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

M. Zscho¨ck1, A. Nesseler1 and I. Sudarwanto2

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

2004/0839: received 20 July 2004, revised 8 September 2004 and accepted 9 September 2004

ABSTRACT

M. ZSCHO¨ C K, A. NESSELER AND I. SUDARWANTO. 2004. 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
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 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 *Smal* macro-restriction 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 (Fossomeric, Aarhus, 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 32Staph (bioMérieux, Nürtingen, Germany). All isolates were additionally investigated by PCR amplification of species-specific parts of the gene encoding the 23S RNA (Akinenden 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 32Staph system (bioMérieux) was used for the species identification.

**Rapid slide agglutination tests**

Six rapid slide agglutination test were included: Mast-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 32Staph 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

<table>
<thead>
<tr>
<th></th>
<th>Mast-Staph® (Mast Diagnostics)</th>
<th>Staphylase® (Oxoid)</th>
<th>Staphytect-Plus® (Oxoid)</th>
<th>Staphylase-Latex® (Becton Dickinson)</th>
<th>Slidex Staph Plus® (bioMérieux)</th>
<th>Dry Spot Staphytect Plus® (Oxoid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>True-positive</td>
<td>78</td>
<td>71</td>
<td>73</td>
<td>71</td>
<td>70</td>
<td>68</td>
</tr>
<tr>
<td>False-positive</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>True-negative</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>48</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>False-negative</td>
<td>12</td>
<td>19</td>
<td>17</td>
<td>19</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Autoagglutination</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>90.1</td>
<td>85.1</td>
<td>86.5</td>
<td>84.4</td>
<td>84.4</td>
<td>83.0</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>86.7</td>
<td>78.4</td>
<td>81.1</td>
<td>77.8</td>
<td>77.8</td>
<td>75.6</td>
</tr>
</tbody>
</table>

*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 (Zschock 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 anti-capsular 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 antcapsular 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, Staphytest-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 Staphytest 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 Griethuyzen 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 1972). As mentioned above only 69.4% of bovine mastitis strains carried the type 5 or 8 polysaccharide capsule antigen (Pourel 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 cultivating 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 \textit{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 \textit{S. aureus} in bovine mastitis samples be aware that some strains may give a false-negative result.

REFERENCES


