

## Evaluation of a Test Strip Used to Monitor Food Processing Sanitation

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### ABSTRACT

A test strip, which contained a small absorbent pad on the end of a plastic strip for detection of microbial contamination of liquids and surfaces, was compared to standard rinse solution and surface contact methods. Bottles and food contact surfaces were unclean, cleaned or cleaned and sanitized before being evaluated with test methods. Results from test strips correlated well with those of the standard rinse solution method for bottles that were clean and/or sterilized, but not for those that were heavily contaminated. When test strips were used on cleaned contact surfaces, counts were one log cycle greater than those of contact plate or swab methods; however, the three surface methods correlated well for surfaces that were cleaned and sanitized. To insure that the contact methods were recovering microorganisms, surfaces were spread with known levels of *Escherichia coli* and *Staphylococcus aureus*. Results correlated well for low levels of contamination, but not for levels greater than  $1 \times 10^4$  organisms/25 cm<sup>2</sup>. Overall, test strips could be used for quick indication of sanitation of cleaned and sanitized food contact surfaces and containers if special precautions and limitations were understood.

Production of high quality food products depends on a well-planned and implemented cleaning and sanitation program. To be effective, this program must be coupled with a regular audit system that detects the presence of microorganisms on surfaces or in rinse solutions. Swab, rinse and agar contact methods have been designed to measure the contamination of surfaces. Several reviews of methods for evaluation of sanitation of food contact surfaces have been published (1,3,5,7,14,17).

Various modifications of these standard methods have been developed. Agar slice methods, which allow agar to be pushed onto the surface, have been proposed, and include the agar syringe method of Litsky (17) and the agar sausage method of TenCate (16). Another modification of the agar contact method involved using adhesive tape that was either transferred to agar for incubation (12) or directly stained for microscopic examination (3). Winter et al. (18) evaluated a microcolony count method by staining a membrane filter

from the rinse method with Janus green. A wipe-rinse technique (moistened cloth was wiped over the surface, placed in buffered dilution water and agitated, and serial dilutions were plated) was evaluated for sampling large surface areas (10).

Methods that are inexpensive, do not require skilled technicians or sophisticated laboratory facilities, and give reliable indications of sanitation are needed by most food processing industries. Recently, microbiological test kits have become commercially available for use in assessing contamination of liquids and surfaces. Bailey and May (2) evaluated some of these kits for microbiological testing of hydrocarbon fuel systems and suggested that they could be used to determine contamination in food processing situations. This research was designed to evaluate the efficacy of a new sanitation test strip for use in food processing situations. The sensitivity of the test strip for detecting microbial contamination was compared to that of the standard swab, rinse and agar contact methods.

### MATERIALS AND METHODS

#### *Food contact surfaces evaluated*

Surfaces in the Purdue University Meat Processing Laboratory (wooden table tops and stainless steel cutting table, saw and scale), Purdue University Pilot Plant (stainless steel tables, cottage cheese vat and vat pasteurizer), and Food Microbiology Laboratory (alberene stone laboratory bench tops) were chosen for test surfaces. The days, times, sites and number of samples were randomly chosen for each test. Sampling was done on surfaces that were unclean, cleaned or cleaned and sanitized; however, some samples were taken a few hours after the cleaning operation was completed.

#### *Test strips*

Test strips consisted of 8.3 × 2 cm plastic strips to which were attached 1.6 × 2 cm pads containing nutrients and growth indicators. Pads of the test strips were moistened with a neutralizer solution to prevent inhibitory effects from residual sanitizers or with distilled water. Pads were then used in the same manner as swabs to sample a defined area by gently pressing the pad to the surface and rotating about 2 to 4 times in a circular motion or drawing the pad across the surface and then reversing the direction. They were immersed into rinse solution for 5 s to obtain a liquid count. Test strips were then put into sterile transparent zip-lock pouches and incubated for 24 h at 30 to 32°C. After incubation, the density of microorganisms on the test strip was compared to a standard color chart (see Table 1 for chart comparison values for surface and liquid counts). These test strips can

be used to rapidly detect problems with the sanitation program, but cannot replace standard microbiological procedures because they provide an estimate of the total microbial count.

