Acta vet. scand. 1991, *32*, 155–161.

Comparison of Three Commercial Rapid Agglutination Test Kits for Identification of Coagulase Positive Staphylococci from Foods and Animals

By *I.-J. R. Holme, O. Rosef* and *S. Ewald*

Department of Food Hygiene, Norwegian College of Veterinary Medicine, Oslo, and Food Inspection Service in Skien, Nome and Siljan, Norway.

Holme, I.-J. R., O. Rosef and S. Ewald: Comparison of three commercial rapid agglutination test kits for identification of coagulase positive staphylococci from foods and animals. Acta vet. scand. 1991, 32, 155–161. – Three rapid agglutination assays for the identification of *Staphylococcus aureus* Monostaph (Bionor A/S, Skien, Norway), Staphyslide-Test (BioMerieux, Lyon, France) and Staph-Rapid-Test (Roche, Basel, Switzerland), were compared. A total of 104 Gram-positive, catalase positive cocci were tested: Nineteen *Staphylococcus* reference strains comprising 15 spp. (4 strains were coagulase positive), and 7 *Micrococcus* reference strains comprising 4 spp.; 22 food isolates comprising 13 *S. aureus,* 8 coagulase positive *Staphylococcus* spp., and 1 *Micrococcus* sp.; 56 animal isolates comprising 11 *S. aureus,* 9 *S. hyicus* subsp. *hyicus,* 2 *S. intermedius,* 15 coagulase positive and 19 coagulase negative *Staphylococcus* spp. Totally 54 strains were coagulase positive.

Considering agglutination of a coagulase positive strain as a correct identification, Monostaph, Staph-Rapid-Test, and Staphyslide-Test correctly identified 52 (96.3 %), 47 (87.0 %) and 48 (89.0 %) of the coagulase positive staphylococci, respectively. Monostaph, Staph-Rapid-Test and Staphyslide-Test showed 1 (2.0 %), 4 (8.0 %) and 4 (8.0 %) false positive reactions respectively. Monostaph, Staph-Rapid-Test and Staphyslide-Test gave 0 (0.0 %), 6 (5.8 %) and 7 (6.7 %) non-interpretable reactions, respectively.

Monostaph may be a good alternative to the tube-coagulase test for rapid and reliable identification of coagulase positive staphylococci from both food and veterinary sources. However, false negative reactions may occur with coagulase positive strains of *S. hyicus* subsp. *hyicus* and *S. intermedius.*

Staphylococcus aureus; food isolates; animal isolates.

Introduction

Staphylococcus aureus can be identified by the tube test for coagulase (TC) or the slide agglutination test for clumping factor (CF) (*Kloos & Schleifer* 1975). The TC test is based on the free coagulase enzyme which clots citrate- or EDTA-stabilized plasma by splitting fibrinogen to fibrin (*Barry et al.* 1973). The CF test is based on a fibrinogen binding protein (CF) on the cell surface of *S. aureus.* Combined with the fibrinogen of certain animals, CF causes clumping (agglutination) of the cells when mixed on a slide. CF was initially named bound coagulase. (Dutchie 1955, *Usui* 1986). Recently *Bodén & Flock* (1989) have demonstrated coagulase present on the cell surface of *S. aureus.* It was concluded that this cell-bound coagu-

lase has affinity for fibrinogen and thus is responsible for the clumping of staphylococci in fibrinogen.

The TC test is regarded as the reference method for discriminating *S. aureus* from other members of the *Micrococcaceae* family (*Anon.* 1965). This method is reliable if carefully performed. However, strains of *S. aureus* giving a weak reaction may easily be interpreted as coagulase negative in a busy routine laboratory (*Dibb et al.* 1983). Furthermore, false positive and negative reactions may occur (*Jeljaszewicz et al.* 1983), and the TC test requires up to 24 h incubation. Therefore there is a need for more rapid identification systems for *S. aureus.*

CF has been found in approximately 97 % of human *S. aureus* isolates (*Jeljaszewiez et al.* 1983). *S. aureus* isolated from cows, swine, sheep and hares were 100 % CF positive. *S. aureus* biotypes E and F (now *S. intermedius*) isolated from dogs were 57 % CF positive, horse 17 %, mink 30 %, and fox 40 % CF-positive. Strains from pigeons were all CF negative (*Hajek & Marsalek* 1976).

