Evaluation of a new *Staphylococcus aureus* latex agglutination kit, Prolex Staph Xtra, against other third-generation kits

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ABSTRACT

Staphylococcus aureus, including methicillin-resistant strains, continues to be a common cause of infection/colonisation, which necessitates accurate and prompt diagnosis in the laboratory. Several rapid agglutination tests that aid this function are available, and some have been modified to improve their performance. One such kit, Prolex Staph Xtra, has been released recently. This study aims to compare this kit with other improved kits (i.e., Pastorex Staph-Plus, Staphaurex Plus and Staphytect Plus) and investigate their ability to confirm the identity of 100 strains of *S. aureus*. Results showed that 50 were resistant to methicillin. Specificity was checked against 30 strains of coagulase-negative staphylococci and 20 *Enterococcus* species isolates. Of the four kits tested, Prolex Staph Xtra and Pastorex Staph-Plus proved superior in terms of sensitivity and speed.

KEY WORDS: Latex fixation tests.
Staphylococcus.
Methicillin resistance.

Introduction

Staphylococcus aureus continues to play an important role in the field of medical microbiology, with a recent report showing that 45% of infections are caused by S. aureus, of which 62% were methicillin-resistant *S. aureus* (MRSA).[1] In 2001 the mandatory reporting of *S. aureus* bacteraemia (including MRSA) was introduced and shows that between April 2005 and March 2006, *S. aureus* accounted for approximately 18,000 bacteraemia cases, of which 40% were MRSA.[2]

The ability to recognise the colonial appearance of *S. aureus* and the use of an accurate and rapid *S. aureus* agglutination test are fundamental in an efficient microbiology laboratory. Previously, the authors evaluated the agglutination ability of several kits when taking colonies from Columbia blood agar, Mannitol-salt agar and Baird-Parker medium.[3] With the routine use of chromogenic media for MRSA now in place, and the introduction of modified rapid agglutination reagents, including the Prolex Staph Xtra method, it was decided to revisit this work.

It has been shown that certain strains of MRSA produce undetectable amounts of clumping factor and protein A, giving false-negative agglutination results.[4-6] One particular antigen, capsular serotype 5, has been particularly associated with this phenotypic phenomenon.[7] However, the latest rapid agglutination kits should take this into account and incorporate additional antigen detection systems.

The present study aims to compare the ability of four commercially available rapid agglutination kits against 100 strains of *S. aureus*, including 50 MRSA strains. All 100 strains were tested from Columbia blood agar (CBA) and the 50 MRSA strains were also tested from a chromogenic medium, MRSA*Select* (Bio-Rad). The two kits from the previous study that produced superior results are also included in the study. All apparent discrepant results are retested on two further occasions to ensure the accuracy of the data produced.

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Materials and methods

The 50 methicillin-sensitive *S. aureus* (MSSA) strains were consecutive routine isolates. The MRSA strains, however, were mainly isolates from previous study collections and included many of the historical epidemic strains. Justification for not solely using routinely isolated MRSA strains was to ensure that a wide range of strains other than the regional EMRSA 15 and 16 strains were tested.

All *S. aureus* strains were identified using a combination of heat-labile DNase and colour on CHROMagar Staph aureus (BioConnections), as well as the rapid agglutination result. Any discrepant results were identified biochemically using API Staph. The MRSA strains were either NCTC strains or previously isolated strains that had been sent to the Staphylococcal Reference Laboratory (HPA, Colindale, UK) for confirmation of identification and epidemiological typing.

The kits tested were Pastorex Staph-Plus (Bio-Rad), Staphaurex Plus (Remel), Staphytect Plus (Oxoid) and the new Prolex Staph Xtra (Pro-Lab Diagnostics). The antigen detection targets are given in Table 1.

All 100 stains were subcultured on and tested from prepoured CBA (Oxoid). The 50 MRSA strains were also subcultured on and tested from the department's routine MRSA medium, prepoured MRSA*Select* (Bio-Rad).

