Acta vet. scand. 1991, *32*, 543–549.

Evaluation of Three Slide Agglutination Tests for Rapid Identification of *Staphylococcus aureus*

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Niskanen A., H. Korkeala, M. Manninen, M. Vuento and P. Kuusela: Evaluation of three agglutination tests for rapid identification of *Staphylococcus aureus*. Acta vet. scand. 1991, 32, 543–549. – Three slide agglutination tests for identification of *Staphylococcus aureus* were compared. The agglutination tests used for evaluation were Staphaurex (Wellcome Diagnostics), Staphyslide-Test (BioMerieux), and ANI *S. aureus* TEST (Ani Biotech Oy). A total of 347 isolates were analyzed, including 288 strains of *S. aureus*, 49 of *S. epidermis*, 11 of *S. intermedius*, 12 strains of other staphylococci and 14 non-staphylococcal strains. One hundred of the *S. aureus* strains were isolates from cases of food poisoning, 129 from mastitis and 59 from other clinical cases. The sensitivities of the tests were also compared using diluted suspensions of *S. aureus* strains and with purified Protein A dilutions.

The results showed that the sensitivities of the tests were 98.6 %, 97.9 % and 99.0 % for Staphaurex, Staphyslide-test and ANI *S. aureus* TEST, respectively. The specificities were 100 % for the Staphyslide test and 98.8 % for both the ANI *S. aureus* TEST and the Staphaurex test. The sensitivities measured with diluted *S. aureus* strain suspensions and Protein A solutions were equal with the Staphaurex and ANI *S. aureus* TEST.

All the agglutination tests studied proved to be practical, easy to use and accurate for the rapid identification of *S. aureus* strains from culture isolates.

latex agglutination test; food poisoning; mastitis.

Introduction

Staphylococcus aureus is a widely distributed microorganism causing serious infections in humans and animals which can be very difficult to treat (*Jeljaszevicz et al.* 1983). It also produces enterotoxins, which are one of the most frequent causes of food poisoning outbreaks (*Niskanen* 1977). An enterotoxin-like substance is also responsible for toxic shock syndrome (*Bergdoll et al.* 1981).

The isolation of *S. aureus* from clinical samples is carried out by cultivation of a clinical specimen on blood agar plates containing 5 % sterile defibrinated sheep blood or on a suitable selective medium (*Kloos & Schleifer* 1986). In the case of food samples the isolation of *S. aureus* is usually performed using selective culture media. When heat-treated, frozen, desiccated or otherwise stressed bacteria are to be identified a primary enrichment procedure is recommended.

The conventional identification of *S. aureus* is based on colony morphology, pigment production, anaerobic growth, acid production from different carbohydrates and production of coagulase, hemolysins and thermonuclease. Many other physiological and

biochemical properties have also been used for identification purposes (*Kloos & Schleifer* 1986).

Coagulase is produced by almost all strains of *S. aureus*. The detection of this enzyme(s) is therefore important in the routine identification of *S. aureus*. The tube test for coagulase detection should be performed under carefully standardized conditions, which makes it rather laborious and time consuming especially when testing only a small number of samples. The tube test has therefore generally been replaced with slide tests detecting clumping factor, present in the cell wall of *S. aureus*.

Practically all *S. aureus* strains contain protein A in their cell wall (*Gronvall et al.* 1972, *Winblad & Ericson* 1973). This protein binds to the Fc-fragment of immunoglobulin G, for which reason some identification tests also contain protein A-binding IgG in the reagent.

The aim of this study was to compare the sensitivities and specificities of 3 commercially available agglutination tests for identification of *S. aureus*,

The new card agglutination test ANI *S. aureus* TEST (Ani Biotech Oy, Helsinki, Finland), which utilizes dried reagents, was compared with the conventional latex agglutination test Staphaurex (Wellcome Diagnostics) and with the hemagglutination test Staphyslide (BioMerieux).