Another characteristic of *S. aureus* is the presence of protein A, which is found on the cell surface of 90–99 % of human *S. aureus* strains (*Forsgren* 1970), 48–100 % of bovine *S. aureus* strains (*Kronvall et al.* 1972, *Jonsson & Holmberg* 1981), and 7 % of canine *S. intermedius* strains (*Cox et al.* 1986). Protein A binds unspecifically to IgG of several species, including man and rabbit.

Recently, slide-agglutination tests have been developed for rapid differentiation between *S. aureus* and other species within the *Micrococcaceae.* These methods are based on sheep erythrocytes or latex particles, coated with fibrinogen or fibrinogen and IgG. Agglutination occurs in the presence of the specific cell wall components of *S. aureus,* CF and protein A (*Essers & Radebold* 1980).

The purpose of this study was to compare 3 commercially available slide agglutination tests for identification of coagulase positive staphylococci isolated from animals and foods. Monostaph (Bionor A/S, Skien, Norway) has proved to be a reliable and rapid test for identification of *S. aureus* from human clinical isolates (*Flesland* 1987). Staph-Rapid-Test (Roche, Basel, Switzerland) and Staphyslide-Test (BioMerieux, Lyon, France) have been evaluated and recommended by *Veers-Pothoff et al.* (1987) for identification of *S. aureus* from human clinical isolates. Staphyslide-Test has proved to be a usefull alternative to the TC test for the characterization of staphylococci isolated from foods (*Schulze & Wernery* 1986). *Orsi et al.* (1989) found that Staph-Rapid-Test and Staphyslide-Test lacked in sensitivity and specificity.

Materials and methods

Bacterial strains

A total of 104 Gram positive, catalase positive cocci were tested.

The following reference strains were used: CCM 2469 *S. aureus,* CCM 2734 *S. capitis,* CCM 3573 *S. caprae,* 2736 *S. cohnii,* CCM 901 *S. epidermidis,* CCM 3512 *S. gallinarum,* CCM 2737 *S. haemolyticus,* CCM 3474 *S. hominis,* CCM 2995 *S. hyicus* subsp. *chromogenes,* CCM 5739, CFDD, 478 and 5066 *S. intermedius,* CCM 2686 *S. saprophyticus,* CCM 3472 *S. sciuri,* ATCC 27848 and S. 217 *S. simulans,* CCM 2730 *S. warneri,* CCM 2438 *S. xylosus,* ATCC 27570 and TM 92 *M. kristinae,* ATCC 4698 and WK 2 *M. luteus,* MAW 332 *M. lylae,* CCM 314 *M. sedentarius.*

Food isolates: Twenty two strains were isolated from foods. Thirteen were identified as *S. aureus,* according to the biochemical

tests previously described (*Ewald* 1987). Nine strains were only tested for coagulase. Eight were positive and 1 negative. The coagulase negative strain did not ferment glucose anaerobically and was thus considered as *Micrococcus* sp.

Animal isolates: Fifty six strains were isolated from animals. Eleven *S. aureus,* 9 *S. hyicus* subsp. *hyicus* (all coagulase negative) and 2 *S. intermedius* strains were kindly supplied by the Department of Microbiology and Immunology, Norwegian College of Veterinary Medicine. Thirty four of the strains from animals were only tested by the coagulase reaction and consisted of 15 coagulase positive and 19 coagulase negative strains. The coagulase negative strains fermented glucose anaerobically, and were thus considered as *Staphylococcus* spp.

