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False-negative test results in the Slidex Staph Plus (bioMérieux) agglutination test are mainly caused by *spa*-type t001 and t001-related strains.

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Abstract

The relative sensitivity of commercial agglutination kits for fast identification of *S. aureus* is usually given to be about 98%. This reported sensitivity has sometimes been questioned. In this study, three collections of molecularly defined, single-copy strains of *S. aureus* were used to compare the sensitivities of agglutination-based identification and the MALDI-TOF mass spectrometry-based identification using the Biotyper 2.0

database to a molecularly defined reference method. Clinical isolates (n=363) of methicillin-susceptible *S. aureus* (MSSA), and 240 clinical isolates of methicillin-resistant *S. aureus* (MRSA) were included. In order to rule out a predominance of local MRSA-strains, a collection of 104 pulsed-field-gel electrophoresis divergent MRSA strains were also tested. MALDI-TOF MS using Biotyper database (Bruker) identified all isolates, whereas the Slidex Staph Plus (bioMérieux) detected only 98.0% of the MSSA, 94.5% of the MRSA and only 70.1% of the MRSA of the molecularly divergent strain collection. Interestingly, strains with a false-negative test result in the agglutination methods were mostly *spa*-type t001 and t001 related. The MALDI-TOF MS based identification can thus be used as an alternative identification method for suspected false-negative results from the agglutination tests - especially if the local prevalence of t001 and t001 related strains is high.

Keywords

S. aureus, *spa*, agglutination, MALDI-TOF MS, t001

Introduction

A rapid and reliable identification of *S. aureus* is crucial to decreasing *S. aureus* associated morbidity. Several commercial agglutination kits are available for identifying *S. aureus*, but their sensitivity has sometimes been questioned. As a result, test accuracy

has been enhanced periodically [1-5] by incorporation of more reagents into the kits. The relative sensitivities of these tests are given by the manufacturers to be between 97.6% and 100%. Recently, Matrix assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS) was introduced as a method for bacterial identification [6-9]. Several studies with more than 80 strains of *S. aureus* have been described. The relative sensitivities of the MALDI-TOF-based *S. aureus* identification ranged from 93.4% to 100% [10-13]. In these studies the sensitivity seems to be dependent on the quality of bacterial characterization, the underlying database and identification system, the investigated proteomic profile range, and on the study design. To our knowledge, ours is the first study in which MALDI-TOF identification was compared to the agglutination kit Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France). In our study, three molecularly characterized *S. aureus* strain collections were included. In the first collection, 363 consecutively collected, molecularly defined, single-copy strains of methicillin-susceptible (MSSA) were included. In a second collection, 240 molecularly defined, single-copy strains of methicillin-resistant *S. aureus* (MRSA) were tested. Further, a molecularly defined MRSA collection (n=104) with a diverse genetic background [14] was included to rule out dominance of certain local MRSA-strain clusters. The sensitivity of MALDI-TOF MS-based identification was compared to the sensitivity of the agglutination kit Slidex Staph Plus. The sensitivity of the agglutination tests was unexpectedly low in the first MRSA collection. To rule out a

problem dependent on a single agglutination test composition, two additional agglutination tests were performed in the pulsed-field gel electrophoresis (PFGE) divergent *S. aureus* strain collection using the commercial Pastorex Staph plus (Bio-Rad, München, Germany) and Staphytest Plus kits (Oxoid, Basingstoke, UK). Moreover, two lots of Slidex Staph Plus (bioMérieux) were tested by different blinded investigators. MRSA strains that were negative in agglutination tests were characterized by *spa*-typing to show if this property was associated with certain lineages.

