Rapid Assay for In Situ Identification of Coagulase-Positive Staphylococci Recovered by Membrane Filtration from Swimming Pool Water

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A rapid, in situ thermonuclease test that identifies colonies of Staphylococcus aureus among staphylococci isolated from swimming pool water by membrane filtration recovery on various selective and differential media is described.

Staphylococci, including many coagulase-positive strains (Staphylococcus aureus), are a major bacterial contaminant of swimming pools and other recreational waters with a high bather density. Thus, enumeration of either total staphylococci or S. aureus appears to provide a useful index of the level of contamination and of the concurrent effectiveness of filtration and disinfection procedures. One of the major obstacles to the use of these organisms as an indicator of potential infection hazards is lack of a recovery system which is sufficiently selective, differential, and reliable for their enumeration. Although each of the past four editions of Standard Methods for the Examination of Water and Wastewater has included a method for the enumeration of total staphylococci or S. aureus in swimming pool water, these methodologies have retained a tentative status due to lack of data documenting their accuracy and precision. Investigators have used a variety of standard selective and differential media (Staphylococcus 110 medium, Chapman-Stone agar, Mannitol Salt agar, Vogel-Johnson agar, Baird-Parker medium) or modifications of these standard formulations for enumerating total staphylococci or S. aureus. The consensus opinion is that none of these media is either sufficiently selective for staphylococcal species or adequately differential for coagulase-positive S. aureus (2, 4).

Although many characteristics (pigmentation, mannitol fermentation, tellurite reduction, gelatin hydrolysis, lipase production) have been reported to be associated with staphylococcal production, the evidence is strongest for its correlation with thermonuclease production (8-11, 14). Toluidine blue O-DNA-agar (TDA) medium has been extensively used for thermonuclease detection by both agar diffusion and replica-plating methods (7, 13). Recently, Lachica (5, 6) used TDA medium in an overlay procedure for rapid (5 h), in situ identification of food-borne S. aureus recovered by direct plating on agar media. This simplified thermonuclease (STN) test is accomplished by overlaying a preheated (2 h in a 60°C oven) plate with TDA medium and incubating it for 3 h at 37°C. S. aureus colonies are identified by a bright pink zone. The following study reports the use of a modified STN test for identification of S. aureus recovered from swimming pool water by membrane filtration on a variety of selective media.

Staphylococcal species were grown overnight in brain heart infusion broth (Difco Laboratorys). After appropriate dilution, each isolate was membrane filtered through Milli-

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TABLE 1. Detection of TDNase-positive staphylococci isolated from swimming pool water

<table>
<thead>
<tr>
<th>Primary isolation medium</th>
<th>Total no. of isolates</th>
<th>No. of Staphylococcus isolates</th>
<th>No. of TDNase-positive isolates identified by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Modified STN</td>
</tr>
<tr>
<td>Chapman-Stone agar</td>
<td>72</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus 110 medium</td>
<td>93</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>Mannitol Salt agar</td>
<td>102</td>
<td>102</td>
<td>2</td>
</tr>
<tr>
<td>Vogel-Johnson agar</td>
<td>30</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>1% Pyruvate-supplemented Vogel-Johnson agar</td>
<td>62</td>
<td>57</td>
<td>0</td>
</tr>
</tbody>
</table>

* Metachromatic agar diffusion technique of Lachica et al. (7).

were tested for coagulase activity by the direct tube method (4). Additionally, a 20% random sample of TDNase-negative isolates from each of the media used was examined for coagulase activity. Of the 359 isolates recovered from swimming pools, the two TDNase-positive isolates were also shown to be coagulase positive, whereas none of the TDNase-negative isolates proved to be coagulase positive. When these plates were subjected to the modified STN test, the two TDNase- and coagulase-positive isolates were identified by a bright pink halo surrounding the colony. No false-positive reactions were observed (Table 1).

There is still no staphylococcus isolation medium or procedure which is the obvious choice of investigators in the field. Currently available media are directed at isolation of total staphylococci and in some instances have the capacity to identify S. aureus based on a variety of differential characteristics which do not appear to correlate well with coagulase production (4). The results of the present study indicate that the STN test modified for use with membrane filters appears to be both sensitive and specific for in situ identification of S. aureus regardless of the primary isolation medium used. The method is rapid, technically simple, and inexpensive and yields easily read results. These qualities appear to offer an advantage over a method for enumerating S. aureus in swimming pools recently proposed by Havelaar and During (3), which depends on an in situ coagulase reaction on rabbit plasma-fibrinogen agar as the primary isolation medium. Because this method is based on membrane filtration instead of direct plating, it is not limited to use only with media which do not contain high salt levels, indicator systems, or both. Lachica (6) noted that the STN test was inoperative for identifying S. aureus recovered by direct plating on Staphylococcus 110 medium and Vogel-Johnson agar. Since the method described here facilitates identification of every isolated colony of S. aureus on all of the recovery media tested, investigators are no longer limited to the use of highly selective or differential media to recover staphylococci which have already been subjected to environmental stresses resulting in possible physiological injury. Instead, efforts can be turned toward the development of media for improved resuscitation of staphylococcal species isolated from swimming pools without giving up the ability to differentiate S. aureus rapidly and accurately.

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LITERATURE CITED