Bacteriological Evaluation of Two Test Methods for Chlorine in Swimming Pools

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It was found that orthotolidine and plastic test strips containing a mixture of syringaldazine and vanillinazine gave different results as to chlorine content of water samples containing urine or large amounts of bacteria while giving consistently similar results with water samples having only sodium hypochlorite added. Orthotolidine was found to give erroneously “safe” readings when in fact viable bacteria were observed on membrane filters after filtration.

Chlorine is commonly used to control bacteria in swimming pools. Free chlorine atoms kill bacteria by chlorinating outer components of the cell and breaking peptide bonds as do other halogens (5), but chlorine bound to protein or amines loses its effectiveness to a large extent (2). It is difficult to determine accurately the level of free chlorine ions in pool water. The standard method of determining chlorine concentrations in pools is the orthotolidine method, which is not selective for free chlorine, but registers bound chlorine as well.

In 1970 a swimming pool water-testing device utilizing indicators was developed as a test for free chlorine and pH (C. O. Rupe et al., Abstr. no. 6, 160th Amer. Chem. Soc. Nat. Meet., 1970). These test strips (Aqua Check; Ames Co., Division of Miles Laboratory, Inc., Elkhart, Ind.) consist of a plastic rectangle (89 by 25 mm) containing absorbent paper treated with a mixture of syringaldazine and vallinazine, which turns from yellow to purple in the presence of free chlorine, and a pH-indicator strip.

This test (syringaldazine and vanillinazine; SV) has been subjected to various laboratory and field tests concerning its chemical accuracy by several workers.

It was found that the SV test gave approximately the same results as standard chemical tests for free chlorine but different results than did orthotolidine when bound chlorine was tested (4; Rupe et al., Abstr. no. 6, Amer. Chem. Soc. Nat. Meet., 1970). However, bactericidal properties of swimming pool water were not tested.

This paper summarizes studies which compared the ability of the orthotolidine and SV test methods to detect potentially dangerous conditions in pools and their relative sensitivities to changes in free chlorine levels.

MATERIALS AND METHODS

Three common bacterial species of medical importance were used in this study. Streptococcus faecalis is usually considered to be one of the best indicators of human fecal contamination of water systems. Escherichia coli has long been considered the standard for indication of fecal contamination of water supplies. Pseudomonas aeruginosa is found in ear infections and some external wounds and is thus of considerable interest in determining the safety of pool water (G. A. Pottz, personal communication). Wild-type strains were used.

Stock cultures of bacteria were maintained on nutrient agar (Difco) and transferred every 3 weeks.

The orthotolidine test was performed by using a Guardex Pool Test Kit, according to the manufacturer's directions (3).

M-Endo Broth MF (Difco) was used as a preferential medium for E. coli, Streptococcus faecalis (SF) Broth (Difco) was the preferential medium for S. faecalis, and nutrient broth (Difco) was used for P. aeruginosa since no preferential medium was available. Absorbent pads (50 mm) and membrane filters (0.45-μm pore size) were obtained from Schleicher and Schuell. All incubations were carried out at 37 C.

Swimming pool water from a community pool was obtained from a depth of 6 to 12 inches (ca. 15.24 to 30.48 cm) below the surface, autoclaved, and used immediately.

Erlenmeyer flasks (500 ml) with 100 ml of water were used to test for bacterial survival. Water was placed in the flasks and sterilized; sodium hypochlorite solution prepared by an approximately 50-fold dilution of Chlorox (Chlorox Co., Oakland, Calif.) was added until the SV test indicated 1.0 to 2.0 μg (ppm) of chlorine per ml which is considered a safe level for pools. The sodium hypochlorite solution was calibrated by the SV test and orthotolidine to insure uniform conditions before each set of flasks was tested.
**Table 1.** Chlorine levels and *Escherichia coli* survival in swimming pool water, after addition of human urine

| Sample | Urine added<sup>a</sup> | Chlorine indicated (μg/ml) | Bacteria<sup>d</sup> |
|--------|-------------------------|---------------------------|---------------------|
|        |                         | Before urine | After urine | SV<sup>b</sup> | OT<sup>c</sup> | SV | OT |
| 1      | 0                       | 1.0          | 1.0         | 1.0          | 1.0         | 0  |
| 2      | 0                       | 1.0          | 1.0         | 1.0          | 1.0         | 0  |
| 3      | 0.05                    | 1.5-2.0      | 1.5         | 1.5          | 1.5         | 0  |
| 4      | 0.05                    | 1.5          | 1.5         | 1.5          | 1.5         | 0  |
| 5      | 0.15                    | 1.5-2.0      | 1.5         | 0            | 1.5         | 0  |
| 6      | 0.25                    | 1.5          | 1.5         | 0            | 1.5         | 1,000 |

<sup>a</sup> Milliliters of fresh human urine added per 100 ml of water after adjustment of chlorine content.
<sup>b</sup> SV test strips.
<sup>c</sup> Orthotolidine pool test kit.
<sup>d</sup> Viable bacteria per 100-ml test flask after equilibration, determined by membrane filter technique. (About $5 \times 10^5$ cells were added to each test flask.)

