

Interpretation of the Tube Coagulase Test for Identification of *Staphylococcus aureus*

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The tube coagulase test is a valid means of identifying *Staphylococcus aureus*, provided that only a firm clot that does not move when the tube is tipped is considered a positive reaction. The widely promulgated interpretation that all degrees of clotting in coagulase plasma are a positive identification of *S. aureus* was disproved by the use of other tests such as anaerobic glucose fermentation, thermonuclease production, and lysostaphin sensitivity. It was found that the source of supply of the coagulase plasma is a factor in the occurrence of false-positive coagulase test results. The use of a mixture of pig and rabbit plasma in the tube coagulase test is also discussed.

All current procedures for the detection of *Staphylococcus aureus* in foods regard a positive tube coagulase test as the definitive identification of *S. aureus* (1, 5, 13). Many of these procedures rely on the interpretation of Turner and Schwartz (12) that any degree of clotting in the coagulase plasma should be considered a positive reaction. This interpretation (Fig. 1) has been widely accepted by regulatory agencies, suppliers of coagulase plasmas, and microbiology laboratories.

In the past 5 years we have noted confusion and difficulty in the interpretation of these reactions, especially the 1+ through 3+ reactions. Further investigation into the identity of several organisms causing these reactions revealed that they were not *S. aureus* but micrococci. These observations prompted us to study the validity of the coagulase test in the identification of *S. aureus* in foods.

MATERIALS AND METHODS

Source of strains. A total of 508 strains were studied (Table 1). We isolated about 100 of these from a wide variety of foodstuffs. The remaining strains are from the culture collections of Dack, Casman, and Bennett and were made available to us by R. W. Bennett.

Coagulase reaction. Strains to be tested for coagulase production were incubated overnight in brain heart infusion broth (Difco) at 35 C. The tube coagulase test was performed by adding 0.2 ml of the overnight brain heart infusion broth culture to 0.5 ml of coagulase plasma ethylenediaminetetraacetate (EDTA [Difco]) in a tube (10 by 75 mm). After gentle mixing, the tests were incubated in an air incubator at 35 C and examined after 2, 4, and 24 h. The reactions

were interpreted according to Fig. 1. The 4+ reaction is a very firm, opaque clot which remains in place when the tube is tipped on its side. The typical 2+ and 3+ reactions we encountered are not as opaque as the 4+ reaction and are surrounded by clear plasma.

The porcine-rabbit plasma medium of Julseth and Dudley (6) was used to detect coagulase production of some strains by the formation of a fibrin halo around colonies on an agar plate.

Thermonuclease production. The toluidine blue O-deoxyribonucleic acid agar of Lachica et al. (7) was used. About 20 ml of toluidine blue O-deoxyribonucleic acid agar was poured into a petri dish (15 by 100 mm). Ten to 20 small wells (about 3 mm in diameter) were cut into the agar with a metal syringe needle or a small pipette. Overnight brain heart infusion broth cultures were steamed at 100 C for 15 min, and 1 drop was dispensed into an agar well. The plates were incubated at 35 C and examined for thermonuclease activity after 4 and 24 h. Positive reactions were bright pink zones, usually with a diameter of 1 to 3 cm.

Lysostaphin sensitivity. The sensitivity of the strains to the activity of lysostaphin was determined by the qualitative assay of Lachica et al. (8). The lysostaphin was obtained from Schwarz/Mann Div. of Becton, Dickinson & Co., Orangeburg, N.Y. The results were recorded after 2, 4, and 24 h of incubation at 35 C.

Carbohydrate fermentations. The ability of the strains to ferment glucose and mannitol anaerobically was determined by using the media and procedures recommended by the Subcommittee on Taxonomy of Staphylococci and Micrococci (10). Anaerobiosis was achieved in GasPak jars (BBL). Results were recorded after 5 days of incubation at 35 C.

Enterotoxin production. The ability of a few of the strains to produce enterotoxins A through F was determined by the microslide assay of Casman and Bennett (3).

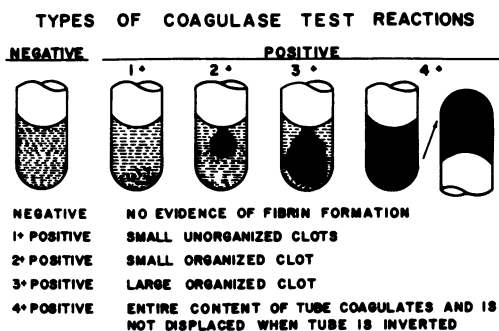


FIG. 1. Interpretation of the coagulase test as proposed by Turner and Schwartz (12).

