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Evaluation of six commercial identification kits for the identification of *Staphylococcus aureus* isolated from bovine mastitis

M. Zschöck[1], A. Nesseler[1] and I. Sudarwanto[2]

[1]Public Investigation Office Hesse, Department of Veterinary Medicine, Giessen, Germany, and [2]Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia

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ABSTRACT

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Aims: Comparison of six commercially available in human medicine well-established slide agglutination systems for the identification of *Staphylococcus aureus.*

Methods and Results: Slide agglutination tests were compared with the conventional tube coagulase test, biochemical identification and with the molecular identification by polymerase chain reaction (PCR) amplification of species-specific parts of the gene encoding the 23S RNA. Systems evaluated included Masta-Staph® (Mast Diagnostics), Staphylase-Test® (Oxoid), Staphytect-Plus® (Oxoid), Staphyloslide Latex® (Becton Dickinson), Slidex Staph Plus® (bioMérieux) and Dry Spot Staphytect Plus® (Oxoid). A total of 141 staphylococcal strains isolated from cases of bovine mastitis including 90 *S. aureus,* 14 *Staphylococcus epidermidis,* 10 *Staphylococcus warneri,* 13 *Staphylococcus xylosus,* 11 *Staphylococcus haemolyticus* and three other coagulase-negative staphylococci were tested with each method. *Staphylococcus aureus* strains were selected by macrorestriction analysis with pulsed field gel electrophoresis (PFGE). Only genetically unrelated strains were included in the study. The sensitivities and specificities of the test were as follows:
Masta-Staph® 86·7 and 90·1%, Staphylase-Test® 78·4 and 85·1%, Staphytect-Plus® 81·1 and 86·5%, Staphyloslide Latex® 77·8 and 84·4%, Slidex Staph Plus® 77·8 and 84·4%, Dry Spot Staphytect Plus® 75·6 and 83·0%.

Conclusions: The results of this evaluation suggest that the six slide agglutination methods tested can provide rapid identification of *S. aureus* also from bovine mastitis. The sensitivity and specificity seems to be less than those reported from human *S. aureus* isolates.

Significance and Impact of the Study: This is one of the first comparative reported investigations about the applicability of different commercially available slide agglutination tests for the detection of *S. aureus* from bovine mastitis using PFGE selected clinical isolates.

Keywords: bovine mastitis, commercial agglutination systems, *Staphylococcus aureus* identification.

INTRODUCTION

For effective prevention and control of bovine mastitis accurate and rapid diagnosis of intramammary infections is essential. New concepts of classification of many udder pathogens were devised and new methods for the identification of many mastitis pathogens were developed. Staphylococcal mastitis remains a major economic problem for the dairy industry throughout the world (Jasper *et al.* 1982). Only *Staphylococcus aureus* was previously considered as a major mammary gland pathogen within the genus

Correspondence to: Michael Zschöck, Public Investigation Office Hesse, Department of Veterinary Medicine, Marburger Str. 54; 35396 Giessen, Germany (e-mail: m.zschoeck@suah.hessen.de).

whereas coagulase-negative staphylococci (CNS) were considered as minor pathogens. *Staphylococcus aureus* has traditionally been identified by the coagulase assay, but the detection of surface proteins such as clumping-factor and/or protein A also enables rapid species identification. Several commercially produced kits are now available to aid diagnostic laboratories in the rapid identification of *S. aureus* providing alternatives to the classic slide and tube coagulase test. They have been extensively validated for use in medical diagnostic bacteriology laboratories in human medicine. Rapid identification methods for *S. aureus* have only rarely been evaluated specifically for bovine mastitis diagnosis (Hogan *et al.* 1986, 1988; Watts and Owens 1988). Separate test validation for the identification of bovine mastitis isolates with these rapid identification kits is necessary as a cause of different *S. aureus* populations from man and cattle (Kapur *et al.* 1995). The slide and tube coagulase test detect free and bound coagulase, respectively, but a considerable proportion of strains deficient in clumping factor activity however was found among isolates from bovine udder (Hummel *et al.* 1992).

In order to improve the sensitivity and specificity of these test kits that employ either latex particles or sheep erythrocytes coated with fibrinogen, many manufacturers have also coated the particles with immunoglobulin G (Grov *et al.* 1970). Additionally, antibodies against capsular types 5 and 8 were attached to latex particles. The staphylococcal capsular polysaccharides type 5 and 8 account for 69·4% of the bovine mammary isolates of *S. aureus* (Poutrel *et al.* 1988).