Methods :

Source of isolates

MSSA

In our study 363 clinical, consecutively collected, single-copy strains of methicillin-susceptible *S. aureus* were included. *S. aureus* was primarily identified by typical colony morphology and Slidex Staph Plus agglutination test (bioMérieux), or by the GPI-card of the Vitek-2 automated identification system (bioMérieux). The species diagnosis was confirmed using the *S. aureus* specific primer, SA442, for methicillin susceptible strains (oxacillin MIC from <0.25 to 2 mg/L), as described previously [15]. All strains were SA442 positive and were included in this study (Table 1). A cefoxitin screen incorporated in the Vitek 2 AST-P549 card was negative in all isolates.

MRSA

S. aureus was primarily identified as described above. Methicillin-resistance determination and cefoxitin screening [16] of all strains were done by the Vitek2 automated system (bioMérieux) and the results were interpreted according to the CLSI guidelines. No differences in the interpretation were observed comparing breakpoints suggested by CLSI and EUCAST (<http://www.Eucast.org>) in our isolates. All isolates identified as MRSA by this method were confirmed by *mecA*-[17] and SA442-PCR, as described previously [15], or by a different PCR method. This PCR used primers between the staphylococcal cassette chromosome *mec* (SCC*mec*) and the *orfX* [18]. As the primer binding in *orfX* is specific for *S. aureus*

A positive PCR was regarded as confirmation of *S. aureus*. Isolates with a negative cefoxitin screening result, or isolates with an oxacillin MIC of ≤ 2 mg/liter were tested by SA442 and *mecA* PCR [15]. SA442 and *mecA* PCR positive strains were defined as MRSA. *OrfX* positive strains with a positive cefoxitin screening test and an oxacillin MIC > 2 mg/liter were defined as a MRSA. *OrfX*, SA442, and *mecA*-PCR positive strains were also defined as a MRSA (irrespective of cefoxitin screening or oxacillin MIC). Overall, 240 consecutively collected single-copy strains of methicillin-resistant *S. aureus* were included. One MRSA strain in this second collection was oxacillin susceptible (oxacillin MIC of 1 mg/L) using a breakpoint of > 2 mg/L oxacillin (Table1). Moreover, a third MRSA collection of 104 PFGE divergent strains were also

included. In this collection, six MRSA strains [14] were oxacillin susceptible using a breakpoint of > 2 mg/L oxacillin. Both MRSA collections were characterized by pulsed-field gel electrophoresis (PFGE), as described earlier [19]. One isolate of each PFGE-group was determined by multi-locus sequence typing (MLST), as described previously [20]. For *spa*-typing, the *spa* gene was amplified by PCR and sequenced. The sequence was compared to the Ridom data base using the BURP (based upon repeat patterns) algorithm [21, 22].

Pulsed-field gel electrophoresis

Pulsed-field-gel-electrophoresis was conducted and interpreted, as described before [19]. Electrophoresis of the *SmaI*-digested DNA was performed with a CHEF-DRIII system (Bio-Rad) using 1.0% agarose gels (Bio-Rad). Subsequently, all PFGE gels were scanned using the Flour-S multiImager system (Bio-Rad) and analyzed using molecular analyst software 1.11 (Bio-Rad).

***S. aureus* identification by latex agglutination kits**

All strains of the MSSA and MRSA collection were tested for agglutination using the commercial staphylococcal agglutination kit Slidex Staph Plus (bioMérieux) in order to compare our local sensitivity data to the data in the literature.

To rule out dominance of certain local prevalent MRSA strains, a MRSA collection of

104 recently described [14], molecularly characterized, methicillin-resistant *S. aureus* strains with diverse genetic backgrounds were also tested. The agglutination results of this collection were compared to two other commercial kits (Pastorex Staph Plus kit Lot ID: 9A2427 [Bio-Rad] and Staphylect Plus kit Lot ID: J61061it). Moreover, two different lots (Lot ID: 0901100771, 0811100478) of Slidex Staph Plus (bioMérieux) were tested by two blinded investigators.