Materials and methods

Organism

A total of 374 isolates were obtained from the reference laboratories detailed in Tables 1 and 5. All the strains except 14 non-staphylococcal strains were identified in the reference laboratories as *Staphylococci* (Table 1) on the basis of the tube coagulase test, thermonuclease production and biochemical and morphological characteristics. Some questionable strains were also analyzed with the API-test. Before the strains were used in the tests they were cultivated on sheep blood agar plates for 18 to 24 h at 37°C.

Twelve *S. aureus* strains originally isolated from outbreaks of food poisoning were also cultivated on Staphylococcus medium no. 110 (Difco) and 5 *S. aureus* strains on Baird-Parker agar (Oxoid) to check the influence of selective effects of the agars on the reactivity of the strains in the tests.

Dilution test

Four *S. aureus* strains (2 rapidly clumping strains 511 and 714 and 2 agglutinating strains 68 and 69) were cultivated on sheep blood agar plates overnight at 37°C. Cells from 10 well-formed colonies were transferred to 1.0 ml of phosphate buffered saline (PBS, pH 7.4) and suspended thoroughly to form stock suspensions (1/1) which were diluted (1/5–1/20) in PBS and analyzed with the tests following the manufacturers' instructions.

Protein A dilutions

Purified protein A (Sigma) was diluted in phosphate buffered saline (PBS, pH 7.4) to give concentrations of 100, 10, 1 and 0.1 mg/ml. One drop (30 µl) of each dilution was mixed with the bacterial suspensions and the reaction times before the appearance of the agglutination reaction were measured.

Commercial reagents and performance of the tests

Staphaurex (Wellcome) and ANI *S. aureus* TEST (Ani Biotech Oy) are latex agglutination tests, in which latex particles are coated with fibrinogen for detection of clumping factor and with immunoglobulin G for the detection of protein A.

In the ANI *S. aureus* TEST the reagent is

applied to dried reagent dots on the card. In the Staphaurex test the reagent is in liquid form.

Staphyslide (BioMerieux) is a hemagglutination test including test reagent (sheep erythrocytes sensitized with fibrinogen) and control reagent (unsensitized sheep erythrocytes).

The comparative tests were performed by following the instructions of the manufacturers.

Results

A total of 374 strains were studied (Table 1). Of the 288 *S. aureus* strains previously identified in the Food Research Laboratory, Technical Research Centre of Finland, using routine methods, 285 were positive in the ANI *S. aureus* TEST and 284 and 282 in the Staphaurex and Staphyslide tests, respectively. The sources of different *S. aureus* strains and the reactions of strains in different groups are presented in Table 5. All the tests worked similarly with strains from clinical (human origin) and mastitic (cow origin) samples. Of the food poisoning strains Staphyslide failed to react with 4, Staphaurex with 2 and ANI *S. aureus* TEST with 1 strain.

All the 49 *Staphylococcus epidermidis* strains were negative in the tests used. One *S. intermedius* strain of 11 studied was positive in the ANI *S. aureus* TEST and Staphaurex test but negative in the Staphyslide test. Other staphylococcal strains, including 1 *S. saprophyticus*, 1 *S. xylosus* and 10 *S. simulans* strains, were all negative in the tests studied. The non-staphylococcal strains *Bacillus cereus, Clostridium perfringens* and 12 *Streptococcus* species were all correctly detected as negative.

The *S. aureus* strains which gave negative results with 1 or 2 agglutination tests were also identified with the API test. All were detected as *S. aureus*. The 1 *S. intermedius* strain which was positive in the ANI *S. aureus* TEST and Staphaurex test and also the other 10 *S. intermedius* strains were also tested with the API test and shown to be *S. intermedius* by this test.

The assay parameters were calculated for each test on the basis of the results displayed in Table 1. From these calculations (Table 2) it can be seen that the sensitivities of the different tests were practically equal. The specificities of the Staphaurex and ANI *S. aureus* TESTs were 98.8 %, whereas that of the Staphyslide test was 100 %. The best positive predictive value was 100 % for Staphyslide but this gave the lowest negative

Table 1. A comparative study of *Staphylococcus aureus* and other bacterial strains with 3 different agglutination tests.