Agglutination tests

The strains were grown aerobically on blood agar plates for 18–24 h before testing by the following slide agglutination tests: (i) Staphyslide-Test (BioMerieux, Lyon, France), which consists of sheep erythrocytes coated with fibrinogen. Non-sensitized sheep erythrocytes are used as control. (ii) Staph-Rapid-Test (Roche, Basel, Switzerland), which is based on sheep erythrocytes coated with both fibrinogen and IgG. Non-sensitized sheep erythrocytes are used as control, (iii) Monostaph (Bionor A/S, Skien, Norway) consisting of 3 micron core/shell-type particle Dynospheres® (Dyno Particles, Oslo, Norway), to which both human IgG (Sigma Chemical Company, MO 63178, USA) are covalently coupled by the tosyl-method (*Nilsson & Mosback* 1980). Other latex agglutination tests are based on smaller particles, normally less than 1 micron, and physical absorption of the proteins. Particles coated with bovine serum albumin are used as control.

Tube coagulase test:

The tube coagulase test (TC) was performed according to the recommendation of *Anon.* (1965). Reactions were read after 2, 4, 6 and 18 h as positive (+) or negative (–). Strains tested for coagulase only were considered as coagulase positive *Staphylococcus* spp. when coagulase positive, and coagulase negative *Staphylococcus* spp. when coagulase negative and positive for anaerobic fermentation of glucose.

Results

The ability of the 3 rapid test kits to differentiate between coagulase positive and negative staphylococci and micrococci is shown in Table 1. Monostaph correctly identified 96.3 % of the coagulase positive staphylococci (n = 54). One of the coagulase negative staphylococci (n = 50) gave a false positive result. No non-interpretable results were recorded.

Staph-Rapid-Test correctly identified 87 % of the coagulase positive staphylococci (n = 54), and 8 % of the coagulase negative strains (n = 50) gave false positive results. Non-interpretable results were observed for 5,8 % of the strain tested (n = 104).

Staphyslide-Test correctly identified 89 % of the coagulase positive staphylococci (n = 54) and 8 % of the coagulase strains (n = 50) gave false positive results. Non-interpretable results were observed for 6,7 % of the strains tested (n = 104).

The six strains of *S. intermedius* included were all coagulase positive (TC). Four were agglutinated with Monostaph, 1 with Staph-Rapid-Test and 3 with Staphyslide-Test. Considering agglutination of all coagulase positive strains (including the *S. intermedius* strains) as correct identifications and reading the non-interpretable reactions as negative. Monostaph gave significantly better identification compared to both Staph-Rapid-Test

Table 1. Comparison of 3 agglutination tests for identification of *Staphylococcus aureus*.

Species	Number tested	TC[a] +	Monostaph +	%	-	%	NI[b]	%	Staph-Rapid-Test +	%	-	%	NI[b]	%	Staphyslide-Test +	%	-	%	NI[b]	%
Coagulase positive																				
Staphylococcus spp.	23	23	23	100	0	0	0	0	23	100	0	0	0	0	22	96	1	4	0	0
S. aureus	25	25	25	100	0	0	0	0	23	92	0	0	2	9	23	92	0	0	2	9
S. intermedius	6	6	4	67	2	33	0	0	1	17	5	83	0	0	3	50	2	33	1	17
Staphylococcus spp.[c]	42	0	1	2	41	98	0	0	3	7	35	83	4	10	3	7	36	87	3	7
Micrococcus spp.[c]	8	0	0	0	8	100	0	0	1	13	7	88	0	0	1	13	6	75	1	13
Total	104	54																		

a TC=tube coagulase

b NI= non-interpretable

c The coagulase negative Staphylococcus spp. and Micrococcus spp. consisted of:
S. epidermidis (n=1), S. capitis (n=1), S. caprae (n=1), S. cohnii (n=1), S. gallinarum (n=1), S. haemolyticus (n=1), S. hominis (n=1), S. hyicus subsp. chromogenes (n=1), S. hyicusus subsp. hyicus (n=9), S. saprophyticus (n=1), S. sciuri (n=1), S. simulang (n=1), S. xylosus (n=1), S. warneri (n=1), Staphylococcus spp. (n=19), M. kristinae (n=2), M. luteus (n=3), M. lylae (n=1), M. sedentarius (n=1), Micrococcus sp. (n=1).

and Staphyslide-Test (P = 0.01) (chi-squared test). There was no statistically significant difference between the Staphyslide-Test and Staph-Rapid-Test. Excluding *S. intermedius* from the material, no significant difference between the 3 tests were found (chi-squared test).

