New Method for Evaluating Antibacterial Activity Directly on Fabric

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Bacteriostasis of treated fabric was evaluated directly by a colony count made on the treated fabric after impregnation with seeded agar containing triphenyltetrazolium chloride and incubation in a horizontal, suspended position in a petri dish.

A number of methods have been described for evaluating antimicrobial activity of treated fabric (1–15). Most of the methods are indirect, requiring the removal of the agent from the fabric before it is measured chemically or biologically. Of these, the agar diffusion method (13) is the most frequently used and has been standardized by the American Association of Textile Chemists and Colorists (AATCC) as an official test method for detecting bacteriostatic activity on fabric (2). The antimicrobial agent diffuses from the fabric into seeded agar, producing a zone of inhibition around the fabric. This test is limited to detection of antimicrobial agents which are easily diffusible in agar or to those not very substantive to fabric. However, the most important objection to all of the indirect methods is that they do not provide realistic appraisals of antimicrobial activity on the fabric surface.

More direct methods were reported, such as the measurement of urease inhibition of inoculated fabric containing urea (10) or the soil burial test for evaluating antifungal activity (1), but they have limited application. A test described by Quinn (12) permits direct enumeration of bacterial colonies on the fabric surface, but the test is not easy to run, colonies are not easily visible, and the possibilities for diffusion of the antimicrobial agent have not been minimized. A simple test is described below which facilitates a direct observation of bacterial colony growth on the surface of a fabric swatch and limits diffusion of the antimicrobial agent to the immediate surface area of the fabric.

A swatch [1 by 1 inch] (2.54 by 2.54 cm) is suspended tautly and horizontally in a petri dish by means of a wire hanger with hooks (Fig. 1). After autoclaving, 0.4 ml of molten AATCC agar (2) containing 20 μg of 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) per ml and 2 million bacterial cells is applied slowly and uniformly to the fabric surface. To prevent drying of the agar, approximately 5 ml of sterile distilled water is added to the petri dish bottom. After 48 hr of incubation at 37 °C, a colony count is made on both sides of the white fabric surface. The TTC is reduced by bacterial dehydrogenase enzymes to form a red insoluble dye (triphenylformazan), which stains bacterial colonies red. A low-power stereoscopic microscope may be used to be certain all colonies are counted. Untreated control swatches with 2 million cells are included in the test, but the colonies which develop are too numerous to count. These swatches appear pinkish due to numerous microscopic red colonies. However, a countable swatch of 200 colonies is obtained by applying a 1:10,000 agar dilution of the seeded agar to untreated fabric (Fig. 2).

Bacterial suspensions of Staphylococcus aureus (ATCC 6538) or Escherichia coli (ATCC 4352) are prepared by washing 24-hr slant cultures grown on AATCC agar (2) with 7 ml of 0.003 M phosphate buffer (pH 7.0) and agitating for 1 min in a test tube by using a Vortex Genie at a no. 6 setting. A 1:100 dilution of the cell suspension in phosphate buffer is diluted 30 to 40% with molten agar (48 C) in a flask and swirled to mix.

The fabric was treated in a launderometer, by the method of Petrocci and Clarke (11), with a treatment solution to fabric ratio of 1:10 and an exposure period of 10 min (25 C). The treatment solution was an aqueous dilution of 50 μg of active Hyamine 3500 per ml (Rohm and Haas Co.; an alkyl(dimethylbenzylammonium chloride antimicrobial agent substantive to fabric) in 0.26% of a laundry detergent-sanitizer formulation. To simulate a home laundry operation and to demonstrate substantive to fabric, the treated fabric was rinsed twice for 2 min each. After rinsing, the fabric was hung to dry overnight at room temperature before evaluating for bacteriostasis by the suspended swatch and zone of inhibition tests.
FIG. 1. Wire hanger for suspending the fabric swatch is constructed of two 23-gauge stainless-steel wires, each approximately 11 inches in length (28 cm), shaped as indicated to fit in a standard petri dish, and silver soldered at right angles to each other at the midpoint. The fabric is pierced with the sharpened hook-ends and held taut by the spring action of the bends.

FIG. 2. Close-up view of control fabric swatch suspended in petri dish, showing colonies of Escherichia coli (ATCC 4352) which had developed on fabric surface after impregnation with agar medium containing TCC and approximately 200 bacterial cells. Red colonies, which appear black in photograph, are found on both sides of swatch.

The surface of treated fabric was shown to be bacteriostatic when evaluated by the new suspended swatch test. Typical data are shown in Table 1. Few colonies of S. aureus and E. coli developed on the treated fabric compared to the too numerous to count results with untreated fabric. Good recovery was obtained on untreated swatches inoculated with 1:10,000 seeded agar dilutions compared to plate counts, confirming the number of viable organisms applied to the treated fabric. The treated fabric produced an inhibition of >99.999%, based on the number of viable cells applied.

Similarly treated fabric also showed bacteriostatic activity when evaluated by the zone of inhibition test, but zone analysis suggests less activity (Table 1). Small zones (1 to 2 mm or less) were produced by all swatch sides of all reps versus S. aureus, and zones versus E. coli were smaller in size, shape, and number.

The suspended swatch test can provide a simple means for direct analysis of bacteriostasis on the surface of treated fabric. The method is more suitable than the agar zone test for evaluating fabric treated with substantive or low-water-soluble antimicrobial agents, or both.

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### Table 1. Bacteriostatic activity of treated fabric as determined by zone of inhibition and suspended swatch tests

| Fabric       | Test organism            | Zone test (2) | No. of cells applied | Suspended swatch test | Avg colony count/swatch after incubation |
|--------------|--------------------------|---------------|----------------------|-----------------------|------------------------------------------|
| Treated      | Staphylococcus aureus    | 20            | 2,120,000            | 1.4                   |
|              | Escherichia coli         |               |                      |                       |
| Untreated    | S. aureus                | None          | 2,120,000            | TNC                   |
|              | E. coli                  | None          | 2,320,000            | TNC                   |

- Number of swatch sides with clear zone out of 20 sides. Results based on five reps. Zone analysis and medium of Petrocci and Clarke (11).
- Determined by plate count.
- Results based on five reps. TNC, too numerous to count.
- Treatment solution: 0.26% detergent-sanitizer formulation containing (w/w), 51% sodium tripolyphosphate, 27% sodium sesquicarbonate, 10% Triton N-101, 8% anhydrous sodium metasilicate, 2.38% Hyamine 3500 (80%), 1.0% CMC 7 LT, 0.30% Tinopal UNPA, and 0.08% Tinopal RBS-200%.
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