Technical methods

Staphaurex reappraised: role of protein A in false positive results and recommendations for use

ALBERT J MIFSUD, SUSAN C PARKER *Department of Medical Microbiology, London Hospital, London*

The production of free coagulase, as detected by the tube coagulase test, is considered to be the definitive means of identifying *Staphylococcus aureus*.¹ This test, however, may take 24 hours before a result is obtained. Although quick, the slide coagulase test has a false negative rate between 3% and 15%.^{2,3} Another commonly used test, the DNase test, rarely gives false negative results but requires 24 hours' incubation.⁴

In an attempt to overcome these problems a new latex agglutination kit (Staphaurex) has been introduced. This consists of latex particles coated with fibrinogen and IgG, which detect clumping factor and protein A, respectively. We tested this reagent with 480 human isolates of staphylococci. We also investigated the role of protein A in causing false positive results with Staphaurex.

Material and methods

Isolates were obtained from routine specimens submitted to our laboratory for analysis. Potentially clinically important isolates of staphylococci were

selected and submitted to tube coagulase,⁵ slide coagulase,⁵ and DNase⁴ testing. Staphaurex testing was also performed in duplicate, once following the manufacturer's instructions and once using only 20 µl of reagent.

Organisms which gave positive results with all three reference tests were identified as *S aureus*. Isolates which gave negative results on tube coagulase and DNase testing and which were negative on slide coagulase testing or which autoagglutinated in saline were classified as non-*S aureus* staphylococci. Organisms which gave conflicting results were identified biochemically by the APIStaph system. These strains were also tested for the presence of protein A by a single, mixed tube agglutination method.⁶

Isolates identified as *S aureus* were then tested for methicillin sensitivity in the routine laboratory by a disc diffusion technique.⁷

Results

Table 1 summarises the results. Table 2 shows the sensitivity, specificity and predictive values of positive and negative results. There was complete correlation between results obtained using the different volumes of Staphaurex reagent.

Discussion

The latex test was extremely sensitive and compared favourably with other tests commonly used for identification of *S aureus*, both methicillin resistant and methicillin sensitive strains found in the North East Thames region. Furthermore, the Staphaurex test gave a clear cut difference between positive and negative reactions without problems of autoagglutination.

Table 1 Comparison of Staphaurex testing with reference methods in 480 isolates of *Staphylococci*

No of strains	Tests					
	Tube coagulase	Slide coagulase	DNase	Staphaurex	Identification	Protein A
135	+	+	+	+	<i>S aureus</i> (Meth S)	
36	+	+	+	+	<i>S aureus</i> (Meth R)	
283	—	—	—	—	Non- <i>S aureus</i> staphylococci	
12	—	AA	—	—	Non- <i>S aureus</i> staphylococci	
2	—	+	+	+	<i>S aureus</i> (Meth S)	+
4	+	—	+	+	<i>S aureus</i> (Meth S)	+
1	—	+	—	—	<i>S epidermidis</i>	—
1	—	+	—	+	<i>S hominis</i>	—
2	—	—	+	—	<i>S simulans</i>	—
1	—	—	—	+	<i>S warneri</i>	—
1	—	—	—	+	<i>S saprophyticus</i>	—
2	—	—	—	+	<i>S saprophyticus</i>	+

+ = positive result; — = negative result.

AA = autoagglutinating strains; Meth S = methicillin sensitive; Meth R = methicillin resistant.

Table 2 Percentage sensitivity, specificity, and predictive values of positive and negative results on Staphaurex testing compared with reference methods

	Tube coagulase test	Slide coagulase test	DNase test	Staphaurex
Sensitivity	98.9	97.7	100	100
Specificity	100	99.3	99.3	98.3
Predictive value of positive result	100	98.9	98.9	97.3
Predictive value of negative result	99.3	98.6	100	100

Although false positive Staphaurex reactions have been described with non-*S aureus* staphylococci, previous workers have not attempted to investigate this further.^{2 8 9} The production of protein A by some isolates of *S saprophyticus* explains some of the false positive results. Three of the five organisms which were falsely positive with Staphaurex in our study were *S saprophyticus*. This limits its use in testing urinary isolates.

