of a test to determine if there was any significant difference between the means of the paddle tests and the Standard Methods analysis data. The following formula was used to generate the statistical values for this test:

$$t = \frac{y_1 - y_2}{s^2/n_1 + s^2/n_2}$$

Results

Paddle Test Data vs. Standard Methods Data

Table 1 details the confidence levels obtained from comparing the means of paddle test data and Standard Methods data. Note that the higher the confidence level, the more likely it is that the paddle testers gave a different result than the Standard Methods analysis. The number in parentheses is the relative standard deviation (RSD), which is found by dividing the standard deviation of a data set by it's mean. The confidence level in this case can be thought of as a measure of a test paddle's accuracy(the lower the confidence level, the higher the accuracy), while the RSD is a measure of the precision, or repeatability of the test paddle measurements.

The trend seen in Table 1 is as the confidence level decreases, the relative standard deviation increases. To put it another way, even though the data sets with a lower confidence level may appear to be more accurate, the RSD reveals such a large spread in the data that the appearance of accuracy is found to be false.

A graphical view of all the paddle test data generated is shown in Figure A. The line through the center of the graph represents a 1:1 relationship between the test paddle data and the Standard Methods data. The unshaded portion of the graph repre-

Bacteria Level (cfu/mL)	Type A Low Nutrient	Type A High Nutrient	Type B Low Nutrient	Type B Neutralizing
≈ 10	99.9% (0.1)*	99.5% (0.3)	90% (1.9)	90% (1.5)
≈ 50	<90% (1.2)	<90% (1.9)	97.5% (1.9)	97.5% (1.2)
≈ 200	99% (0.2)	95% (0.5)	99.9% (0.4)	99.9% (1.0)
≈ 300	99.9% (0.1)	99.5% (0.1)	99.9% (0.8)	99.9% (0.8)

Table 1 – Confidence levels for means testing of paddle testers vs. standard methods data. (RSD in parentheses)