MALDI-TOF MS

All strains were examined with MALDI-TOF MS using Microflex LT instrument (Bruker Daltonics, Bremen , Germany). Formic acid was used for the sample preparation and the Flexcontrol 3.0 software and Biotyper 2.0 database (Bruker Daltonics) were used to calculate and process the analytical data, as described previously [13]. Identification was performed in duplicate and the identification score cut-off was applied on each measurement, according to the manufacturer's instructions.

Statistical analysis

A Fisher's exact probability test was used to calculate the P-value for the experimental data. P-values of <0.05 were considered to be statistically significant.

Results :

In the MSSA collection, the Biotyper 2.0 database gave a correct species diagnosis in all

(363 out of 363) strains. The analytical sensitivity of the latex agglutination Slidex Staph Plus kit for the identification of *S. aureus* was 98.0% (356 out of 363 strains). The seven MSSA isolates, which tested negative using the Slidex Staph Plus (bioMérieux) kit, were characterized using *spa*-typing. One (MSSA 88) of the seven strains was *spa*-type t001. The strains MSSA 101 (t015), MSSA 118 (t078), MSSA 166 (t550), MSSA 486 (t495), MSSA 545 (t026) and MSSA 622 (new *spa*-type with *spa*-repeat pattern: 04-34-17-32-17) were singletons with regard to the *spa*-type. The new *spa*-type was assigned to be *spa*-type t6091 and deposited in the Ridom database [21]. The *spa*-type t001 is often thought to be associated with MRSA; therefore, agglutination negative MSSA strains were tested for the presence of SCCmec using primers between the SCCmec and the *orfX* region, as described earlier [18, 23]. Six out of seven agglutination-negative MSSA strains (*spa* types t015, t026, t078, t495, t550, t6091) tested negative in the aforementioned PCRs. This indicates that a SCCmec is not present in these isolates. In one oxacillin-susceptible (oxacillin MIC = 1 mg/L, cefoxitin screen negative) and agglutination-negative strain (*spa* type t001) the PCRs using primers between the SCCmec element and the *orfX* region were positive. As expected, the strain was also *mecA* positive (Table 1). Our isolate was tested for the presence of Pantone Valentine leukocidin (PVL) and was PVL-negative.

The relative sensitivity of the Slidex Staph Plus agglutination test in the MSSA collection was similar to sensitivities reported in the literature [3, 4].

In the MRSA collection, a correct species diagnosis was achieved using the Biotyper 2.0 database in all (240 out of 240) strains. Interestingly, only 94.5% (227 out of 240 strains, $p=0.035$) of the molecularly defined and consecutively collected single-copy MRSA strains were identified as *S. aureus* by the Slidex Staph Plus (bioMérieux) agglutination kit. Thirteen molecularly defined MRSA isolates tested negative and were further characterized by *spa*-typing. Ten of thirteen agglutination negative strains were *spa*-type t001: 3236, 3239, 3249, 3250, 3295, 3311, 3327, 3328, 3334, and 3351. The other MRSA strains were singletons: 3216b was t003, 3254 was t587, and 3255 was t480. The sensitivity of the Slidex Staph Plus agglutination results in the MRSA strain collection was lower compared to the MSSA collection and the difference was statistically significant ($p=0.035$). Forty-nine percent of these strains were of PFGE group 35 (ST 022), 13% were of PFGE group 11 (ST 225), 13% were of PFGE group 13 (ST 008), 2% were of PFGE groups 1 and 7 (ST 228), 4% were of PFGE group 16 (ST 045), and 6% were of other PFGE groups. The distribution of the sequence types was similar to previous reports. To exclude a bias introduced by locally prevalent strains, a molecularly divergent (PFGE) strain collection was also tested. All of the additionally tested 104 MRSA strains were identified as *S. aureus* using the Biotyper 2.0 database. In this strain collection a sensitivity of only 70.1% (73 out of 104) was observed using agglutination by the Slidex Staph Plus kit (bioMérieux). Four strains tested weakly positive after 30-60 s (4 out of 104). If these isolates were counted as questionable then the sensitivity

was 66.3% (69 out of 104). Two different Slidex Staph Plus kit (bioMérieux) Lot IDs were tested by two different blinded investigators to rule out possible differences in test interpretation. The test results were virtually identical and independent of the lot and examiner (data not shown).

