Short reports

Comparison of commercial slide agglutination kits with a tube coagulase test for the rapid identification of *Staphylococcus aureus* from blood culture

R P D Cooke, C T Jenkins

Abstract

Eighty clinical specimens of BACTEC 9240 blood culture vials, culture positive for staphylococci (38 *Staphylococcus aureus* and 42 coagulase negative staphylococci), were tested directly for the presence of clumping factor/protein A and free coagulase. Seven commercial slide agglutination kits were compared with a direct-tube coagulase (DTC) method. All tests were performed on blood culture pellets. Sensitivity, specificity, and negative and positive predictive values for the seven commercial kits were extremely variable, whereas a two-hour DTC test was highly predictive of *S aureus*. There was no significant difference between a two-, six- or 24-hour DTC test. Three (8%) *S aureus* isolates remained DTC negative even after 24 hours' incubation. Staphylococcal slide agglutination kits should not be used directly on blood culture broths. In contrast, a two-hour DTC test is a useful, rapid screening test for *S aureus* bacteraemia, provided isolates from DTC negative blood culture broths are re-tested using standard laboratory techniques.

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Keywords: blood culture; *Staphylococcus aureus*; slide agglutination; direct-tube coagulase.

Staphylococci are one of the most common isolates from blood culture specimens,¹ and may be reliably distinguished from streptococci by Gram stain of the blood culture broth.² Though the early distinction between *Staphylococcus aureus* and coagulase negative staphylococci (CNS) may be helpful in initiating appropriate antimicrobial treatment, few laboratories in the UK proceed to rapid identification tests if staphylococci are suspected in blood culture media.³ Those who do test directly usually prefer a tube coagulase method to detect free coagulase rather than a slide agglutination test which detects clumping factor or protein A, or both.³

Commercial slide agglutination kits for the identification of *S aureus* are widely used as they are particularly convenient and technically simple. However, they have been principally designed for isolates grown on solid culture media.⁴ Previous reports have suggested that sensitivity, though not specificity, may be reduced if slide agglutination tests are performed directly from blood culture broths.⁵⁻¹⁰ Such studies have often excluded many of the commercial kits commonly available in the UK. We have therefore re-evaluated the role of the slide agglutination method for the rapid identification of *S aureus* from patients with bacteraemia and compared its performance with a direct-tube coagulase (DTC) test.

Methods

BACTEC Plus/F blood culture vials from patients with suspected bacteraemia were studied retrospectively using the BACTEC 9240 system. Vials, culture positive for *S aureus* or CNS, were stored at -20°C pending analysis.

Blood culture broth (8-10 ml) was centrifuged at 700 rpm for 10 minutes to remove red blood cells. The supernatant was then centrifuged at 3000 rpm for a further 10 minutes. The pellet was suspended in 0.5 ml sterile saline.^{7,8} Slide agglutination tests were performed using one drop of the pellet suspension. The following commercial slide agglutination kits were tested according to the manufacturers' protocols: Prolex Staph Latex Kit (Pro-Lab Diagnostics, Neston, UK), Slidex Staph Kit (bioMérieux, Basingstoke, UK), Staphyslide-Test (bioMérieux), Staphylococcus aureus Latex (The Binding Site Ltd, Birmingham, UK), Staphaurex (Murex Diagnostics Ltd, Dartford, UK), Staphytect (Unipath Ltd, Basingstoke, UK), and Bacto Staph Latex (Difco Laboratories, West Molesey, UK). All the kits detect clumping factor and protein A except Staphyslide (clumping factor only). Apart from Bacto Staph Latex and Staphaurex, manufacturers include control reagents with their kits. Results were recorded

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Table 1 Performance of slide agglutination kits

Kit	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	PPV	NPV
Prolex Staph Latex	8	38	4	30	21	91	67	56
Slidex Staph	8	37	5	30	21	88	67	55
Staphyslide test	2	38	4	36	5	91	33	51
Staphylococcus aureus Latex	13	16	26	25	34	38	33	39
Staphaurex	14	35	7	24	37	83	67	59
Staphytest	8	38	4	30	21	91	67	56
Bacto Staph Latex test	28	33	9	10	74	79	76	77

PPV = positive predictive value; NPV = negative predictive value.

as positive or negative (including non-specific reactions). Non-specific reactions were noted if agglutination was granular/stringy or recorded in the control reagent. False positive reactions were defined as a positive agglutination reaction from a blood culture which subsequently grew CNS. Each manufacturer's product information insert was also analysed to determine whether the identification of *S. aureus* direct from blood culture was recommended or commented upon.

A DTC test was performed by adding four drops of pellet suspension to nutrient broth containing 10% human plasma (Blood Transfusion Service). Broths were incubated at 37°C and examined for clot formation at one, two, six, and 24 hours. Results were recorded as either positive or negative. Findings were evaluated statistically using the χ^2 test.

All blood culture broths were subcultured to check for viability and purity. Identification of *S. aureus* and CNS was confirmed using standard laboratory protocols including Gram stain, presence of clumping factor/protein A (Staphytest) and DNase activity. *S. aureus* was defined as a staphylococcus which was clumping factor/protein A positive, DNase positive, and DTC positive (or tube coagulase positive from plate culture if the DTC test was negative).