Discussion

The results of this study demonstrated some variation in the sensitivity of the 3 tests for rapid identification of coagulase positive staphylococci.

Apart from one strain, Monostaph did not agglutinate any of the coagulase negative strains included in this study. This particular strain agglutinated in all the tree tests. One explanation of this phenomenon may be that the strain actually is *S. aureus* and only CF is produced. This strain also produced thermostable DNAse, which is a characteristic of *S. aureus, S. hyicus* and *S. intermedius,* but not the coagulase negative staphylococci.

The TC test has been regarded as the reference method for discrimination between *S. aureus* and other members of the *Micrococcaceae* family. However, other species, such as *S. hyicus* subsp. *hyicus* and *S. intermedius* are able to produce coagulase. Like *S. aureus* more than 90 % of *S. intermedius* strains produce coagulase detected by the TC test. Some 24–56 % of *S. hyicus* subsp. *hyicus* produce coagulase, although it is often weak and may require 18–24 h for detection by the TC test (*Schleifer* 1986). CF is not produced by *S. hyicus* (*Schleifer* 1986).

S. intermedius was formerly designated *S. aureus* biotype E and F and some strains produce varying amounts of CF (*Devriese et al.* 1978). Cell wall-bound protein A also has been detected in *S. intermedius* isolates. *Cox et al.* (1986) detected protein A in 6 of 139 *S. intermedius* isolates from dogs and cats, whereas *Fehrer et al.* (1988) detected cell-bound protein A in 48 of 50 isolates from canine skin. Therefore it is not surprising that 4 of the 6 strains tested agglutinated with Monostaph, 3 with Staphyslide-Test and 1 with Staph-Rapid-Test.

Most of the coagulase positive staphylococci isolated from dogs are *S. intermedius,* and about 25 % of these strains produce enterotoxin (*Hirooka et al.* 1988). Dogs are in close contact with humans and *S. intermedius* has been isolated from food (*Hirooka & Müller* 1982). Mishandling of food in which these bacteria are present could lead to staphylococcal food poisoning.

Both Staphyslide-Test and Staph-Rapid-Test gave non-interpretable results. Out of the 104 strains tested the former test gave 7 and the latter gave 6, respectively. This may have several explanations. Staphyslide-Test consists of erythrocytes coated with fibrinogen. This test will therefore only identify strains producing CF. Staph-Rapid-Test is based on erythrocytes coated with both IgG and fibrinogen. Remaining plasma proteins will probably also be bound to the erythrocytes and unspecific reactions due to the interaction between bacterial components and plasma proteins may therefore occur. Biological particles such as erythrocytes contain many different biochemical structures that may interact with bacterial structure giving unspecific agglutination. Monostaph was easy to read and no non-interpretable results were observed. *Flesland* (1987) tested human *S. aureus* routine isolates and found both Monostaph and Staphaurex (Welcome) which is a test based on latex particles coated with IgG and fibrinogen, equally reliable and in complete aggreement with the TC test.

Recently *Johne et al.* (1989) have demonstrated that *S. aureus* separated from mastitis milk samples without cultivation were encapsulated with an exopolysaccaride masking surface protein A and *S. aureus*-specific cell wall components. The authors did not investigate whether CF was also masked. The polysaccaride layer on in vivo bacteria was reduced markedly after just 1 transfer from milk to blood agar plates. *Fournier et al.* (1989) tested recent human clinical *S. aureus* isolates by Staphyslide-Test and 2 latex agglutination tests after subcultivation on Columbia agar (Difco), followed by cultivation on Mueller-Hinton agar plates (Difco). These 3 agglutination tests are based on both fibrinogen and IgG. Seven of 183 *S. aureus* isolates did not agglutinate with any of the agglutination tests, thus indicating masking of both CF and protein A. All these 7 isolates were of capsular polysaccaride serotype 5. The authors suggest that these agglutination kits can be improved by the use of antibodies reactive with *S. aureus* capsular polysaccaride.