To exclude a single manufacturer's antibody composition problem, the Pastorex Staph Plus (Bio-Rad) and Staphytest kits (Oxoid) were additionally tested (Table 2). In the collection of PFGE-divergent strains, a sensitivity of 83.6% (87 out of 104, $p=0.035$) was observed using agglutination by the Pastorex Staph plus kit (Bio-Rad). Eleven strains tested weakly positive after 30-60 s (11 out of 104). If these isolates were counted as questionable (not positive) then the sensitivities were even lower (68 out of 104, $p=1$). The sensitivity was 78.8% (82 out of 104, $p=0.59$) using the Staphytest Plus Kit (Oxoid). Thirteen percent (14 out of 104) tested weakly-positive after 30-60 s (76 out of 104, $p=0.66$). The sensitivity of all of the tested agglutination kits was thus significantly lower compared to the sensitivity archived by MALDI-TOF MS and the Biotyper 2.0 database. Despite the differences between the agglutination tests, a statistical difference was only observed between the Slidex Staph Plus kit and the Pastorex Staph Plus kit (Bio-Rad) when weak positive results (agglutination after 30 s) were interpreted as true positive results. MRSA strains that had tested negative in the agglutination tests were further characterized using *spa*-typing [21] in addition to PFGE-typing. About 71% of these strains were *spa*-type t001 (22 out of 31), 12.9% of the

strains were t480 (4 out of 31), and one strain each was t013, t109 and a new *spa*-type with a repeat pattern (26-30-17-34-17-20-17-12-16). This new *spa*-type was assigned to be *spa*-type t5712 and deposited in the Ridom database [21]. The *spa*-types t001, t013, t109, t480, and t5712 were related to each other. Two other *spa*-types were detected as singletons (t032 and t064). Interestingly, one strain of t008 tested weakly positive or negative (MRSA strain 1565) and one tested positive in the agglutination test (MRSA strain 2748). In the PFGE-divergent MRSA collection the majority (18 of 22) of the *spa*-type t001 characterized strains in the PFGE groups 1 to 10, which corresponds to t001 and t001-relates *spa*-types, were agglutination negative using the Slidex Staph Plus kit. Two isolates each were weak positive and positive. Some strains (2 of 10) clustered in PFGE-group 13, which corresponds to t008 and t064. The majority of the latter isolates were agglutination positive.

Discussion:

Most of the fast agglutination tests for the identification of *S. aureus* lack sensitivity [3, 5, 24] compared to highly accurate, molecularly-based identification approaches [15, 25]. The agglutination kits differ in antibody composition against targets like protein A, certain capsule types, and fibrinogen(Fg)- or fibronectin(Fn)-binding proteins. A comprehensive description of the antibody composition is not given by the manufacturers. Alternative molecularly based methods, however, are time consuming

and a hindrance to a fast identification of *S. aureus*. Previous studies have shown that the MALDI-TOF MS based identification [7, 10-13] could be an alternative method to molecularly-based methods. The sensitivity of MALDI-TOF identification using the Biotyper 2.0 database in a mass-to-charge ratio of 3000 to 15000 Da was high (707 out of 707) and equal to a molecular method (Table 3). In contrast to this there have been controversial discussions about whether the MALDI-TOF MS technology could serve as a surrogate method for the detection of Pantone Valentine Toxin [26, 27]. Interestingly, the sensitivity of the Slidex Staph Plus kit was lower in MRSA strains than in MSSA (98.0% versus 94.5%, $p=0.035$). To rule out an overrepresentation of locally predominant strains, a collection of 104 MRSA with different PFGE patterns were tested. In this collection, the sensitivity of the Slidex Staph Plus kit was even lower (Table 3). To rule out a kit-specific problem, two other agglutination tests were also investigated. These kits showed a sensitivity comparable to that of the Slidex Staph Plus kit. If the weakly positive agglutination results were interpreted as a correct identification, our data suggest that the sensitivity of the Pastorex Staph plus kit (Bio-Rad) was slightly higher. The relative sensitivities were given in the manufacturers' data sheet as 99.2% (n=441) for the Slidex Staph Plus kit, 100% (n=439) for the Pastorex Staph Plus kit, and 97.6% for the Staphytest Plus kit. The tube test for free coagulase was used as the reference method in these studies. Moreover, non-*S. aureus* free coagulase test positive strains and auto-agglutination positive isolates were usually