This study was designed to compare the performance characteristics of one older single (fibrinogen) and two double (fibrinogen and IgG) commercial detection systems and three of these newer in bacteriology of human medicine well-established detection kits containing fibrinogen, immunoglobulin G and anti-staphylococcal capsular antibodies with the classical tube coagulase test, the biochemical identification and a PCR method for species identification of *S. aureus*.

Ninety *S. aureus* isolates from bovine clinical and subclinical mastitis which, according to their *SmaI* macrorestriction pattern are different to each other, were included. In addition, 51 CNS from different herds at different locations were also tested.

MATERIALS AND METHODS

Bacterial strains

Clinical staphylococci isolates collected after cultivation (IDF 1981) of quarter milk samples from cows suffering from clinical or subclinical mastitis [somatic cell count (Fossomatic, Aarhous, Denmark) ≥100 000 cells ml^{-1} or macroscopic signs of mastitis] during the period of 1998–2000 included *S. aureus* ($n = 90$), *Staphylococcus epidermidis* ($n = 14$), *Staphylococcus warneri* ($n = 10$), *Staphylococcus xylosus* ($n = 13$), *Staphylococcus haemolyticus* ($n = 11$) and nontypable CNS ($n = 3$).

Selection of clinical *S. aureus* strains was carried out according to their genetic relatedness. It was based on the results of a DNA macrorestriction analysis (Toshkova *et al.* 1997) and defined as published by Tenover *et al.* (1994). Only genetically unrelated isolates identified by pulsed field gel electrophoresis (PFGE) were used in this study. They were classified as genetically unrelated if they showed a six band or greater difference and a dice coefficient of correlation of 60% or less. Banding pattern were compared visually by two independent observers.

Further, only epidemiologically unrelated (isolated from milk samples of different flocks) CNS field strains were used.

As positive control *S. aureus* (ATCC 25 923) standard strain was used.

Staphylococcus aureus identification

All isolates were cultured on 5% Columbia sheep blood agar (Merck, Darmstadt, Germany) and chosen on the basis of their colonial and Gram stain morphologies and a positive catalase reaction for 24 h at 37°C. A tube coagulase test was performed on each isolate with EDTA-treated rabbit plasma (Merck) using the direct tube method (Harmon *et al.* 1990) and evaluated after 4 and 24 h incubation at 37°C.

Furthermore, biochemical properties for species identification were detected by using the commercial identification system API 32_{Staph} (bioMérieux, Nürtingen, Germany).

All isolates were additionally investigated by PCR amplification of species-specific parts of the gene encoding the 23S RNA (Akineden *et al.* 2001). As *S. aureus* identified isolates were selected by macro restriction analysis using PFGE as described above.

Identification of CNS

CNS were identified by the negative tube coagulase test and the negative 23S RNA PCR.

Additionally the API 32_{Staph} system (bioMérieux) was used for the species identification.

Rapid slide agglutination tests

Six rapid slide agglutination test were included: Masta-Staph® (Mast Diagnostics), Staphylase-Test® (Oxoid), Staphytect-Plus® (Oxoid), Staphyloslide Latex Test®

(Becton Dickinson), Slidex Staph Plus® (bioMérieux) and Dry Spot Staphytect Plus® (Oxoid).

The Staphylase-Test® (Oxoid) is a first generation test using sensitized sheep erythrocytes with rabbit fibrinogen. Masta-Staph® (Mast Diagnostics) and Staphyloslide Latex Test® (Becton Dickinson) are second generation tests consisting of latex particles coated with human fibrinogen and IgG. Tests of the third generation are Staphytect-Plus® (Oxoid), Dry Spot Staphytect Plus® (Oxoid) and Slidex Staph Plus® (Merieux). These products identify *S. aureus* by the detection of clumping factor (fibrinogen), protein A (IgG) and *S. aureus* external structures (capsular polysaccharides).

Commercial agglutination tests were performed as described by the manufacturers.

Each of the staphylococcal strains on Columbia sheep blood agar was coded and tested blindly with each method on the same day.

All six tests were performed by one observer on each of the 141 isolates. Strains with discrepant results among the six test systems were retested by the same observer. All isolates with discrepant test results from both investigations were retested with the tube coagulase test.