TABLE 1. Source of isolation and number of strains

Source	No.
Frozen foods (not incriminated in food poisoning)	144
Throat and nose of healthy humans	111
Mastitic cows	37
Foods incriminated in food poisonings; general laboratory isolates	216

RESULTS

The identification of the 508 strains is presented in Table 2. The 439 strains which gave a 4+ coagulase reaction also produced thermonuclease and were sensitive to lysostaphin. On the basis of these tests, these strains are indeed *S. aureus*. Ninety-seven of the strains in this group were tested for glucose and mannitol fermentation. Sixty-seven of the strains fermented both sugars, whereas the remaining 30 fermented glucose but not mannitol. It does not appear that mannitol fermentation is a reliable characteristic in the identification of *S. aureus*, an observation reported by other researchers (4, 14, 15).

A group of 69 strains gave a 2+ or 3+ coagulase reaction and would have been identified as *S. aureus* according to Fig. 1. However, these were found not to be *S. aureus* on the basis of other characteristics (Table 2). All of the strains in this group were catalase-positive cocci. None of these strains produced thermonuclease. Within this group, 49 strains were not sensitive to lysostaphin and also did not ferment glucose or mannitol. These most likely belong to the genus *Micrococcus*. The remaining 20 strains fermented glucose but not mannitol and were fully or partially sensitive to lysostaphin. These are most likely *S. epidermidis*. Most of these 69 strains could also be differentiated from *S. aureus* on the basis of their cell diameter (1 to 1.5 μm). Furthermore,

none of these 69 strains produced a fibrin halo on the porcine-rabbit plasma medium of Julseth and Dudley (6).

About one-third of the 439 *S. aureus* strains are known to produce one or more types of enterotoxin. Examination of 15 of the 69 non-*S. aureus* strains revealed that they did not produce enterotoxins A through F.

During this study the tube coagulase reaction of several of the strains was also tested in pig coagulase plasma EDTA and in coagulase plasma EDTA (BBL). The results are presented in Table 3. Ten *S. aureus* strains give a 4+ reaction in all three plasmas. An additional 96 strains were 4+ in pig and Difco rabbit plasma, but were not tested in BBL rabbit plasma. However, 55 non-*S. aureus* strains which produced a 2+ to 3+ clot in Difco rabbit plasma were negative in pig and BBL rabbit plasma. The observed difference in coagulase reaction between the various plasmas undoubt-

TABLE 2. Identification of strains by the coagulase reaction and other biochemical characteristics

Biochemical characteristic	Identification of strains				
	<i>S. aureus</i>			Non- <i>S. aureus</i>	
	67 ^a	30	342	49 ^a	20
Coagulase reaction ^b	4+	4+	4+	2-3+	2-3+
Thermonuclease production	+	+	+	-	-
Lysostaphin sensitivity	+	+	+	-	±
Anaerobic fermentation					
Glucose	+	+	NT ^c	-	+
Mannitol	+	-	NT	-	-

^a Number of strains.

^b Using coagulase plasma EDTA (Difco).

^c NT, Not tested.

TABLE 3. Effect of various coagulase plasmas (with EDTA) on the coagulase test reaction

No. of strains	Coagulase test reaction		
	Rabbit plasma (Difco)	Rabbit plasma (BBL)	Pig plasma
<i>S. aureus</i>			
10	4+	4+	4+
96	4+	NT ^a	4+
Non- <i>S. aureus</i>			
55	2-3+	-	-

^a NT, Not tested.

edly contributes to the difficulty and confusion in interpreting the test reaction.

The 2+ and 3+ reactions in Difco rabbit plasma could be eliminated by adding about 30% pig plasma to it. This was verified on only a few of the non-*S. aureus* strains.

These results do demonstrate that the tube coagulase test is a valid means of identifying *S. aureus*. Only a complete, firm clot which does not move when the tube is tipped on its side can be considered a positive coagulase test for the purpose of identifying *S. aureus*. Until workers become proficient and confident in this interpretation and reagents are standardized, the identity of strains which yield a 1+ to 3+ coagulase test result should be confirmed by other tests such as anaerobic glucose fermentation, lysostaphin sensitivity, and thermonuclease production.