excluded. The relative specificities were given in the manufacturers' data sheet as 98.9% (n=438) for the Slidex Staph Plus kit, 100% (n=138) for the Pastorex Staph-Plus kit and 97.6% for the Staphytest Plus kit. Compared to our study, little information was given with regard to the reference method of *S. aureus* and non-*S. aureus* isolates by all three manufacturers. A previous study described a relative sensitivity of 93% and a relative specificity of 72.7% for a MRSA collection [28]. Many agglutination tests were therefore enhanced periodically by incorporation of more reagents into the kits. In recently published studies, high relative sensitivities of about 97.9% [3, 4, 29] were published for MSSA and MRSA strain collections. In addition, the relative specificities were given to range from 91.3% (Slidex Staph kit) to 99.1% (Slidex Staph Plus kit) [3]. Several factors could be addressed by the phenomenon of a agglutination negative result. In all agglutination reagents, irrespective of the manufacturer, certain surface located targets were detected with specific antibodies. Well characterized surface proteins e.g. immunoglobulin(Ig) G-Fc-binding protein and von Willebrand factor-binding protein A (SpA), fibronectin (Fn)-binding proteins such as FnBP A and FnBP B, the Fg-binding clumping factor (Clf)-A and ClfB [30] could serve as targets for this approach. In addition, major prevalent capsule types, such as capsule type 5 (CP5) and 8 (CP8) [31], can mask other surface proteins, such as ClfA [32]. Recently, new non-typeable capsular types were described [33-35]. It is likely that these new capsular types, or a modified composition of the capsular types, could also impair agglutination tests.

Moreover, a 230 kDa surface protein has been recently described [36], which was cleaved by plasminogen into to fragments of 175- and 68 kDa respectively, and given the name plasminogen sensitive protein (Pls). It was found that Pls-positive isolates give negative test results in agglutination tests with reagents designed to detect Fn- and Fg-binding proteins and SpA [36]. This protein also interferes with adherence and internalization into eukaryotic cells, indicating that the expression of Fn-binding proteins on the surface in these isolates was reduced [30]. Seven out of 37 isolates were Pls positive [37]. Interestingly, two of these isolates were t001, and both were positive for the gene *pls*. One isolate, however, was not expressing the Pls protein. Nevertheless, the *spa*-type deduced clonal complex was not able to predict impaired invasiveness, which was argued to be associated with the presence of Pls [37]. The difference between our sensitivity data and previously published sensitivities argue for a local emergence of a new MRSA clone, which seems to be associated with *spa*-type t001. Moreover, it seems to be likely that these isolates were different concerning the expression of surface proteins or capsular types, which were targeted by the agglutination tests. Nevertheless, the underlying mechanism remains to be elucidated.