The present study shows that Monostaph may be a good alternative to the tube-coagulase test for rapid and reliable identification of coagulase positive staphylococci from both food and veterinary sources. However, false negative reactions may occur with coagulase positive strains of *S. hyicus* subsp. *hyicus, S. intermedius* and primary clinical isolates of *S. aureus.*

Acknowledgement

The author are grateful to Professor K. H. Schleifer, Technische Universität München, Germany, and to Dr. W. E. Kloos, North Carolina State University, Raleigh, North Carolina, USA for kindly providing the reference strains for this study.

References

Anonymous: Subcommittee on Taxonomy of Staphylococci and Micrococci. Recommendations. Int. Bull. Bact. Nomencl. Taxon. 1965, *15,* 109–110.

Barry AL, Lachica RVF, Atchinson FW: Identification of *Staphylococcus aureus* by simultaneous use of tube coagulase and thermonuclease tests. Appl. Microbiol. 1973, *25,* 496–497.

Bodén MK, Flock J-I: Fibrinogen-binding protein/Clumping factor from *Staphylococcus aureus.* Infect. Immun. 1989, *57,* 2358–2363.

Cox HU, Schmeer N, Newmann SS: Protein A in *Staphylococcus intermedius* isolates from dogs and cats. Amer. J. vet. Res. 1986, *9*, 1881–1884.

Devriese LA, Hajek V, Oeding P, Meyer SA, Schleifer KH: Staphylococcus hyicus (Sompolinsky 1953) comb. nov. and *Staphylococcus hyicus* subsp. *chromogenes* subsp. nov. Int. J. syst. Bacteriol. 1978, *28*, 482–490.

Dibb WL, Hellum KB, Østervold B, Oeding P: Comparison of four methods for differentiation of *Staphylococcus aureus* from other *Micrococcaceae* in routine laboratory. Acta Path. Microbiol. Immunol. Scand. Sect. B. 1983, *91*, 307–310.

Duthie ES: The action of fibrinogen on certain pathogenic cocci. J. gen. Microbiol. 1955, *13*, 83–393.

Essers L, Radebold K: Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. J. clin. Microbiol. 1980, *12*, 641–643.

Ewald S: Enteroxin production by *Staphylococcus aureus* strains isolated from Danish foods. Int. J. Food Microbiol. 1987, *4*, 207–214.

Fehrer SL, Boyle MDP, Halliwell REW: Identification of protein A from *Staphylococcus intermedius* isolated from canine skin. Amer. J. vet. Res. 1988, *49*, 697–701.

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

Forsgren A: Significance of protein A production by Staphylococci. Infect. Immun. 1970, *2*, 672–673.

Fournier JM, Boutonier A, Bouvet A: Staphylococcus aureus strains which are not identified by rapid agglutination methods are of capsular serotype 5. J. clin. Microbiol. 1989, *27*, 1372–1374.

Hajek V, Marsalek E: Evaluation of classificatory criteria for staphylococci. In: Jeljaszewicz J, ed. Staphylococci and staphylococcal disease. Gustav Fisher Verlag, Stuttgart 1976, p. 11–21.

Hirooka EY, Müller EE: Isolamento, caracterizacad e isolados de productos arneos comercializados em Londrina-PR. (Isolation and characterization of isolates from commercial meat products in Londrina-PR). Cien. Technol. Aliment, 1982, *2*, 123–133.

Hirooka EY, Müller EE, Freitas JC, Vicente E, Yoshimoto Y, Bergdoll MS: Enterotoxigenicity of *Staphylococcus intermedius* of canine origin. Int. J. Food Microbiol. 1988, *7*, 185–191.

Jeljaszewicz J, Switalski LM, Adlam C: Staphylocoagulase and clumping factor. In: Easmon CSF, Adlam C, eds. Staphylococci and staphylococcal infections. Academic Press, London 1983, p. 525–558.

Johne B, Jarp J, Haaheim LR: Staphylococcus aureus exopolysaccaride in vivo demonstrated by immunomagnetic separation and electron microscopi. J. clin. Microbiol. 1989, *27*, 1631–1635.