Table 1. Detection methods employed by the various rapid agglutination kits.

	Antibody label	Clumping factor	Protein A	Other factors
Prolex Staph Xtra	Blue latex	Y	Y	Capsular polysaccharides
Pastorex Staph-Plus	Red latex	Y	Y	Capsular polysaccharide
Staphaurex Plus	Yellow latex	Y	Y	Specific IgG and cell surface antigens
Staphytect Plus	Blue latex	Y	Y	Capsular polysaccharides

Table 2. Manufacturers' procedural instructions.

	Number of colonies	Maximum rocking time (sec)
Prolex Staph Xtra	2	20
Pastorex Staph-Plus	1–3	30
Staphaurex Plus	6	30
Staphtect Plus	5	20

In addition, 30 routine strains of coagulase-negative staphylococci (CNS) and 20 routine strains of *Enterococcus* species were tested to determine whether or not the kits had specificity problems and were prone to false-positive results.

All tests were carried out according to the manufacturers' recommendations (Table 2).

Results

Using CBA, all kits confirmed the identification of the 50 strains of MSSA within the allotted manufacturers' rocking period. Pastorex Staph-Plus and Prolex Staph Xtra also detected the 50 MRSA strains within the recommended rocking period. However, Staphaurex Plus and Staphytect Plus failed to detect five and three strains, respectively. Of the five false-negative strains with Staphaurex Plus, two gave agglutination reactions in 30–60 sec, one in 60–90 sec and the final two strains showed no agglutination within 120 sec of rocking. Of the three false-negative results with Staphytect Plus, all gave agglutination in 30–60 sec.

Using MRSA*Select*, 48 of the 50 strains grew, indicating that although the new cefoxitin-containing chromogenic medium performs admirably with current MRSA strains, the methodology is not 100% foolproof. Pastorex Staph-Plus and Prolex Staph Xtra gave the anticipated results, whereas Staphaurex Plus and Staphytect Plus gave false-negative results with four and three strains, respectively. The detailed breakdown of time taken to agglutination for all the kits is given in Table 3. With Staphaurex Plus and Staphytect Plus, the occasional MRSA strain that was detectable with colonies taken from CBA became undetectable when taken from MRSA*Select*. Surprisingly, the reverse scenario also occurred, where strains that previously did not give agglutination within the recommended time with colonies taken from CBA became detectable when taken from MRSA*Select*. This suggests that medium constituents have an effect on the amount of antigen produced, although this was not apparent from the results obtained with Pastorex Staph-Plus and Prolex Staph Xtra.

The specificity of the kits is also worthy of mention. Pastorex Staph-Plus and Prolex Staph Xtra performed similarly. None of the 30 CNS strains gave false-positive reactions, but cross-reactions were seen with the *Enterococcus* species. Prolex Staph Xtra gave seven apparent false-positive results within the recommended rocking period. On six occasions, the strains gave reasonable agglutination with the negative control latex, thus invalidating the test. On one occasion, however, the latex negative control did not agglutinate, suggesting a slight problem with antibody specificity. Pastorex Staph-Plus gave nine false-positive results, six of which were invalidated as above; however, on three occasions the latex negative control did not agglutinate, providing true false-positive results. Staphytect Plus gave cross- reactions with one CNS and one *Enterococcus* species, and on each occasion this was an antibody specificity problem. Staphaurex Plus gave no cross-reactions.

Discussion

As suggested earlier, the ability to confirm quickly the identity of *S. aureus* is fundamental to all microbiology departments. An ideal combination of accuracy and speed is required. Reading the product inserts confirms that all the manufacturers have attempted to achieve such goals. All kits

Table 3. Time to detection of MRSA strains taken from Columbia blood agar ($n=50$) and MRSA*Select* ($n=48$).