Statistical analysis

The statistical significance of differences in sensitivity and specificity was determined by the Fisher's exact test and the chi-squared test.

RESULTS

Principally all investigated rapid slide agglutination test are easily to perform with results being obtained after 20–60 s in all systems.

Some of the investigated strains were not easily to interpret with all test systems. Slidex Staph Plus® was most difficult to read, often showing weak reactive results for isolates, which were strongly positive within the other test systems. Test evaluation was obtained after 20–60 s in all systems.

The results of the 141 tested staphylococci by six commercially available rapid slide agglutination tests are summarized in Table 1. No single commercial kit identified all of the by tube coagulase, API 32_{Staph} and 23S PCR-positive *S. aureus* clinical isolates. One hundred and sixteen of the 141 staphylococcal field strains including 68 *S. aureus* isolates selected by macrorestriction analysis and 48 CNS field strains were correctly detected by all investigated slide agglutination test systems. True-positive reactions for *S. aureus* were obtained by Slidex Staph Plus® ($n = 70$), Staphyloslide-Latex® and Staphylase® ($n = 71$), Staphytect-Plus® ($n = 73$) and Masta-Staph® ($n = 78$). Masta-Staph® missed 12 (13·3%), Staphytect-Plus® 17 (18·9%), Staphylase® and Staphyloslide-Latex® 19 (21·1%), Slidex Staph Plus® 20 (22·2%) and Dry Spot Staphytect Plus® 22 (24·4%) bovine *S. aureus* mastitis clinical isolates.

Of 51 CNS strains except the Staphyloslide-Latex® test with 48 true-negative results, all remaining tests detected 49 of these strains correctly. Interestingly, false-positive results for the same *S. epidermidis* isolate were obtained by all commercial test systems. Furthermore one *S. epidermidis* strain gave positive results in Masta-Staph®, Dry Spot Staphytect Plus® and Slidex Staph Plus®. One *S. haemolyticus* isolate showed a false-positive reaction with Staphytect® and Staphyloslide-Latex®.

One *S. aureus* field strain gave an unemployable result by Staphylase® test showing autoagglutination with the control reagent.

Masta-Staph® (86·7%) was the test system with the highest sensitivity, followed by Staphytect-Plus® (81·1%) and Dry Spot Staphytect Plus® (75·6%). The specificity of the Masta-Staph® test (90·1%) was also higher than the other investigated tests ranging from 86·5% (Staphytect-Plus®) to 83·0% (Dry Spot Staphytect Plus®).

Differences in sensitivity and specificity were statistically ($P < 0·05$) not significant.

Table 1 Results of testing 141 staphylococci by six commercial slide agglutination identification systems

	Rapid slide agglutination test					
$n = 141$*	Masta-Staph® (Mast Diagnostics)	Staphylase® (Oxoid)	Staphytect-Plus® (Oxoid)	Staphyloslide-Latex® (Becton Dickinson)	Slidex Staph Plus® (bioMérieux)	Dry Spot Staphytect Plus® (Oxoid)
True-positive	78	71	73	71	70	68
False-positive	2	1	3	3	2	2
True-negative	49	49	49	48	49	49
False-negative	12	19	17	19	20	22
Autoagglutination	–	1	–	–	–	–
Specificity (%)	90·1	85·1	86·5	84·4	84·4	83·0
Sensitivity (%)	86·7	78·4	81·1	77·8	77·8	75·6

**S. aureus* ($n = 90$), *S. epidermidis* ($n = 14$), *S. warneri* ($n = 10$), *S. xylosus* ($n = 13$), *S. haemolyticus* ($n = 11$), other CNS ($n = 3$).

DISCUSSION

As described in IDF-Bulletin 132 (IDF 1981) occurrence of a β-haemolytic zone on bovine blood agar surrounding staphylococcal colonies leads to the diagnosis of *S. aureus* bovine mastitis although former studies reported the occurrence of haemolysin-negative *S. aureus* strains (Aarestrup *et al.* 1999). Therefore commercially supplied test kits for the rapid identification of *S. aureus* are widely used in clinical microbiological laboratories, because the tube coagulase test, generally accepted as the 'gold standard' for the identification of *S. aureus,* needs up to 24 h for a final negative test result. New tests based on molecular biology, for example the PCR are also time consuming and not suitable in routine bacteriological mastitis laboratory. So commercially available agglutination tests became an attractive alternative for *S. aureus* species identification in clinical routine laboratory. Tests can be performed directly from the primary culture plate and results are available within a few seconds. Studies on the evaluation of test kits for *S. aureus* identification are usually performed with freshly collected staphylococcal isolates submitted to the clinical laboratory. To avoid the usage of duplicate isolates in such a comparative study of different test systems, as *S. aureus* mastitis problems are often epidemic in a given flock (Zschöck *et al.* 2000) and in a distinct area only a few bacterial clones are responsible for bovine *S. aureus* mastitis (Annemüller *et al.* 1999), the present study was performed by genetically unrelated *S. aureus* bovine mastitis isolates which were selected previously by macrorestriction analysis through PFGE.