Although infrequent isolates are relatively overrepresented in this collection, our data also caution against the unreflected use of agglutination tests. Interestingly, about 93.5% of the strains, which were false-negative in our agglutination tests, were also *spa*-type t001 or a related *spa*-type (t013, t109, t480 and the new *spa*-type t5712). In the PFGE-

divergent MRSA collection, the majority (18 of 22) of the agglutination false-negative and *spa*-type t001 strains clustered in PFGE-group 1 to 10, which corresponds to t001 and t001-related strains. The majority of strains (8 of 10), which clustered in PFGE-group 13 (t008 and t064), were agglutination positive. Two out of four isolates previously characterized as t008 were described to be Pls-positive [37]. Single strains, characterized as t001 and t001-related, were agglutination positive and indicate that this phenomenon is not strictly associated with the *spa*-type. The distribution of the *spa*-types of agglutination negative strains in the MSSA collection seems to be different from that of the MRSA collections. As a limitation of the MSSA and first MRSA strain collection (consecutively collected) no molecular epidemiology data, such as *spa*-types were available for all isolates. No data thus could be given with regard to the prevalence of *spa*-type t001 and agglutination results in these collections. In the PFGE-divergent MRSA collection the majority (82%) of the *spa*-type t001 characterized strains in the PFGE groups 1 to 10 were agglutination negative using the Slidex Staph Plus kit. One strain, which was primarily included as MSSA using the EUCAST cut-off value for oxacillin of ≤ 2 and a negative cefoxitin screening, was also characterized as *spa*-type t001 (Table 1). This strain was positive for the *mecA*-gene and also the SCCmec/orfX-based PCR to amplify the orfX-region [23]. In the literature SCCmec/orfX negative and *mecA*-positive MRSA strains due to for example variation of the SCCmec/orfX-region, were described. SCCmec/orfX positive and *mecA*-negative MSSA strains, which have

lost the *mecA*-gene, were described also [38]. Moreover, fewer mutations within the FemXAB protein family were described for phenotypic oxacillin susceptible phenotypes in *mecA*-positive *S. aureus* isolates [39].

In conclusion, our results argue for an epidemic emergence of a MRSA-strain with an altered surface structure, thus allowing for the agglutination tests to produce false-negative results. Our results indicate that latex agglutination could miss a significant amount of MRSA depending on the local prevalence of t001 and related strains.

Moreover, our data support testing oxacillin-susceptible (oxacillin MIC ≤ 2 mg/L), Slidex Staph Plus kit agglutination negative *S. aureus* isolates for the presence of *mecA* to rule out MRSA-isolates with a phenotypic oxacillin MIC below the cut-off value given by EUCAST guidelines. MALDI-TOF MS based identification required more time than agglutination based methods, but the identification was clearly more accurate. Our results suggest using an alternative identification method in suspected false-negative agglutination tests to increase sensitivity of *S. aureus* identification.

Conflict of interests statement

The authors certify that there is no actual nor potential conflict in relation to this article.

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Table 1: Characterization of *S. aureus* strains used in this study

S. aureus strains used in this study were identified by molecular methods and MALDI-TOF MS-based identification. The phenotypic characterization results for oxacillin MIC, the Vitek2 cefoxitin screening test result (AST P459 card) and the PCR results for the detection of *mecA*-gene and SCCmec (*orfX*) were given together with the Slidex Staph Plus (bioMérieux) identification test results.

Results were given as + for positive and - for negative test results. * isolate was misclassified as MSSA using phenotypic tests. ND = Not determined

Table 2: Sensitivity of the *S. aureus* agglutination kits in a collection of 104 pulsed-field-gel electrophoresis divergent strains.

Three agglutination kits were investigated in a MRSA strain collection of 104 pulsed-field-gel electrophoresis divergently tested MRSA strains using the Slidex Staph Plus (bioMérieux), Pastorex Staph Plus (Bio-Rad) and Staphytest Plus kits (Oxoid). Results were given as p (positive), neg (negative) and w (weakly) positive after 30-60 s. The other strains not given in the table were consistent test positive in all agglutination kits. PFGE group “34” represents a group of singletons. Most of the agglutination negatively tested strains were *spa*-type t001 (22 out of 31 strains) and t001 related (t013, t109, t480 and a new *spa*-type t5712 ; 7 out of 31 strains).