Bacterial strains studied	Number of strains	Reaction of different agglutination tests					
		ANI *S. aureus* TEST		Staphaurex		Staphyslide	
		+	−	+	−	+	−
S. aureus[a]	288	285	3	284	4	282	6
S. epidermidis	49	0	49	0	49	0	49
S. intermedius	11	1	10	1	10	0	11
Other staphylococci	12	0	12	0	12	0	12
Non staphylococci	14	0	14	0	14	0	14
Total	374						

[a] All the strains used, were identified in approved laboratories.

Table 2. Comparison ot test results of different staphylococcal and other bacterial strains with different agglutination tests.

Parameter	ANI *S. aureus* TEST	Staphaurex	Staphyslide
Sensitivity:	99.0 %	98,6 %	97,9 %
Specificity:	98.8 %	98.8 %	100 %
Efficiency:	98.9 %	98.1 %	98.4 %

predictive value, 93.5 %. The overall efficiency was best for the ANI *S. aureus* TEST (98.9 %) and slightly lower for the Staphyslide and Staphaurex tests (98.4 and 98.1 % respectively).

The sensitivities of the 2 latex agglutination tests, ANI *S. aureus* TEST and Staphaurex, were compared using the dilutions of 2 intensively clumping strains and with typical agglutinating strains (Table 3). The results revealed no differences between the 2 assays (Table 3).

Table 3. Sensitivity of 2 different latex agglutination tests.

Test	Strain*	Dilution studied			
		1/1	1/5	1/10	1/20
ANI *S. aureus* TEST	714	+[a]	+	+	–[b]
	511	+	+	+	–
	68	+	+	–	–
	69	+	–	–	–
Staphaurex	714	+	+	+	–
	511	+	+	+	–
	68	+	+	–	–
	69	+	–	–	–

[a] + reaction detected in 30 sec as mentioned in the instructions of use.

[b] – reaction or weak positive reaction in more than 30 sec.

* *S. aureus* strains cultivated on sheep blood agar plates and 5–6 colonies picked to 500 µl of phosphate buffered saline (PBS) giving approximately 10 cfu/ml in first 1/1 dilution. Strains 714 and 511: strong clumping reaction. Strains 68 and 69: ordinary aglutination reaction.

Table 4. Comparison of minimum detectable levels and reaction times of Protein A with ANI *S. aureus* TEST and Staphaurex test.

Test	Protein A concentration (µg/ml)[a]			
	100	10	1.0	0.1
ANI *S. aureus* TEST	+< 10 s	+15 s	+30 s	±3 min
Staphaurex	–3 min	±3 min	+1 min	±3 min

[a] Protein A (Sigma) diluted to Phosphate buffered saline (PBS), pH 7.4.

+ clear agglutination; ± weak agglutination; – no visible agglutination.

The minimum levels of purified Protein A detected with the different tests are presented in Table 4. The results showed that ANI *S. aureus* TEST could detect 1.0 µg/ml of Protein A within 30 sec. The same concentration reacted with Staphaurex in 1 min. The higher concentrations of Protein A gave faster and stronger reactions in the ANI *S. aureus* TEST but the Staphaurex test gave an indefinite reaction in 3 min with the Protein A concentration of 10 µg/ml and at a Protein A concentration of 100 µg/ml the test was negative.

All the strains cultivated on selective agars normally used in food microbiology gave clear positive reactions in all the tests studied.

Discussion

The results show that the agglutination tests have sufficient sensitivity to detect *S. aureus*. The sensitivities of the different tests were practically equal. Similar results have also been found in earlier studies (*Essers & Radebold* 1980, *Baker et al.* 1985, *Berke & Tilton* 1986, *Brown* 1986). The appearance of the agglutination reactions showed considerable variation. The most typical reaction is total clumping of the reagent when mixed

Table 5. Performance of 3 agglutination tests in the detection of *S. aureus* strains isolated from different sources.

S. aureus strains isolated from	Number of strains	Agglutination tests					
		ANI *S. aureus* TEST		Staphaurex		Staphyslide	
		+	−	+	−	+	−
Food poisoning cases[a]	100	99	1	98	2	97	4
Mastitis cases[b]	129	128	1	128	1	128	1
Clinical isolates[c]	59	58	1	58	1	58	1
Total	228	285	3	284	4	282	6

[a] *S. aureus* strains isolated from poisoning outbreak in Technical Research Centre of Finland, Food Research Laboratory, Espoo, Finland.