#### Techniques evaluated

Rinse solution and surface contact methods were used to evaluate test strips supplied by Diversey-Wyandotte Corporation, Wyandotte, Michigan (Fig. 1) against the standard rinse, swab and agar contact methods (4). Sterilized and non-sterilized 250-ml glass bottles were



Figure 1. Test strips used to evaluate sanitation of bottles and surfaces show (left to right) no contamination to contamination, indicated by appearance of colored areas.

used for the rinse solution method (4). The test strips were immersed into the well-mixed diluent for 5 s, removed, and incubated at 32°C for 24 h in a Precision Scientific gravity convection incubator and at 30°C for 24 h in a Microstix incubator (Ames Company, Division Miles Laboratory, Inc., Elkhart, Indiana 46514), as shown in Fig. 2.

The surface contact methods were randomly sampled over a 324-cm<sup>2</sup> test area as shown in Fig. 3. Two test strips (Diversey-Wyandotte Corporation, Wyandotte, Michigan 48192) and a cotton swab (Puritan Cotton-tipped Applicators, Hardwood Products Co., Guilford, Maine 04443) were rubbed over a 25-cm<sup>2</sup> surface marked off by a sterilized cardboard template (Fig. 3). A RODAC plate (Falcon Rodac Plates, Fisher Scientific Company, Itasca, Illinois 60143) was pressed into the remaining exposed surface area covering approximately 25 cm<sup>2</sup> (Fig. 3). The position within the sampling area was randomly chosen for the test strips, swab and contact plates. Standard testing procedures using Plate Count Agar (Difco Laboratories, Detroit, Michigan 48232) were done for the swab and RODAC contact plates (4); however, the sampling area, normally about 50 cm<sup>2</sup>, was changed to 25 cm<sup>2</sup> so that data from each method could be easily compared.

#### Variability testing

To assess variability of the test methods, the laboratory bench top and a stainless steel table were spread with the following two levels each of *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229): less than 100 organisms/25 cm<sup>2</sup> and greater than  $1 \times 10^4$  organisms/25 cm<sup>2</sup>. Plate counts were done to determine the levels of these bacteria. These two bacteria were chosen because they are recommended for use in evaluating germicidal and detergent sanitizers (8). The test organisms were grown in trypticase soy broth (BBL, Division Becton Dickinson and Company, Cockeysville, Maryland 21030) at 35°C for 24 h, diluted in sterile trypticase soy broth, applied to the surface by a Pasteur pipette, and allowed to air dry. The surface area was randomly sampled using test strips, swabs, and RODAC plates as described above.

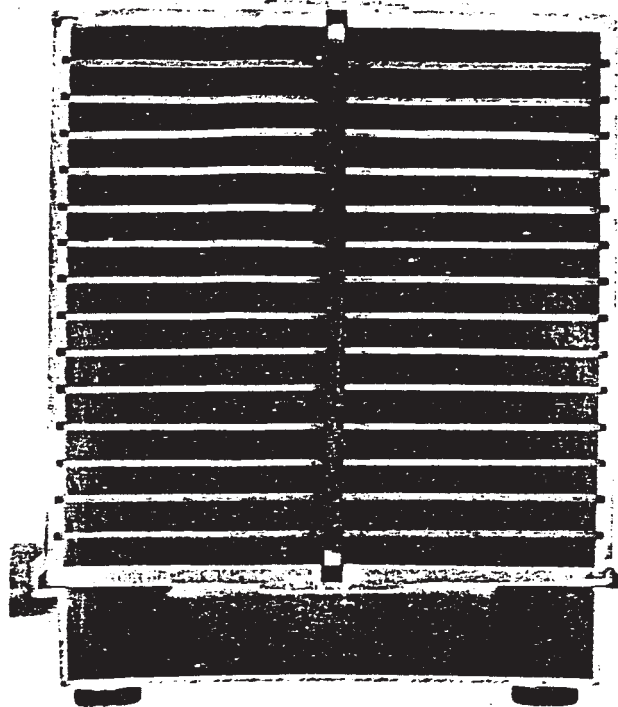


Figure 2. Table-top Microstix incubator can conveniently hold 30 test strips and be used by industries who do not have microbiological incubators.