Table 3: Agglutination test and MALDI-TOF MS identification results of three different *S. aureus* strain collections

The sensitivity of the Slidex Staph Plus (bioMérieux) was tested in a strain collection of molecularly defined MSSA (1) and MRSA (2). To rule out a dominance local strains, a third collection of 104 pulsed-field-gel electrophoresis (PFGE) divergent strains were included and tested with the Pastorex Staph Plus (Bio-Rad) and Staphylect Plus kit (Oxoid) and the Slidex Staph Plus (bioMérieux) agglutination test. The relative sensitivity of MALDI-TOF MS based *S. aureus* identification was superior to all tested agglutination kits.

Strain collection	Isolates n=	Slidex Staph plus n=	OrfX-PCR MecA-PCR		Oxacillin MIC	Oxacillin MIC	Cefoxitin screen
			OrfX-PCR	MecA-PCR	≤ 2	> 2	
MSSA	356	+	ND	ND	+		-
	6	-	-	ND	+		-
(MRSA)*	1*	-	+	+	+		-
MRSA	225	+	+	ND		+	ND
consecutive collected	1	+	+	+	+		+
	13	-	+	+		+	+
MRSA	66	+	ND	+		+	+
molecularly divergent	1	+	ND	+		+	-
	5	+	ND	+	+		+
	1	+	ND	+	+		-
	31	-	ND	+		+	+

strain	species	PFGE-group	subgroup	spa-type	Slidex Staph plus	Staphylect Plus	Pastorex Staph Plus
1497	MRSA	01	2	001	neg	neg	p
2397	MRSA	02	1	001	neg	neg	neg
1352	MRSA	02	4	001	neg	neg	neg
2387	MRSA	02	7	001	neg	neg	neg
1583	MRSA	02	8	480	neg	neg	neg
2052	MRSA	02	9	480	neg	w	neg
1867	MRSA	02		001	neg	w	p
2615	MRSA	03	1	001	neg	neg	w
1874	MRSA	03		013	neg	neg	neg
0680	MRSA	05		001	neg	neg	w
2021	MRSA	06		001	neg	neg	p
2682	MRSA	07	10	001	neg	neg	w
2029	MRSA	07	11	001	neg	w	neg
1827	MRSA	07	3	001	neg	w	p
1655	MRSA	07	4	001	neg	neg	neg
2131	MRSA	07	6	001	neg	p	neg
1343	MRSA	07	7	001	neg	neg	neg
2610	MRSA	07		001	neg	w	p
2770	MRSA	09		001	neg	neg	w
2293	MRSA	10		001	w	w	w
1496	MRSA	11	1	001	neg	w	p
1565	MRSA	13	3	008	w	neg	neg
1651	MRSA	13	9	064	neg	neg	neg
1578	MRSA	14	3	109	neg	neg	w
1321	MRSA	22	2	001	neg	neg	neg
2239	MRSA	22	3	001	w	w	neg
0673	MRSA	29		480	neg	w	w
1518	MRSA	34		480	neg	neg	p
2450	MRSA	34		001	neg	neg	neg
2487	MRSA	35	13	032	neg	neg	neg
1452	MRSA	36		001	neg	neg	neg
0992	MRSA	43		5712	neg	neg	neg
1367	MRSA	45		001	neg	w	w
2351	MRSA	34		001	neg	w	w

Strain collection	Number of strains	Agglutination test results	Correct identification by MALDI-TOF MS n=	Sensitivity %	Slidex Staph plus n=	Sensitivity %	Staphylect Plus n=	Sensitivity %	Pastorex Staph Plus n=	Sensitivity %
1) MSSA	363	positive weakly positive negative	363	100	356 0 7	98	ND ND ND		ND ND ND	
2) MRSA	240	positive weakly positive negative	240	100	227 0 13	93.5	ND ND ND		ND ND ND	
3) MRSA	104	positive weakly positive negative relative sensitivity	104	100	69 4 31	66.3 3.8 70.1	68 14 22	65.4 13.4 78.8	75 11 18	72.1 10.6 82.7