Results

Eighty culture positive vials were studied, of which 38 grew *S. aureus* and 42 CNS. For slide agglutination kits, overall sensitivities were low and specificities high with considerable variation between the performances of individual kits. Neither positive nor negative predictive values of any of the kits tested exceeded 80%. Ten non-specific reactions were noted (two Staphaurex, three Staphylococcus aureus Latex, one Prolex Staph Latex, two Staphyslide, two Slidex Staph). In contrast, a two-hour DTC test was highly predictive of *S. aureus*. Sensitivity, specificity, and positive and negative predictive values were 87%, 100%, 100%, and 90% respectively. Three (8%) *S. aureus* isolates remained DTC negative even after 24 hours' incubation. All were tube coagulase positive from plate cultures. The negative pre-

dictive values of a two-, six- and 24-hour DTC test were not significantly different ($p > 0.05$). An analysis of the results is presented in tables 1 and 2.

Apart from bioMérieux, manufacturers did not comment on the use of their kits direct from blood culture.

Discussion

This study confirms the view that slide agglutination kits should not be used for the rapid identification of *S. aureus* direct from BACTEC blood culture media.⁹ Of the seven commercial kits evaluated, only two (Staphaurex and Bacto Staph Latex) have been previously studied as rapid identification tests. Bacto Staph Latex has been examined only once before and performed less well than in this report: sensitivity 59% and negative predictive value 64%.⁸ Staphaurex is a commonly used staphylococcal identification kit in the UK. Previous reports have produced sensitivities varying from 12.8%¹⁰ to 77.3%.⁸ Staphaurex's previously quoted high sensitivity may explain why four UK laboratories in a 1992 survey opted to use a staphylococcal latex kit for the direct identification of positive blood cultures.³

The design of previous clinical evaluations of commercial slide agglutination kits has been extremely variable. Some workers have used seeded blood cultures, others clinical cultures. All the evaluations have used limited numbers of isolates (precluding statistical analysis of the results), variable inocula, different incubation times, and diverse media types. Nevertheless, all have reached the conclusion that staphylococcal agglutination kits have not been designed for direct use on blood culture broths and lack sensitivity. This should be made clear in the kits' product insert information. From our survey, only one manufacturer (bioMérieux) currently gives such advice.

In contrast to slide agglutination methods, this study has shown that a two-hour DTC test is highly predictive of *S. aureus*. Using BACTEC NR blood culture media, Claxton *et al* found a relatively low sensitivity of 65.5% but a high negative predictive value of 90.6%.⁹ However, other workers using BACTEC NR

Table 2 Performance of direct-tube coagulase test

True positive	Time (hours)	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	PPV	NPV
26	1	42	0	12	68	100	100	78
33	2	42	0	5	87	100	100	89
34	6	42	0	4	90	100	100	91
35	24	42	0	3	92	100	100	93

PPV = positive predictive value; NPV = negative predictive value.

media and the DTC test have shown higher sensitivities, 98%⁵ and 86.2%,¹⁰ figures comparable with our own results. Furthermore, a two-hour reading is as reliable as a test read at six or 24 hours' incubation.⁵⁻¹⁰ Staphylococci cultured from screen negative blood culture broths should, however, be re-tested for free coagulase and/or clumping factor/protein A plus DNase.

If the DTC test is routinely combined with tests for clumping factor/protein A and DNase from plate cultures, mis-identification of coagulase-positive staphylococci should be avoided. *Staphylococcus intermedius*, a potentially invasive pathogen from dog-bite wounds, is usually tube coagulase positive (rabbit plasma positive, human plasma variable), clumping factor/protein A negative but DNase positive. *Staphylococcus cugdumensis*, associated with a variety of infections including endocarditis, is typically tube coagulase negative, clumping factor positive and weakly DNase positive. *S. aureus* should be positive for all three tests.

Of other potential rapid diagnostic methods for *S. aureus* bacteraemia, the rapid thermonuclease test seems to be as insensitive as slide agglutination,⁵ whereas a DNA probe method is currently not cost-effective. The aurease test is rarely used.³ The DTC test remains a simple, cheap, rapid, and reliable method available to all diagnostic laboratories. Medical microbiology departments may be deterred from its use (only 22 of 287 UK laboratories performed a DTC test in 1992³) because of the commonly used, but labour intensive, double centrifuga-

tion step.⁶⁻⁹ However two recent reports have indicated no loss of sensitivity when the DTC test is performed directly on uncentrifuged blood culture broths.⁵⁻¹⁰ This finding should encourage laboratories to use the DTC test routinely for the rapid identification of *S. aureus* from positive blood cultures.

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Bone marrow granulomas in infiltrating lobular breast cancer

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Abstract

A 50 year old woman with a history of infiltrating lobular breast carcinoma presented with back pain. Bone scan and magnetic resonance imaging were not conclusive. A bone marrow aspirate appeared normal. A routine trephine biopsy specimen showed granulomas but no obvious infiltration by carcinoma. Immunohistochemical staining with epithelial markers demonstrated carcinoma cells in the trephine specimen. This case illustrates the difficulty of detecting infiltrating lobular carcinoma in bone marrow and the value of immunological techniques in this context. It also describes the

development of bone marrow granulomas as a response to infiltration by carcinoma. (*J Clin Pathol* 1997;50:166-168)

Keywords: bone marrow granulomas; lobular breast cancer; immunohistology.

Granuloma formation is well recognised in lymph nodes draining an area of carcinoma. Granuloma formation in bone marrow has a wide differential diagnosis, including granulomatous infections, sarcoïd, drug hypersensitivity, lymphoid, and haemopoietic malignancy.¹ Metastatic carcinoma does not seem to be a widely recognised cause.