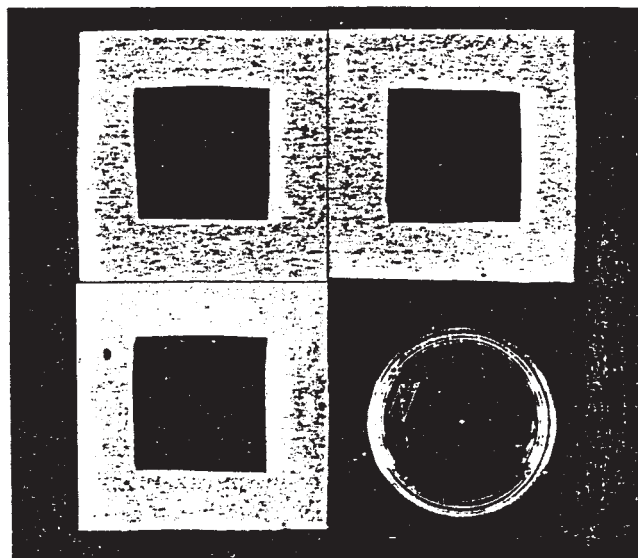


Figure 3. Randomly chosen 324-cm<sup>2</sup> areas were marked off using three sterilized cardboard templates for two test strips and one swab sample and an unbordered area for contact plates. This design facilitated the sampling of similar areas by all methods.

#### Statistical analysis

Data were analyzed by nonparametric statistical methods because the results of the test strip were expressed in range values and those of the standard tests, in actual numbers. The Kruskal-Wallis Test, which involves ranking all values in ascending order and using these ranked

valued for the analysis, was done on all data (9). The Multiple Comparison Test was used to evaluate the differences among treatments (9).

## RESULTS AND DISCUSSION

Because results of the test strip being evaluated could only be reported in range values based on color interpretation charts, all values were eventually converted to range values for statistical analysis (Table 1). Although these range values were used for all analyses, the actual values for the rinse bottle, contact plate and swab methods are reported in subsequent tables.

### Rinse solution method

Bottles that were unclean, clean and sterilized were used to test the efficacy of the test strip in relation to that of the rinse solution method for detecting sanitation of containers (Table 2). Data collected from the test strips incubated at 32°C in a gravity convection incubator were significantly different from those of the rinse solution method for bottles that were not cleaned (Table 2). However, the bottles would be considered unsatisfactory for use at this point. Although the test strip was not as

effective for detecting a similar level of contamination as that of the rinse solution method for counts of around  $5 \times 10^4$  organisms/ml, it is doubtful that this level would be encountered in most operations. For these experiments, the test strip picked up fewer organisms than the rinse method, usually 1 to 2 log cycles lower. This could give the processor a false indication of sanitation. Because the data obtained when using the test strips to evaluate bottles that were cleaned, and cleaned and sanitized correlated well with those of the rinse solution method (Table 2), these strips could be used to indicate the degree of sanitation of the containers. These test strips could be compared to the dip-pad and dipslide kits evaluated by Bailey and May (2). These kits were easy to use and gave good agreement with the laboratory standards, but had an endpoint sensitivity of 150 microorganisms/ml. From the results reported in Table 2, it appears that the test strip may have a higher endpoint sensitivity than these other test kits.