**Table 2.** Chlorine levels and *Pseudomonas aeruginosa* survival in swimming pool water after addition of human urine

| Sample | Urine added<sup>a</sup> | Chlorine indicated (μg/ml) | Bacteria |
|--------|-------------------------|---------------------------|----------|
|        |                         | Before urine | After urine | SV | OT |
| 1      | 0                       | 2.0          | 1.5         | 2.0 | 1.5 | 0  |
| 2      | 0                       | 2.0          | 1.5         | 2.0 | 1.5 | 0  |
| 3      | 0.05                    | 2.0          | 1.5         | 1.0 | 1.5 | 0  |
| 4      | 0.05                    | 2.0          | 1.5         | 1.0 | 1.5 | 0  |
| 5      | 0.15                    | 2.0          | 1.5         | 0   | 1.5 | 0  |
| 6      | 0.25                    | 2.0          | 1.5         | 0   | 1.5 | 2,700 |

<sup>a</sup> See footnotes for Table 1.

**Table 3.** Chlorine levels and *Streptococcus faecalis* survival in swimming pool water after the addition of human urine

| Sample | Urine added<sup>a</sup> | Chlorine indicated (μg/ml) | Bacteria |
|--------|-------------------------|---------------------------|----------|
|        |                         | Before urine | After urine | SV | OT |
| 1      | 0                       | 2.0          | 1.5         | 2.0 | 1.5 | 0  |
| 2      | 0                       | 2.0          | 1.5         | 2.0 | 1.5 | 0  |
| 3      | 0.05                    | 2.0          | 1.5         | 1.0 | 1.5 | 0  |
| 4      | 0.05                    | 2.0          | 1.5         | 1.0 | 1.5 | 0  |
| 5      | 0.15                    | 2.0          | 1.5         | 0   | 1.5 | 250 |
| 6      | 0.25                    | 2.0          | 1.5         | 0   | 1.5 | 1,600 |

<sup>a</sup> See footnotes for Table 1.

Urine and the bacteria to be tested (0.005 ml of an overnight culture with $10^9$ to $2 \times 10^9$ cells per ml) were added sequentially to the flasks with omissions as indicated in the tables. Flasks were mixed and allowed to equilibrate for 15 min after each addition. After final equilibration, the number of viable bacteria was determined by the membrane filter technique.

**RESULTS AND DISCUSSION**

When sodium hypochlorite solution alone was added to the flasks, the test results of orthotoli-
Fig. 1. Variation of indicated free chlorine levels in the presence of bound chlorine with time for otho-
tolidine and SV test methods.

dine and the SV test showed good agreement. It appeared that when only free chlorine was present the test results of these two methods were consistent.

Urine and bacteria contain amines which bind chlorine and lower its effective concentration. When testing with SV, it was found that the initial chlorine level of 1.5 μg/ml dropped after the addition of 0.05 to 0.25 ml of urine to flasks containing 100 ml of water (Table 1–3). Similar tests with the orthotolidine method showed little or no change in the indicated level of free chlorine after addition of the urine. Viable E. coli in these samples ranged from 640 to 1,000 per 100 ml (Table 1). Under the same condition, P. aeruginosa (Table 2) varied from 520 to 2,700 bacteria per 100 ml, and S. faecalis ranged from 250 to 1,600 colonies (Table 3).

With a larger (0.1 ml) inoculation of an overnight culture (10⁸ to 2 × 10⁸ cells per ml) of E. coli, the solution had final SV test readings of zero and final orthotolidine readings of approximately 1.5 μg per ml of chlorine. These samples were found to contain from 2.0 × 10⁸ to 1.8 × 10⁹ viable E. coli per 100 ml.

In every instance in which SV gave a "safe" reading, there were no viable bacteria detectable by growth on M-Endo, SF, or nutrient broth. However, in all instances of "unsafe" SV readings and "safe" orthotolidine readings shown in Table 1–3, the bacteria counts per 100 ml were above the standards of the South Carolina Board of Health (four coliforms per 100 ml).

Similar tests were carried out starting with distilled water. The results obtained were identical although more sodium hypochlorite solution had to be added initially to obtain the necessary 1.0 to 2.0 μg of free chlorine per ml level. This is because the swimming pool water already had some active chlorine.

It has been reported by Black et al. (1) that orthotolidine testing must be done at 0 to 1 C and within 5 sec to obtain accurate free chlorine results in the presence of chloramines. Due to the difficulty of obtaining readings within this time interval under normal pool testing conditions, we investigated the effects of time on the results obtained. These are shown in Fig. 1, and indicate that readings at somewhat longer times (10 to 20 sec) give inaccurate results under conditions where bound chlorine is present (0.25 ml of fresh urine added at zero time).

The SV test provides a more precise measurement of effective antimicrobial activity of chlorinated water than does the orthotolidine test method commonly used for swimming pool testing. Orthotolidine fails to detect possible dangerous situations which are easily found with the SV test.

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LITERATURE CITED

1. Black, A. P., M. A. Kerrin, J. J. Smith, G. M. Dykes, and W. E. Harlan. 1970. The disinfection of swimming pool water. II. A field study of the disinfection of public swimming pools. J. Pub. Health 60:740–750.
2. Fetner, R. H., and R. S. Ingols. 1956. A comparison of the bactericidal activity of ozone and chlorine against Escherichia coli at 1°. J. Gen. Microbiol. 15:381–385.
3. Field, C. A. Guardex instruction booklet. Purex Corporation, Ltd.
4. Geeting, D. G., C. B. Hager, and A. H. Free. 1970. Aqua check—a swimming pool information system. A bulletin of Ames Technical Services Laboratory, Ames Co., Division of Miles Laboratories, Inc., Elkhart, Ind.
5. McKee, J. E., C. J. Brokaw, and R. T. McLaughlin. 1960. Chemical and colocal effects of halogen in sewage. J. Water Pollut. Control Fed. 32:795–819.