Previous evaluations of commercial agglutination systems for the identification of staphylococci demonstrated slightly lower accuracy levels with animal isolates than those reported from human clinical isolates (Weber and Wachowitz 1989; Boerlin *et al.* 2003).

In our investigation we focused on six commercially available testkits for the rapid identification of *S. aureus*. We used a simple 'first generation'-test system consisting of rabbit fibrinogen sensitized sheep red blood cells (Staphylase®) and two latex reagent tests containing particles coated with human fibrinogen and IgG (Masta-Staph® and Staphyloslide Latex®).

Our special intention was to check the hypothetic benefit of the newer diagnostic systems with the addition of anticapsular antibodies against the capsule types 5 and 8 of *S. aureus*. Such strains, common in human isolates, were also found in bovine *S. aureus* strains isolated from bovine mastitis (Poutrel *et al.* 1988). The use of these anticapsular antibodies should, theoretically, improve the detection of *S. aureus* isolates particularly such strains deficient in clumping factor and protein A. Instead of Slidex Staph Plus® test with the usage of monoclonal antibodies raised against the polysaccharide capsule types 5 and 8, Staphytect-Plus® is working with rabbit polyclonal antibodies against these *S. aureus* surface structures. The Fc portion of rabbit IgG simultaneously is able to detect cell wall bound protein A. Additionally porcine fibrinogen is added for detection of clumping factor activity. Dry Spot Staphytect Plus® uses the same reagent which is dried onto a reaction card.

Slidex Staph Plus® is also based on a triple detection system. Latex particles sensitized with human fibrinogen and murine monoclonal antibodies enable simultaneous detection of clumping factor, protein A and group-specific antigens of capsule types 5 and 8 of *S. aureus.*

In contrast to former investigations from human diagnostic laboratories (Essers and Radebold 1980; Personne *et al.* 1997; Wilkerson *et al.* 1997; Smole *et al.* 1998; Van Griethuysen *et al.* 2001) the sensitivity of *S. aureus* identification of some of the investigated systems was only between 77·6 and 86·9%.

The lower sensitivities could be mainly explained by the lack of detection of atypical strains of *S. aureus* defective in clumping factor, protein A or capsule type 5 or 8. Such strains are not uncommon in bovine mastitis. Hummel *et al.* (1992) reported the lack of clumping factor activity in *S. aureus* mastitis strains of more than 50% of the investigated field strains. Laevens *et al.* (1996) isolated an atypical clumping factor negative *S. aureus* strain as a cause of intramammary infection in a dairy herd. Protein A occurrence was reported only being between 50 and 60% of strains isolated from bovine mastitis in contrast to human strains being between 90 to 99% (Forsgren 1970; Kronvall *et al.* 1972). As mentioned above only 69·4% of bovine mastitis strains carried the type 5 or 8 polysaccharide capsule antigen (Poutrel *et al.* 1988) in contrast to 70–80% of human clinical isolates (Fournier *et al.* 1989). However we actually did not compare directly human and bovine mastitis strains. Slight difference in methodologies (e.g. type of media or culturing conditions) may contribute to the performance of the tests. The results of this study demonstrated that there is a statistically not significant variation in sensitivity and specificity between the investigated commercially available slide agglutination systems. This could be explained as a result of different fibrinogen and immunoglobulin G types used in these systems. For example in contrast to porcine or murine IgG, human IgG was reported to have a significantly higher binding activity to protein A of *S. aureus* (Müller *et al.* 1983). The drying of reagents onto special reaction cards seems to decrease sensitivity and specificity.