### Surface methods

Cleaned, and cleaned and sanitized surfaces were used to test the efficacy of using the test strips to evaluate sanitation of food contact surfaces (Table 3). Unlike the rinse method that was reporting low counts by the test strip method, the test strips gave counts higher than those of the contact plate and the swab methods for some samples. Although these counts were generally one log cycle greater than those reported for the standard methods, the information could give the processor a quick indication that the cleaning and sanitizing operations are unsatisfactory. The Public Health Service recommends that a standard of less than 100 microorganisms per surface area swabbed (approximately 50 cm<sup>2</sup>) be used to assess the acceptability of equipment sanitation (19). Because the test area was only 25 cm<sup>2</sup>, the microbial count should be less than 50 microorganisms for adequate sanitation. Most values for the contact and swab methods are greater than this

TABLE 1. Values used to arrange data from standard tests and test strip analyses into equivalent ranges for statistical comparisons.

Chart comparison values	Surface range count	Liquid range count
1 <sup>a</sup>	0 - 10	0 - 10 <sup>2</sup>
2 <sup>b</sup>	10 - 10 <sup>2</sup>	10 <sup>2</sup> - 10 <sup>3</sup>
3	10 <sup>2</sup> - 10 <sup>3</sup>	10 <sup>3</sup> - 10 <sup>4</sup>
4	10 <sup>3</sup> - 10 <sup>4</sup>	10 <sup>4</sup> - 10 <sup>5</sup>
5	10 <sup>4</sup> - 10 <sup>5</sup>	10 <sup>5</sup> - 10 <sup>6</sup>
6	10 <sup>5</sup> - 10 <sup>6</sup>	10 <sup>6</sup> - 10 <sup>7</sup>

<sup>a</sup>Values for test strips are satisfactory for cleaned and sanitized surfaces. All other values are unacceptable.

<sup>b</sup>Values for test strips are satisfactory for cleaned surfaces. All other values are unacceptable.

TABLE 2. Microbial counts per container for 250-ml unclean, clean, and sterilized glass bottles rinsed with 20 ml of phosphate buffered water.

Bottles	Test strip (30°C)	Test strip (32°C)	Rinse method (32°C)
	Organisms/container (number) <sup>a</sup>		
Unclean	0 - 10 <sup>2</sup> (3)	0 - 10 <sup>2</sup> (3) <sup>b</sup>	1.1 - 8.4 × 10 <sup>4</sup> (20) <sup>b</sup>
	10 <sup>2</sup> - 10 <sup>3</sup> (1)	10 <sup>2</sup> - 10 <sup>3</sup> (1)	
	10 <sup>3</sup> - 10 <sup>4</sup> (4)	10 <sup>3</sup> - 10 <sup>4</sup> (6)	
	10 <sup>4</sup> - 10 <sup>5</sup> (12)	10 <sup>4</sup> - 10 <sup>5</sup> (10)	
Clean	0 - 10 <sup>2</sup> (8)	0 - 10 <sup>2</sup> (8)	4 - 54 (6)
	10 <sup>2</sup> - 10 <sup>3</sup> (2)	10 <sup>2</sup> - 10 <sup>3</sup> (2)	114 - 660 (4)
Sterilized	0 - 10 <sup>2</sup> (10)	0 - 10 <sup>2</sup> (10)	<2 - 4 (10)

<sup>a</sup>Number of samples with the indicated range values.

<sup>b</sup>Data for the test strip at 32°C are significantly different from those for the rinse method at 32°C for  $\alpha = 0.05$ .

recommended limit. Therefore, use of the test strip would give an indication that the sanitation is inadequate despite the higher count that is detected by this method. Once the count exceeds 50 organisms/25 cm<sup>2</sup>, the surface is considered unsanitary for food contact regardless of the absolute number of microorganisms present.

For surface areas that were adequately cleaned and sanitized, the test strips correlated well with the contact plate and swab methods for detecting similar levels of contamination. These test strips could then be used to give an indication of the level of contamination after cleaning and sanitizing operations. Since the results can be obtained within 24 h as opposed to 48 h for the contact plate and swab methods, food processors will know what areas need attention the following day.

