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# NOTES

## Simplified Thermonuclease Test for Rapid Identification of *Staphylococcus aureus* Recovered on Agar Media

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A simplified thermonuclease test that identifies colonies of *Staphylococcus aureus* 5 h after recovery on various agar media is described.

Current methods for the detection of *Staphylococcus aureus* in foods consider a positive tube coagulase test as definitive identification of *S. aureus* (1, 10). Confusion and difficulty were reported recently in the interpretation of this confirmatory test (8, 9). In contrast, the thermonuclease test for broth cultures (5) was observed to be easier to interpret in addition to its being relatively inexpensive, simple to perform, and reliable (8, 9). The need for a confirmatory test, however, requires an additional day or two in most diagnostic schemes (1, 10). In efforts to develop a more rapid test, the incorporation of plasma to plating media has been proposed (7). However, in some instances the plate and tube reactions do not correlate (2, 6) and, in addition, the cost of routine testing is considerable.

A selective heat treatment approach was attempted to distinguish thermonucleases produced by *S. aureus* colonies on plates of agar media (4). This involved heating the plates in the oven prior to nuclease determination. The attempt was unsuccessful as a result of the heat-induced distortions of the solid media. The approach is reinvestigated in the present study, using lower heating temperatures. The detection of thermonuclease activity is done by the colony overlay procedure by using toluidine blue O-deoxyribonucleic acid-agar (TDA) medium (5). Preheated plates with grown colonies are overlaid with 10 ml of molten TDA. An *S. aureus* colony is identified by a bright pink zone in 3 h at 37°C. The method is referred to as the simplified thermonuclease (STN) test.

After preliminary experiments with varying time-temperature schedules, the heating of agar plates for 2 h in a 60°C oven (mechanical convection type) was satisfactory. This heating schedule ensured the maintenance of 60°C for at least 60 min at the center of each plate when plates were stacked no more than three deep. When isolates were needed for further reference, colonies were picked before the heat treatment.

Various nuclease-producing bacteria isolated from food were investigated. The 60°C, 2-h heat treatment was adequate to inactivate the nucleases produced by the non-*S. aureus* bacteria growing on Standard Methods agar (Difco). Table 1 shows that all of these bacteria exhibited nuclease activity before the heat treatment, as indicated by the bright pink halos around the colonies 3 h after the TDA overlay. Only the *S. aureus* nucleases resisted the heat treatment. The halo of enzyme activity varied in size among the different strains of *S. aureus*.

The STN test detected thermonucleases produced by the *S. aureus* isolates growing on the following selective media: Baird-Parker medium (Difco), tellurite-polymyxin-egg yolk agar (Difco), tellurite-glycine agar (Difco), and egg yolk-sodium azide agar (3). The pH indicator in the Vogel and Johnson medium (Difco) precluded the use of the STN test. The test was likewise inoperative for identifying *S. aureus* recovered on Staphylococcus medium 110 (Difco). Apparently, the high salt content in the medium inhibited the activity but not the production of the thermonucleases. This is indicated by the detection of the enzyme when agar blocks supporting the colonies were cut from the medium, boiled for 15 min, and transferred to TDA microslides (5).

When four naturally contaminated food samples (ham, a frankfurter, and two cheeses) were examined by the surface-plating technique, the STN test facilitated the identification of every *S. aureus* colony in each countable plate (20 to 200 colonies) of Baird-Parker medium, tellurite-glycine agar, and egg yolk-sodium azide agar. For additional studies colonies were subcultured before heat treatment. There was no problem in seeing that a picked colony was

TABLE 1. *Detection of nucleases produced by bacterial cultures on Standard Methods agar plates before and after heating in a 60°C oven for 2 h*

| Bacteria | No. of isolates | Nuclease activity[a] | |
|---|---|---|---|
| | | Unheated | Heated |
| *Staphylococcus aureus* | 25 | + | + |
| *Staphylococcus* sp. | 5 | + | − |
| *Aeromonas hydrophila* | 3 | + | − |
| *Serratia marcescens* | 2 | + | − |
| *Serratia liquefaciens* | 7 | + | − |
| *Bacillus* sp. | 20 | + | − |

[a] Nuclease detection by overlaying agar cultures with TDA medium (5). Bright pink zones are indicative of nuclease activity after 3 h at 37°C.

thermonuclease positive since there was sufficient enzyme activity remaining in the agar. Only representative colonies were determined with the tube coagulase and thermonuclease tests. From food samples analyzed during the past 12 months, all suspected colonies confirmed as *S. aureus* were positive with the STN test.

The results of the present study indicate that the STN test is an improvement over the tube coagulase and thermonuclease tests in identifying *S. aureus* recovered from food. It facilitates the identification of every isolated colony of *S. aureus* on a recovery meedium in 5 h at minimal cost.

## LITERATURE CITED


1. **Association of Official Analytical Chemists.** 1975. Section 46.040. *In* Methods of analysis of the A.O.A.C., 12th ed. Association of Official Analytical Chemists, Washington, D.C.
2. **Elek, S. D.** 1959. *Staphylococcus pyogenes*. E. and S. Livingston Ltd., London.
3. **Hopton, J.** 1961. A selective medium for the isolation and enumeration of coagulase positive staphylococci from foods. J. Appl. Bacteriol. 24:121–124.
4. **Lachica, R. V. F., and R. H. Deibel.** 1969. Detection of nuclease activity in semisolid and broth cultures. Appl. Microbiol. 18:174–176.
5. **Lachica, R. V. F., C. Genigeorgis, and P. D. Hoeprich.** 1971. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. 21:585–587.
6. **Lotter, L. P., and B. S. M. Horstman.** 1967. Comparison of a tube method and a plate method for detecting coagulase production. Am. J. Clin. Pathol. 48:153–155.
7. **Parisi, J. T., J. N. Baldwin, and M. Sottile.** 1973. Pour-plate method for the detection of coagulase production by *Staphylococcus aureus*. Appl. Microbiol. 25:558–561.
8. **Rayman, M. K., C. E. Park, J. Philpott, and E. C. D. Todd.** 1975. Reassessment of the coagulase and thermostable nuclease tests as means of identifying *Staphylococcus aureus*. Appl. Microbiol. 29:451–454.
9. **Sperber, W. H., and S. R. Tatini.** 1975. Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*. Appl. Microbiol. 29:502–505.
10. **Thatcher, F. S., and D. S. Clark (ed.).** 1968. Microorganisms in foods: their significance and methods of enumeration. University of Toronto Press, Toronto, Canada.