	Within kit time limit		30–60 sec		60–120 sec		Negative at 120 sec	
	CBA	*Select*	CBA	*Select*	CBA	*Select*	CBA	*Select*
Prolex Staph Xtra	50	48	0	0	0	0	0	0
Pastorex Staph-Plus	50	48	0	0	0	0	0	0
Staphaurex Plus	45	44	2	3	1	0	2	1
Staphytect Plus	47	45	3	1	0	1	0	1

gave 100% antigen detection rates for the 50 MSSA strains tested. Two of the kits, Pastorex Staph-Plus and the new Prolex Staph Xtra, achieved this goal with the 50 MRSA strains tested, producing consistently reliable results with colonies taken from either CBA or MRSA*Select*.

Staphytect Plus and Staphaurex Plus gave good results for MRSA but failed to detect some strains within the recommended rocking period from both CBA and MRSA*Select*. Staphytect Plus gave a sensitivity of 94% with both media, whereas Staphaurex Plus achieved a sensitivity of 90% and 92% with colonies taken from CBA and MRSA*Select*, respectively. Reasons why some strains could be detected on CBA and not on MRSA*Select*, and vice versa, needs further investigation. However, these discrepancies were double-checked to confirm their accuracy.

Traditionally, latex agglutination procedures involve an antibody (or antigen) coating on the surface of latex particles, resulting in sensitised latex particles. When a sample containing the specific antigen is mixed with the milky sensitised latex, it causes visible agglutination. The degree and visual standard of agglutination is dependent on the quality of the antibody or antigen used, its method of coating to the latex particle, and the initial quality of the latex particle.

Approximately 100 agglutinants (clumps) are required to produce agglutination and form an aggregate that is visible to the human eye. The selection of the appropriate size and quality of latex particle, and the method employed to bind the antibody or antigen, is of paramount importance in this process.

Prolex Staph Xtra uses carboxylate-modified microparticles (CM-MP), which are thought to offer advantages over traditional latex reactions. Reagent stability and reactivity are enhanced by covalently attaching antigens and antibody to functionalised microparticles.[8] Several approaches have been developed to achieve this goal, the most common of which employs CM-MP. The CM-MP used in Prolex Staph Xtra incorporates a distinct blue dye that is not surface-bound and hence should not interfere with the functionality of the particle. It is 1 µm in diameter, aiding rapid visual recognition and in theory giving faster reaction times.

In the present study, Prolex Staph Xtra kit performed excellently, as did Pastorex Staph-Plus. The fact that the manufacturer recommends the use of two colonies and that positive reactions should take less than 20 seconds illustrates confidence in the test.

In this study, the kits were tested in accordance with the manufacturers' instructions. An alternative method of determining the sensitivity of a procedure is to use a minimum number of colonies for all the kits tested. If two colonies are used, as recommended for Prolex Staph Xtra and Pastorex Staph-Plus (1–3 colonies), the difference in the efficiency of the above two kits against Staphytect Plus and Staphaurex Plus is even more pronounced. Once again, Prolex Staph Xtra and Pastorex Staph-Plus performed similarly.

The 50 MSSA strains needed a total of approximately 375 seconds rocking time for agglutination (average: 7.5 sec per strain) and the 50 MRSA strains needed a total of approximately 750 seconds rocking time (average: 15 seconds) with colonies taken from either medium. Staphaurex Plus equivalent rocking times were approximately double these values, as were times for the MRSA strains with Staphytect Plus. Staphytect Plus performed better with the 50 MSSA strains, needing 530 seconds rocking time for visible agglutination to occur (average: 10.6 sec).

The cross-reaction seen with enterococci is not a major issue and can be overcome easily by using the negative latex control (following the manufacturer's instructions) and observing the different colonial appearances, even with colonies taken from MRSA*Select*.

Overall, using current MSSA strains and both current and historical MRSA strains, Prolex Staph Xtra performed very well, as did Pastorex Staph-Plus. Thus, the use of either of these rapid agglutination methods is strongly recommended for the confirmation of S. aureus, whether MRSA or MSSA. □

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