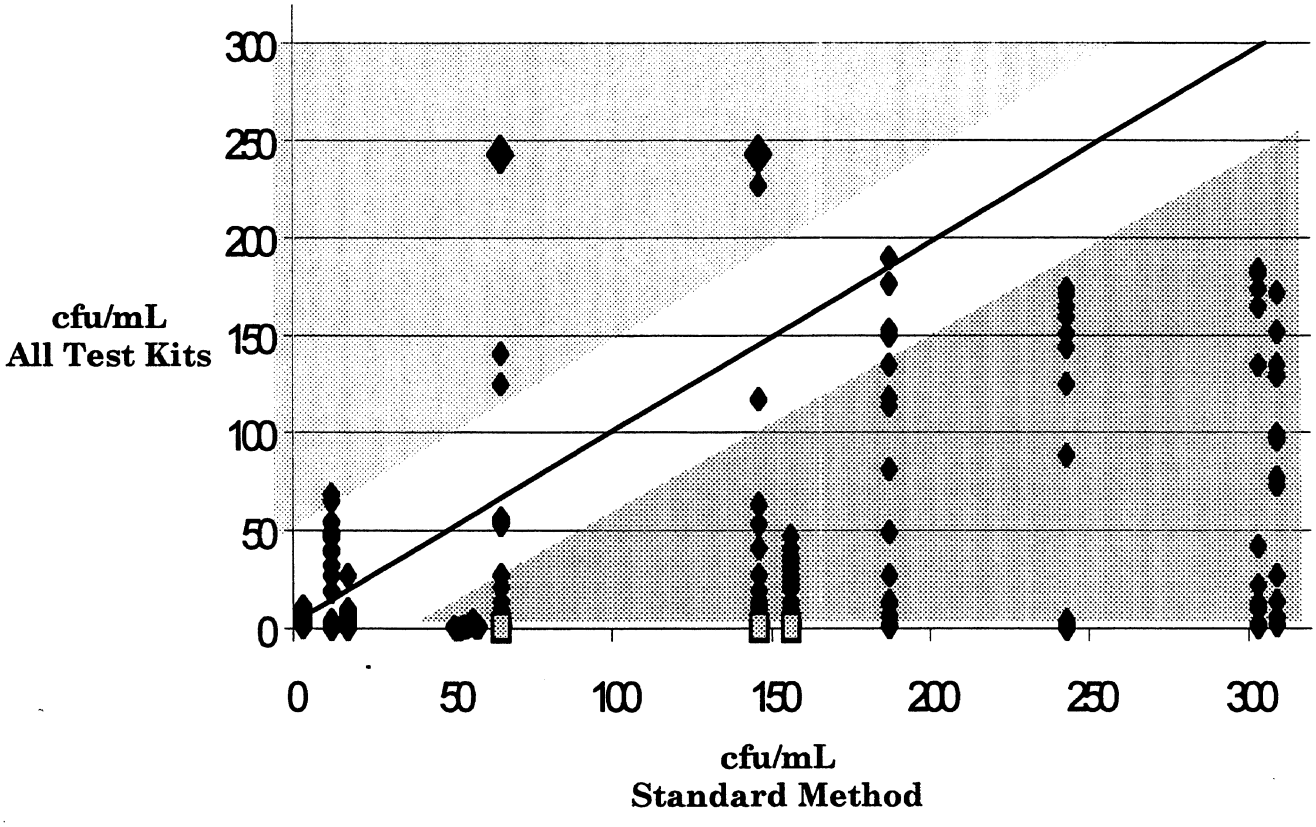

Figure A – Bacteria Levels
Standard Methods vs. All Paddles

Bacteria Level (cfu/mL)	Brand A Low Nutrient	Brand A High Nutrient	Brand B High Nutrient	Brand B Neutralizing
<1 cfu/mL	μ = 33 cfu/mL	μ = 42 cfu/mL	μ < 1 cfu/mL	μ < 1 cfu/mL

Table 2 – Contamination From Poor Sampling Technique

Actual Bacteria Levels	Type A Low Nutrient	Type A High Nutrient
> 1,000 cfu/mL	μ = 375 cfu/mL	μ = 350 cfu/mL
>10,000 cfu/mL	μ = 45 cfu/mL	μ = 20 cfu/mL

Table 3 – Measurement of High Bacteria Counts

sents the area where we could reasonably expect to find 99.7% of the data we could consider statistically accurate. Any point falling in the shaded area above the line is considered an overestimate of true bacteria numbers. By the same logic, any point falling in the shaded area below the line is considered an underestimate of true bacteria numbers. It is clear from the graph that, as a whole, the test paddles we looked at tended to underestimate the true bacteria numbers.

Contamination From Poor Sampling Technique

Type A paddle testers proved more susceptible to poor sampling technique than did Type B as can be seen in Table 2.

Measurement of High Bacteria Counts

One other characteristic of the Type A paddles tested was found to be a severe underestimation of extremely large numbers of bacteria. As Table 3 shows, contamination of a test spa led to a bacteria level of >1,000 cfu/mL, an unacceptable number for pools and spas. The Type A paddles, however gave very low counts compared with the Standard Methods analysis. The underestimation is even more pronounced at >10,000 cfu/mL.

Conclusions

The paddle testers investigated exhibited a tendency to underestimate bacteria numbers in tests conducted under controlled conditions. Under consumer use conditions this type of inaccuracy could engender a false sense of security on the part of pool owners. Of greater concern than the trend seen at lower bacteria levels is the serious underestimation of extremely high bacteria counts which could result in health problems. The sensitivity of these tests to improper sampling technique also presents a problem. Even well meaning consumers don't always follow instructions exactly, a prerequisite for obtaining the best results from any analytical test. Consumer reliance on these tests for accurate information regarding the microbiological quality of their water is problematic. A wiser course of action would be to maintain water balance according to industry standards and follow the manufacturer's instructions carefully for whichever sanitizing system is being used.

About the Authors

Joe Grenier is a Research Microbiologist for Zodiac North American Pool Care Sector. Mr. Grenier holds a degree in Biology from the University of Rhode Island. His primary research interests include the kinetics and mechanisms of various antimicrobial systems.

Ray Denkewicz Jr. is currently Vice President, Technology for **Zodiac North American Pool Care Sector**. Ray holds BS and MS degrees in chemical engineering from Worchester Polytechnic Institute and has completed additional graduate studies in both chemical engineering and material science. He is the author of several publications and holds both US and foreign patents in the area of novel catalysts and water treatment chemicals. He is also currently a member of NSPI's Chemical Treatment and Process Subcommittee.

References

Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Pollution Control Federation, 16th Edition, 1985.