Although results obtained from the contact plate and swab methods did not significantly differ from each other, the swab method generally detected fewer microorganisms per surface area. This is in agreement with the work of Niskanen and Pohja (13) who reported slightly higher counts for the contact plate than for the swab method when used on stainless steel and plastic. However, Stinson and Tiwari (15) concluded that agar contact methods could be used only as quick indicators of bacterial numbers because they gave consistently lower numbers than the swab method. There are a number of reasons why the swab might not be as efficient in recovering microorganisms: (a) amount of pressure applied on swab differs from one person to another (7,14), (b) removal of organisms from the swab may not be complete (7), (c) cotton swabs may retain some microorganisms and cause low counts (14), (d) surfaces examined differ from laboratory to laboratory (7,14) and (e) reproducibility of swab technique is questionable (7).

#### Variability testing

To insure that the contact methods were recovering the microorganisms from the surface, control tests were done using low and high levels of *E. coli* and *S. aureus* spread over a cleaned and sanitized surface. These analyses showed that the tests were reproducible and, for the most part, the test strip results correlated well with those of the standard methods (Table 4). For *S. aureus*, the test strips incubated at 30°C produced results that were 1 to 2 log cycles higher than those of the swab method for high levels of contamination. Because this high level of contamination would be unacceptable for food contact surfaces, the lack of correlation with standard methods would not be as critical as a lack of correlation at a lower level. The results from both the test strips and the swab and contact methods for around 100 microorganisms/25 cm<sup>2</sup> showed acceptable reproducibility and confirmed earlier evidence that these test strips can be used to detect low levels of contamination on contact surfaces. The contact plates for *E. coli* at approximately 1 × 10<sup>4</sup> organisms/25 cm<sup>2</sup> and those for *S. aureus* at both levels of contamination were not countable due to overcrowding and spreading colonies. This phenomenon is one of the drawbacks of using agar contact or agar sausage methods (3,6,14).

#### General observations

The discussion would not be complete unless some general observations on the test strips were noted. Several precautions had to be followed when using the test strips: (a) the pad had a tendency to rub off the plastic strip depending on the pressure applied, (b) the pad could only be used on smooth surfaces since the plastic was not flexible, (c) the corners of the pad frayed

TABLE 3. Microbial counts per 25 cm<sup>2</sup> obtained by random sampling cleaned and cleaned and sanitized stainless steel food contact surfaces in the Purdue University Pilot Plant and alberene stone laboratory bench tops.

Surface	Test strip (30°C)	Test strip (32°C)	Contact plate (32°C)	Swab (32°C)
	Organisms/25 cm <sup>2</sup> (number) <sup>a</sup>			
Clean stainless <sup>b</sup> steel	10 - 10 <sup>2</sup> (1)	10 - 10 <sup>2</sup> (2)	40 - 90 (16)	25 - 90 (16)
	10 <sup>2</sup> - 10 <sup>3</sup> (19)	10 <sup>2</sup> - 10 <sup>3</sup> (18)	110 - 200 (4)	116 - 140 (4)
Clean alberene stone	0 - 10 (7)	0 - 10 (4)	5 - 10 (8)	2 - 6 (9)
	10 - 10 <sup>2</sup> (3)	10 - 10 <sup>2</sup> (6)	11 - 18 (2)	31 (1)
Clean stainless <sup>c</sup> steel	0 - 10 (1)	0 - 10 (1)		4 - 9 (3)
	10 - 10 <sup>2</sup> (1)	10 - 10 <sup>2</sup> (1)	43 - 100 (8)	46 - 100 (9)
	10 <sup>2</sup> - 10 <sup>3</sup> (10)	10 <sup>2</sup> - 10 <sup>3</sup> (11)	102 - 130 (5)	132 (1)
	10 <sup>3</sup> - 10 <sup>4</sup> (1)			
Cleaned and sanitized stainless steel	0 - 10 (12)	0 - 10 (12)	<1 - 3 (13)	<1 - 1 (12)
	10 - 10 <sup>2</sup> (1)	10 - 10 <sup>2</sup> (1)		20 (1)

<sup>a</sup>Number of samples with the indicated range values.