DISCUSSION

In view of the coagulase reactions reported in Table 3, it is likely that the 2+ and 3+ reactions found in the Difco plasma are not coagulase reactions at all, but rather a nonspecific reaction between the plasma and some component of the cells and/or spent medium. We found these 2+ and 3+ reactions would increase almost to the point of being a 4+ reaction when aged (several weeks) rehydrated plasma was used. As a consequence, we do not store rehydrated coagulase plasma more than 5 days under refrigerated conditions before use.

The results in Table 3 were attained by using dozens of lots of Difco plasma, but we have used only two lots of BBL plasma and two lots of pig plasma. It is possible that by using still other lots of Difco plasma and other sources of plasma that the reverse might be true, i.e., a strain giving a negative reaction in Difco plasma might give a nonspecific 2+ or 3+ reaction in another plasma. Furthermore, the nonspecific 2+ and 3+ reactions in Difco plasma should not create concern, since the above results have shown that only a complete (4+) clot can be considered a positive reaction in the identification of *S. aureus* when only the coagulase test is used as a definitive criterion. In this regard all three plasmas appeared comparable.

With regard to the research of Turner and Schwartz (12), it must be pointed out that their work was performed chiefly with another type of plasma (human) and with strains isolated from clinical sources. Their interpretation of the coagulase test (Fig. 1) is probably valid for their work and may have been invalidated by the many subsequent changes in the coagulase

plasmas and the methods used to isolate staphylococci from foods. It is not to their discredit that others have appropriated their interpretation for vastly different reagents and methods.

The 69 2+ and 3+ strains represent, for the most part, isolations from separate food products or ingredients. All of the strains we isolated, and presumably a large proportion of those strains received from R. W. Bennett, had passed through a routine procedure for the isolation of *S. aureus* from foods, such as incubation in Trypticase soy broth with 10% NaCl followed by plating on a selective medium or by direct plating on a selective medium such as Baird-Parker agar, Vogel-Johnson agar, etc. However, quite different isolation procedures are used in the clinical laboratory. From some indications in the literature (2) and contact with clinicians (R. V. F. Lachica, personal communication), it appears possible that acceptance of only a 4+ coagulase test reaction as definitive identification of *S. aureus* may also hold true in the clinical laboratory.

All coagulase reactions were observed after 2, 4, and 24 h of incubation at 35 C. With 99% of the strains, the reaction was complete within 2 h, the remainder reaching completion within 4 h. Therefore, 4 h would be an ample incubation time for a coagulase test performed in the above manner. Since staphylococci produce proteases that may dissolve the fibrin clot, the test should be read periodically so that false-negative reactions are avoided. We recommend that the test not be unduly agitated during this time as this can cause the clot to shrink, also yielding a false-negative result.

Several researchers have reported that a mixture of pig and rabbit plasmas in plating media gives an optimum combination of coagulase-reacting factors for detection of coagulase production by *S. aureus* colonies (6, 9). On a limited number of non-*S. aureus* strains, we found that the 2+ and 3+ tube coagulase reactions were negative when about 30% or more pig plasma was added to the Difco (rabbit) plasma. This mixture of pig and rabbit plasmas may prove to be better than either plasma alone for use in the tube coagulase test. Though not completely understood, the clotting of plasma in the coagulase test seems to involve the conversion of fibrinogen to fibrin by the enzyme complex, coagulase plus coagulase-reacting factor (11). If the plasma used in the tube coagulase test is to be standardized, considerable attention should be devoted to assuring adequate amounts of coagulase-reacting factor, the

quantity of which varies with the source of plasma, rabbit, bovine, pig, human, etc., optimal amounts of fibrinogen, and minimizing the influence of inhibitory factors present in the plasma (9, 11).

We do not advocate replacing the tube coagulase test with other tests such as thermonuclease production, lysostaphin sensitivity, or carbohydrate fermentations. Assuming an organism is shown to have the proper morphology and to be catalase positive, the 4+ coagulase test reaction should stand alone as the definitive identification of *S. aureus*. The supplemental test used herein can be of assistance in identifying strains which do not give a definitive (4+) coagulase test reaction.

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