[b] *S. aureus* strains isolated from mastitis cases of cows in State Veterinary Clinical Institute, Kuopio Regional Laboratory, Kuopio, Finland.

[c] *S. aureus* strains isolated from humans in Helsinki University, Department of Bacteriology and Immunology.

with *S. aureus* strains. This type of reaction was very clear with the ANI *S. aureus* TEST and with the Staphyslide test. The Staphyslide test, however, was more difficult to interpret. Some *S. aureus* strains gave a finely particulated agglutination reaction, which was practically identical with the reaction with purified Protein A (not shown).

Because some *S. aureus* strains completely or partly lack Protein A in their cell walls (*Ruane et al.* 1986), the reagent used for identification of *S. aureus* should contain both fibrinogen and immunoglobulin G as recommended by *Essers & Radebold* (1980). It has also been shown that oxacillin resistant *S. aureus* strains which were resistant to trimethoprim-sulfamethoxazole and rifampicin could not be detected adequately with agglutination tests (*Ruane et al.* 1986).

The different results of the ANI *S. aureus* TEST and the Staphaurex test in detecting different concentrations of purified Protein A may have been due to different coating concentrations of the latex particles with IgG molecules. The detection limits of Protein A with both tests were however similar, ca. 10 µg/ml.

The specificity of the tests was very good. Although the number of negative strains was relatively small, only 86, the result was in agreement with those of earlier studies (*Baker et al.* 1985, *Berke & Tilton* 1986, *Flesland* 1987). Only 1 *S. intermedius* strain was positive in the ANI *S. aureus* TEST and the Staphaurex test. The Staphyslide did not react with this strain. According to Bergeys manual (*Kloos & Schleifer* 1986) 11–89 % of *S. intermedius* strains may contain the clumping factor. However, *S. intermedius* lacks Protein A in the cell wall (*Kloos & Schleifer* 1986), thus providing a theoretical possibility of distinguishing between these 2 bacterial species. In this study 1 *S. intermedius* strain gave a positive result in the latex agglutination test but it was negative in the hemagglutination test. Because it is obvious that this strain contained the clumping factor, the difference may have been due to the different sensitivities of the tests.

The slime production of coagulase-negative staphylococci, particularly *S. epidermidis*, may cause difficulties for an inexperienced person to interpretate the test results. How-

ever, with some experience the clumped bacterial aggregates are easily distinguished from the true clumping reaction of the latex particles. The distinction is more clear with coloured latex than with white latex reagent. The use of selective culture media for detection of *S. aureus* is common in food microbiology. The media most often used, such as Staphylococcus medium no. 110 (Difco) and Baird Parker medium (Oxoid), have no effects on the specificity and sensitivity of the tests.

The use of conventional coagulase tests is laborious and time consuming. Furthermore, coagulase positive animal pathogen strains of *S. intermedius* and *S. hyicus* are also known. Even if the coagulase test is carried out under carefully standardized conditions, some coagulase-negative strains may clot plasmas by producing proteases (pseudocoagulase) (*Kloos & Schleifer* 1986). If citrated plasmas are used, the citrate utilizing bacteria (e.g. fecal streptococci and some members of the *Enterobacteriacea*) may cause false positive reactions (*Kloos & Schleifer* 1986). Therefore it would appear to be most practical and also reliable to use slide agglutination tests for rapid identification of *S. aureus* from culture media in routine work in clinical and food microbiological laboratories. The new card agglutination test is accurate, easy to use and has storable, predosed reagent dots.

Acknowledgement

We thank Dr. Mirja Hedlund of the State Veterinary Medical Institute, Kuopio regional laboratory for providing the mastitis staphylococcal strains. We also thank Mrs. Mirja Eerikäinen of the College of Veterinary Medicine, Department of Food and Environmental Hygiene and Mrs. Helena Tulenheimo of the Technical Research Centre, Food Research Laboratory for technical assistance.