<sup>b</sup>Data generated from both test strips (30 + 32°C) are significantly different from those of both the contact plates and the swab method at  $\alpha = 0.05$ .

<sup>c</sup>Data from the test strips incubated at both temperatures are significantly different from the swab method for  $\alpha = 0.05$ .

TABLE 4. Microbial counts per 25 cm<sup>2</sup> obtained by randomly sampling an alberene stone laboratory bench top spread with approximately 100 or 1 × 10<sup>4</sup> E. coli or S. aureus per 25 cm<sup>2</sup>.

Sample	Test strip (30°C)	Test strip (32°C)	Contact plate (32°C)	Swab (32°C)
	Organisms/25 cm <sup>2</sup> (number) <sup>a</sup>			
100 <i>E. coli</i> per 25 cm <sup>2</sup>	10 - 10 <sup>2</sup> (6)	10 - 10 <sup>2</sup> (6)	80 - 97 (6)	4 - 6 (3) 10 - 33 (3)
1 × 10 <sup>4</sup> <i>E. coli</i> per 25 cm <sup>2</sup>	10 <sup>2</sup> - 10 <sup>3</sup> (2) 10 <sup>3</sup> - 10 <sup>4</sup> (1) 10 <sup>5</sup> - 10 <sup>6</sup> (5)	10 <sup>2</sup> - 10 <sup>3</sup> (2) 10 <sup>3</sup> - 10 <sup>4</sup> (1) 10 <sup>5</sup> - 10 <sup>6</sup> (5)		1.2 × 10 <sup>3</sup> - 2.3 × 10 <sup>4</sup> (8)
100 <i>S. aureus</i> per 25 cm <sup>2</sup>	0 - 10 (1) 10 - 10 <sup>2</sup> (5)	10 - 10 <sup>2</sup> (6)		70 - 180 (6)
1 × 10 <sup>4</sup> <i>S. aureus</i> per 25 cm <sup>2</sup>	10 <sup>5</sup> - 10 <sup>6</sup> (7) <sup>b</sup>	10 <sup>4</sup> - 10 <sup>5</sup> (2) 10 <sup>5</sup> - 10 <sup>6</sup> (5)		1.9 - 3.6 × 10 <sup>4</sup> (7) <sup>b</sup>

<sup>a</sup>Number of samples with the indicated range values.

<sup>b</sup>Data generated from test strips at 30°C are significantly different than those from the swab method for  $\alpha = 0.05$ .

when they came in contact with the template or other obstacles and (d) lint came off the pad on certain surfaces, such as wood. Because of the problems encountered with wooden surfaces, the data collected from the meat laboratory could not be used.

The interpretation of the color chart, designed to produce dark pinkish-red spots on a pink background, created some concern. Any dark pinkish-red spot was read as being indicative of microbial growth. However, the pink background at times interfered with interpretation, especially for the chart values 3 and 4 (Table 1). At times, arbitrary decisions had to be made on which level of contamination was recorded. Also, the incubation times of 24 or 36 h could result in different chart readings because the longer incubation would result in reading a higher chart value. Therefore, one incubation time should be strictly followed for the samples.

The test strip has several possibilities for use for liquid samples, solid surfaces and rinse methods. It could be compared to the Agar Immersion Plating and Contact slides described by Mossel et al (11) for microbial monitoring of food equipment and environments. If molds are present in the environment, they can be detected by this test strip since they will grow very well on the moistened pad (Fig. 1). Detection of molds after 24 to 36 h could be very useful for sanitation practices in some industries.

### CONCLUSIONS

Use of these test strips in place of the swab of contact plate methods can give a quick indication of the sanitation of food contact areas. If all precautions are followed, they can be used by unskilled plant or laboratory personnel with a minimum expenditure of time, energy or space. Since all results in these experiments were not reproducible, the test strip should be viewed as providing an approximate indication of the sanitation of the processing plant and equipment.

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