While the presence of clumping factor activity is a defining characteristic of *S. aureus,* single strains of CNS were also reported to be positive in this reaction (Kloos and Bannermann 1999). One isolate of *S. haemolyticus* and two isolates of *S. epidermidis* of this study did show false-positive reactions. The six agglutination tests varied in the frequency

with which this occurred. These false-positive reactions could be due to other antigens on these isolates that cross-react with any particular antibodies utilized in these assays. Protein A for example was demonstrated to be present in up to 2% of CNS (Winblad and Ericson 1973; Maxim *et al.* 1976).

In conclusion, agglutination systems are also suitable for *S. aureus* identification from bovine mastitis samples. Sensitivity and specificity was less than those reported from strains of human origin. The theoretically imagined benefit of using anticapsular antibodies could not be confirmed. The highest sensitivity and specificity was obtained using a two-phase agglutination system with human fibrinogen and IgG (Masta-Staph®). The other tests demonstrated less optimal results.

Based on these results we recommend that laboratories that choose to use commercial agglutination systems as a method in routine diagnostic bacteriology for identifying *S. aureus* in bovine mastitis samples be aware that some strains may give a false-negative result.

REFERENCES

Aarestrup, F.M., Larsen, H.D., Eriksen, N.H., Elsberg, C.S. and Jensen, N.E. (1999) Frequency of alpha- and beta-haemolysin in *Staphylococcus aureus* of bovine and human origin. A comparison between pheno- and genotype and variation in phenotypic expression. *Acta Pathologica Microbiologica Immunologica Scandinavia* **107**, 425–430.

Akineden, O., Annemüller, C., Hassan, A.A., Lämmler, C., Wolter, W. and Zschöck, M. (2001) Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. *Clinical Diagnostic Laboratory Immunology* **8**, 959–964.

Annemüller, C., Lämmler, C. and Zschöck, M. (1999) Genotyping of *Staphylococcus aureus* isolated from bovine mastitis. *Veterinary Microbiology* **69**, 217–224.

Boerlin, P., Kuhnert, P., Hussy, D. and Schaellibaum, M. (2003) Methods for identification of *Staphylococcus aureus* isolates in cases of bovine mastitis. *Journal Clinical Microbiology* **41**, 767–771.

Essers, L. and Radebold, K. (1980) Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. *Journal Clinical Microbiology* **12**, 641–643.

Forsgren, A. (1970) Significance of protein A production of staphylococci. *Infection Immunity* **2**, 672–673.

Fournier, J.M., Boutonnier, A. and Bouvet, A. (1989) *Staphylococcus aureus* strains which are not identified by rapid agglutination methods are of capsular serotype 5. *Journal Clinical Microbiology* **27**, 1372–1374.

Grov, A., Oeding, P., Myklestad, B. and Aason, J. (1970) Reactions of staphylococcal antigens with normal serum je-globulins and j-globulin fragments of various species origin. *Acta Pathologica Microbilogica Scandinavia* **378**, 106–111.

Harmon, R.J., Eberhart, R.J., Jasper, D.E., Langlois, B.E. and Wilson, R.A. (1990) *Microbiological procedures of the diagnosis of bovine udder infections*, 3rd edn. Arlington, USA: Bulletin National Mastitis Council.

Hogan, J.S., Cornetta, A. and Pankey, J.W. (1986) Comparison of four test procedures to identify *Staphylococcus aureus* isolated from bovine intramammary infections. *American Journal Veterinary Research* **47**, 2017–2019.

Hogan, J.S., Smith, K.L., Todhunter, D.A. and Schoenberger, P.S. (1988) Sensitivity and specificity of latex agglutination tests used to identify *Streptococcus agalactiae* and *Staphylococcus aureus* isolated from bulk tank milk. *American Journal Veterinary Research* **49**, 1537–1539.

Hummel, L., Devriese, A. and Lehmann, G. (1992) Characteristics of bovine *Staphylococcus aureus* with special regard to clumping factor activity. *Zentralblatt Bakteriologie* **276**, 487–492.

International Dairy Federation (IDF) (1981) *Laboratory Methods for Use in Mastitis Work. Document 132*. Brussels: IDF.

Jasper, D.E., Mac Donald, J.S., Mochrie, R.D., Philpato, W.A., Farnsworth, R.J. and Spender, S.B. (1982) Bovine mastitis research: needs, funding and sources of support. In *Proceedings of the 21st Annual Meeting of the National Mastitis Council*, pp. 184–193. Louisville, KY: National Mastitis Council.