Jonsson P, Holmberg O: Staphylococcus aureus isolated from acute and chronic mastitis. Comparative studies on bacterial surface characteristics, biochemical properties and phage typing. In: Jeljaszewicz J, ed. Staphylococci and staphylococcal infections. Stuttgart, New York: Zbl. Bact. Suppl. 10, Gustav Fisher Verlag 1981, p. 793–801.

Kloos WE, Schleifer KH: Simplified scheme for routine identification of human *Staphylococcus* species. J. clin. Microbiol. 1975, *1*, 82–88.

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

Nilsson K, Mosback K: p-Toluenesulfonyl chloride as an activating agent of agarose for the preparation of immobilized affinity ligands and proteins. Eur. J. Biochem. 1980, *112*, 397–402.

Orsi A, Bartoloni A, Martelli L, Colao MG: Evaluation of six different agglutination methods for rapid identification of *Staphylococcus aureus.* Eur. J. clin. Microbiol. 1989, *8*, 655–656.

Schleifer KH: Micrococcae. In: Sneath PHA, Mair NS, Sharpe ME, eds. Bergey's Manual of Sy-

stematic Bacteriology, vol. 2. Williams & Wilkins, Baltimore 1986, p. 1003–1035.

Schulze K, Wernery V: Prüfung von Staphylokokken stämmen mittels Fibrinogenaffinitäts-Test und Vergleich mit anderen Parametern. (Testing staphylococcal strains with the fibrinogen affinity test and a comparison with other parameters). Fleischwirtsch. 1986, *66,* 852–857.

Usui Y: Biochemical properties of fibrinogen binding protein (clumping factor) of the staphylococcal cell surface. Zbl. Bakt. Hyg. A. 1986, *262,* 287–297.

Weers-Pothoff G, Moolhuijzen CEM, Bongaerts GPA: Comparison of seven coagulase tests for identification of *Staphylococcus aureus.* Eur. J. clin. Microbiol. 1987, *6,* 589–591.

Sammendrag

Sammenligning av tre kommersielle agglutinasjons hurtigtester til identifisering av koagulasepositive stafylokokker fra næringsmidler og dyr.

Tre kommersielt tilgjengelige agglutinasjonstester til identifisering af *S. aureus* Monostaph (Bionor A/S, Skien, Norge), Staphyslide-test (BioMerieux, Lyon, Frankrike) og Staph-Rapid-Test (Roche, Basel, Sveits) ble sammenlignet. Materialet bestod av 104 Gram-positive, katalase positive kokker. Av referansestammene var 9 *Staphylococcus* representert med 15 arter mens 7 var *Micrococcus* med 4 arter. Toogtyve stammer var isolert fra næringsmidler. Av disse var 13 *S. aureus,* 8 koagulase positive *Staphylococcus* spp. og en *Micrococcus* sp. Av de 56 isolatene fra dyr var 11 *S. aureus,* 9 *S. hyicus* subsp. *hyicus,* 2 *S. intermedius,* 15 koagulase positive og 19 koagulase negative *Staphylococcus* spp.

Idet det forutsettes att agglutinasjon av en koagulase positiv stamme regnes som korrekt identifikasjon, identifiserte Monostaph, Staph-Rapid-Test og Staphyslide-Test korrekt henholdsvis 96 %, 87 % og 89 % av de koagulase positive stammene (n = 54). Monostaph. Staph-Rapid-Test og Staphyslide-test ga henholdsvis 2 %, 8 % og 8 % falske positive resultater (n = 50), mens de ikke tolkbare resultatene for testene (n = 104) var henholdsvis 0 %, 5,8 % og 6,7 %. Monostaph er et godt alternativ til rørkoagulasetesten for en rask og sikker identifikasjon av koagulase positive stafylokokker fra dyr og mennesker. Det kan imidlertid forekomme falske negative reaksjoner med koagulase positive *S. hyicus* subsp. *hyicus* og *S. intermedius* stammer, samt kliniske isolater av *S. aureus* ved primær isolasjon.

(Received January 8, 1990; accepted May 4, 1990).

Reprints may be requested from: Steen Ewald, Department of Food Hygiene,
Norwegian College of Veterinary Medicine, P. O. Box 8146 Dep., N-0033 Oslo 1, Norway.