References

Baker JS, Bormann MA, Boudreau DH: Evaluation of various rapid agglutination methods for the identification of *Staphylococcus aureus.* J. clin. Microbiol. 1985, *21,* 726–729.

Bergdoll MS, Cross BA, Reiser RF, Robbins RN, Davis JP: A new staphylococcal enterotoxin, enterotoxin F, associated with toxic shock syn drome in *Staphylococcus aureus* isolates. Lancet 1981, *i,* 1017–1021.

Berke A, Tilton RC: Evaluation of rapid coagulase methods for the identification of *Staphylococcus aureus.* J. clin. Microbiol. 1986, *23,* 916–919.

Brown WJ: Comparison of a yellow latex reagent with other agglutination methods for the identification of *Staphylococcus aureus.* J. clin. Microbiol. 1986, *23,* 640–642.

Essers L, Radebold K: Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. J. clin. Microbiol. 1980, *12,* 641–642.

Flesland O: Comparison of two agglutination tests for differentiation between coagulase positive and coagulase negative staphylococci. Acta path. microbiol. scand. Sect. B 1987, *95,* 83–84.

Gronvall G, Holmberg O, Ripa T: Protein A in *Staphylococcus aureus* strains of human and bovine origin. Acta pathol. microbiol. scand. Sect. B 1972, *80,* 735–742.

Jeljaszevicz J, Switalski LM, Adlam C: Staphylococci and staphylococcal infections, Vol. 2. In: Easmon CSF, Adlam C (Eds.). Academic Press, London 1983, 525–557.

Kloos WE, Schleifer KH: Genus IV. *Staphylococcus.* In: Holt JG (Ed.): Bergeys Manual of Systematic Bacteriology vol 2. Williams & Wilkins, Baltimore 1986, 1013–1035.

Niskanen A: Staphylococcal enterotoxins and food poisoning. Production, properties and detection of enterotoxins. Technical Research Centre of Finland, Material and Processing Technology, Publication no. 19, 1977, 83 p.

Ruane PJ, Morgan MA, Citron DM, Mulligan ME: Failure of rapid agglutination methods to

detect Oxacillin-resistant *Staphylococcus aureus*. J. clin. Microbiol. 1986, *24*, 490–492.

Winblad S, Ericson C: Sensitized sheep red cells as a reactant for *Staphylococcus aureus* protein A. Acta pathol. microbial. scand. Sect. B 1973, *81*, 150–156.

Sammanfattning

Bedömning av tre "slide" agglutinationstester för snabb identifiering av Staphylococcus aureus. Tre slide agglutinationstester för identifiering av *Staphylococcus aureus* jämfördes. Följande 3 tester undersöktes: Staphaurex (Wellcome Diagnostics), Staphyslide-Test (Bio Merieux) och ANI *S. aureus* Test (Ani Biotech Oy).

Sammanlagt 347 isolationer analyserades innefattande 288 stammar av *S. aureus*, 49 av *S. epidermis*, och 11 av *S. intermedius*, 12 stammar av andra staphylococci och 14 non-staphylococci stammar. Hundrade av *S. aureus* stammarna var isolerade ur matförgiftningsfall, 129 ur mastitis och 59 ur andra kliniska fall. Även testernas känslighet jämfördes genom att använda diluterade suspensioner av *S. aureus* stammar och med renade Protein A dilutioner.

Resultaten visade att testkänsligheten var 98,6 %, 97,9 % och 99,0 % för Staphaurex, Staphyslide-Test och ANI *S. aureus* Test i nämnd ordning. Specifiteten för Staphyslide Testet var 100 % och för ANI *S. aureus* Testet 98,8 % vardera. Känslighet mätt med diluterade suspensioner av *S. aureus* stammar och Protein A lösningar var lika för Staphaurex och ANI *S. aureus* Test.

Alla undersökta tester visade sig vara praktiska, lätta att använda och exakta för snabb identifiering av *S. aureus* stammar i odlingsprov.

(Received January 22, 1990; accepted April 19, 1991).

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