Staphaurex reagent may be expensive. The use of 20 µl for each test instead of the 50 µl dispensed by the droppers supplied in the kit should, however, substantially reduce the cost of this useful test.

References

- 1 Parker MT. Staphylococcus and Micrococcus. In: Parker MT, ed. *Topley and Wilson's principles of bacteriology, virology and immunology*, 2. London: Edward Arnold, 1984:228.
- 2 Berke A, Tilton RC. Evaluation of rapid coagulase methods for the identification of *S aureus*. *J Clin Microbiol* 1986;23:916-9.
- 3 Kloos WE, Jorgensen JH. Staphylococci. In: Lenette EH, ed. *Manual of clinical microbiology*. Washington DC: American

- Society for Microbiology, 1985:143-53.
- 4 MacFaddin JF. DNase and thermonuclease tests. In: *Biochemical tests for identification of medical bacteria*. Baltimore: Williams and Wilkins, 1980:99.
- 5 Cowan ST. Characterisation test methods. In: *Cowan and Steel's manual for the identification of medical bacteria*. Cambridge: Cambridge University Press, 1974:171.
- 6 Maxim PE, Matthews HL, Mengoli HF. Single tube mixed agglutination test for the detection of Staphylococcal protein A. *J Clin Microbiol* 1976;4:418-22.
- 7 Barry AL, Thornsberry C. Susceptibility Tests: Diffusion test procedures. In: Lenette EH, ed. *Manual of clinical microbiology*, 4. Washington DC: American Society for Microbiology, 1985:978-87.
- 8 Smith SM, Berezny C. Comparative evaluation of identification systems for testing methicillin resistant strains of *Staphylococcus aureus*. *J Clin Microbiol* 1986;24:173-6.
- 9 Dickson JJ, Marples RR. Coagulase production by strains of *Staphylococcus aureus* of differing resistance characters: a comparison of two traditional methods with a latex agglutination system detecting both clumping factor and Protein A. *J Clin Pathol* 1986;39:371-5.

Requests for reprints to: Dr AJ Mifsud, Department of Medical Microbiology, The London Hospital, Whitechapel, London E1 1BB, England.

Storage and recovery of small aliquots of frozen red cells for anti-D boosting

G S GABRA, E A MCLAREN, W MUIR, R MITCHELL
Glasgow and West of Scotland Blood Transfusion
Service, Law Hospital, Carlisle, Scotland

The boosting of volunteers with frozen recovered red cells from accredited donors was recommended by the World Health Organisation in 1981.¹ Frozen recovered red cells were successfully used in routine boosting of eight Rh D negative women previously immunised by pregnancy,² and for immunising and boosting Rh negative male volunteers.³ In view of the lack of specific markers for non-A non-B hepatitis and the possibility of transmitting human immunodeficiency virus to donors who volunteer to join the boosting programme, it is now even more important to implement all available safety measures. The safety

of immunisation and boosting programmes could be improved by storing frozen red cells from accredited donors in small aliquots. Each volunteer could then be boosted using the same donor cells during the whole programme so as to minimise antigenic exposure.

Red cell donations were selected from a panel of R2R2 longstanding regular donors and were phenotyped for the following antigens: ABO, rhesus, M, N, S, s, K, k, P, Le^a, Le^b, Fy^a, Fy^b, Lu^a, Jk^a, Jk^b. This allowed selection of suitable boosting cells and avoided the development of unwanted irregular antibodies.³ At each donation tests of liver function and for hepatitis B virus markers (hepatitis B surface antigen, hepatitis B surface antibody, and hepatitis B core antibody) were performed. Archive serum samples were used to test for antibody to human immunodeficiency virus to ensure that all available frozen cells were negative.

We describe a method using polyolefin freezing bags for storing and recovering Rh positive red cells in small aliquots for boosting. This method satisfies safety requirements and conforms with criteria of good manufacturing practice.⁴