Kapur, V., Sischo, W.M., Greer, R.S., Whittam, T.S. and Musser, J.M. (1995) Molecular population genetic analysis of *Staphylococcus aureus* recovered from cows. *Journal Clinical Microbiology* **33**, 376–380.

Kloos, W.E. and Bannermann, T.L. (1999) *Staphylococcus* and *Micrococcus* sp. In *Manual of Clinical Microbiology*, 6th edn. ed. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. pp. 282–298. Washington, DC: ASM Press.

Kronvall, G., Homberg, O. and Ripa, T. (1972) Protein A in *Staphylococcus aureus* strains of human and bovine origin. *Acta Pathologica Microbiologica Scandinavia (B)* **80**, 735–742.

Laevens, H., Devriese, L.A., Deluyker, H., Hommez, J. and de Kruif, A. (1996) An atypical *Staphylococcus aureus* intramammary infection in a dairy herd. *Veterinary Microbiology* **52**, 271–275.

Maxim, P.E., Mathews, H.L. and Mengoli, H.F. (1976) Single-tube mixed agglutination test for the detection of staphylococcal protein A. *Journal Clinical Microbiology* **4**, 418–422.

Müller, H.P., Litke, O.M. and Blobel, H. (1983) Protein A activity of staphylococci of different animal origins with immunoglobulin G from domestic and experimental animals and from man. *Zentralblatt Veterinärmedizin B* **30**, 305–312.

Personne, P., Bes, M., Lina, G., Vandenesch, F., Brun, Y. and Etienne, J. (1997) Comparative performances of six agglutination kits assessed by using typical and atypical strains of *Staphylococcus aureus*. *Journal Clinical Microbiology* **35**, 1138–1140.

Poutrel, B., Boutonnier, A., Sutra, L. and Fournier, J.M. (1988) Prevalence of capsular polysaccharide types 5 and 8 among *Staphylococcus aureus* isolates from cow, goat and ewe milk. *Journal Clinical Microbiology* **26**, 38–40.

Smole, S.C., Aronson, E., Durbin, A., Brecher, S.M. and Arbeit, R.D. (1998) Sensitivity and specificity of an improved rapid latex agglutination test for identification of methicillin-sensitive and -resistant *Staphylococcus aureus* isolates. *Journal of Clinical Microbiology* **36**, 1109–1112.

Tenover, F.C., Arbeit, R., Archer, G., Biddle, J., Byrne, S., Goerding, R., Hancock, G., Herbert, A. *et al.* (1994) Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *Journal of Clinical Microbiology* **32**, 407–415.

Toshkova, K., Savov, E., Soedarmanto, I., Lämmler, Ch., Chankova, D., van Belkum, A., Verbrugh, H.A. and van Leeuwen, W. (1997) Typing of *Staphylococcus aureus* isolated from nasal carriers. *Zentralblatt Bakteriologie* **286**, 547–559.

Van Griethuysen, A., Bes, M., Etienne, J., Zbinden, R. and Kluytmans, J. (2001) International multicenter evaluation of latex agglutination tests for identification of *Staphylococcus aureus*. *Journal Clinical Microbiology* **39**, 86–89.

Watts, J.L. and Owens, W.E. (1988) Evaluation of the Rapid Mastitis Test for identification of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine mammary glands. *Journal Clinical Microbiology* **26**, 672–674.

Weber, A. and Wachowitz, R. (1989) The usefulness of three rapid commercially-available tests for the identification of *Staphylococcus aureus* strains isolated from animals. *Berliner Münchner Tierarztliche Wochenschrift* **102**, 149–151.

Wilkerson, M., McAllister, S., Miller, J.M., Heiter, B.J. and Bourbeau, P.P. (1997) Comparison of five agglutination tests for identification of *Staphylococcus aureus*. *Journal Clinical Microbiology* **35**, 148–151.

Winblad, S. and Ericson, C. (1973) Sensitized sheep red cells as a reactant for *Staphylococcus aureus* protein A. Methodology and epidemiology with special reference to weakly reacting methicillin-resistant strains. *Acta Pathologica Microbiological Scandinavia [B] Microbiology Immunology* **81**, 150–156.

Zschöck, M., Sommerhäuser, J. and Castaneda, H. (2000) Relatedness of *Staphylococcus aureus* isolates from bovine mammary gland suffering from mastitis in a single herd. *Journal Dairy Research* **67**, 429–435.