Rapid identification of bacterial antigen in blood cultures and cerebrospinal fluid

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SUMMARY The ability of antibody coated latex particle suspensions to aid the diagnosis of septicaemia and meningitis in children was assessed in 272 blood culture sets and 85 cerebrospinal fluid specimens. This was also assessed in cases caused by *Neisseria meningitidis* group B infection.

The method was 100% sensitive and 99% specific, with good predictive values (range 63% to 100%) and provided valuable support to the routine bacteriological examination of blood culture and cerebrospinal fluid.

The production of a clinically useful bacteriological report depends on the time it takes for the organism to grow under test and may result in a delay of 18 hours or longer. Even after such a delay, cultures may fail to yield growth because of previous antimicrobial treatment. Rapid reporting is important when the patient has septicaemia or meningitis to facilitate prompt and appropriate antimicrobial treatment.

Several methods exist for the rapid detection of bacterial growth in blood cultures—for example, radiometric,¹⁻³ impedimetric,^{3,4} or infrared^{5,6} techniques. If these are used in conjunction with a Gram stained film any of these techniques will quickly confirm the presence of bacteria and may give some indication of the family or genus of the infecting organism. Clinical guidance could be made more accurate if it were possible to identify the bacterium without awaiting growth. This can be achieved using immunological methods based on antigen detection, such as counterimmunoelectrophoresis (CIE),⁷ coagglutination (COA)⁸ using staphylococci bearing protein A, and latex particle agglutination (LPA). The lack of an effective reagent for the detection of *Neisseria meningitidis* group B has previously been an important omission in all of the above methods.

Increasing use of CIE and COA has indicated various problems, including low sensitivity, lack of specificity, and cross reactions (personal observation).^{3,9-12} We evaluated a latex particle agglutination system which uses latex particles coated with monovalent or polyvalent antibodies to detect bacterial antigens in blood cultures and cerebrospinal fluid from children.

Patients and methods

Samples were received from children admitted to the Royal Liverpool Children's Hospital (comprising Alder Hey and Myrtle Street units) with suspected bacterial septicaemia or meningitis, and from severely ill children with fever of unknown origin between February 1984 and May 1986.

Suspensions of latex particles coated respectively with antibodies to *Streptococcus pneumoniae*, *Haemophilus influenzae* (Pitman type b), and *Neisseria meningitidis* (groups A, C, Y, W135) were obtained from Wellcome Diagnostics (Beckenham, Kent). Later in the study latex particles coated with a monoclonal antibody to *N meningitidis* group B were also made available to us. Suspensions of known antigen were used as positive controls and uncoated latex particles were used as a negative control.

Blood cultures were collected in two bottles of brain heart infusion, one incorporating para-aminobenzoic acid (PABA) and sodium polyanethanol sulphate, and the other PABA, vitamin K, and haemin (Gibco, Paisley). A 2 ml aliquot was aseptically removed from each bottle after 18 hours of incubation and centrifuged at 1000 × g for 10 minutes. The supernatant was subsequently used for tests.

Following routine bacteriological examination of cerebrospinal fluid (cell count, Gram stain, and culture) the supernatant fluid from centrifuged specimens was tested.

The antibody coated and control latex suspensions (25 µl) were pipetted on to individual areas of a black tile. Blood culture or cerebrospinal fluid supernatant (40 µl) was added to each suspension and mixed with separate wooden sticks. The tile was then rocked

gently for three minutes, while looking for macroscopic agglutination. Agglutination was compared with that obtained with the negative control and was assessed as negative, positive, or weakly positive. All supernatant samples showing agglutination were then heated in a boiling water bath for five minutes, centrifuged, and retested as above. Only those which still showed agglutination were recorded as positive.

Two sources of false positive results were postulated: the presence of protein A and degeneration of blood cells. To investigate these possibilities two blood culture sets (different batch numbers) were inoculated with the Cowan strain of *Staphylococcus aureus* and tested after 18 hours of incubation as above. In addition, protein A (Sigma UK) was mixed with the latex suspensions and examined for agglutination after rocking for three minutes. A series of 26 latex particle agglutination negative blood culture sets was stored at room temperature and retested seven and 21 days later.

The performance characteristics of the four antibody coated latex suspensions were calculated according to the methods of Galen and Gambino.¹³

Results

Two hundred and seventy two blood culture sets and 85 cerebrospinal fluid specimens from severely ill children were examined by latex particle agglutination. One hundred and forty eight (54%) and 22 (26%), respectively, were found to contain bacteria by routine culture—a total of 170. Of these, 64 contained bacteria for which appropriate latex suspensions were available (table 1). Sixty three (98%) gave an appropriate latex particle agglutination only and one (2%) agglutinated with both monovalent and polyvalent *N meningitidis* latex suspensions (*N menin-*

gitidis group B15 was grown). There were no false negative results.

One hundred and six culture positive specimens contained "other" isolates for which antibody coated latex suspensions were not available (table 2): 10 (9%) gave false positive results. Two of these were blood cultures growing *Escherichia coli* K1 which agglutinated only with *N meningitidis* group B latex suspension. As *E coli* K1 and *N meningitidis* group B share a common antigen these two specimens should be considered as having given true positive results. One was a blood culture growing *Klebsiella oxytoca*, which gave a weak though consistent agglutination with *H influenzae* type b latex. Seven were blood cultures growing *S aureus* either in pure growth or as part of a mixture. There were no false positive results among the cerebrospinal fluid specimens.

Table 2 "Other" isolates for which appropriate antibody coated latex suspension was not available

Isolate	No of cases
<i>Escherichia coli</i>	8
<i>Klebsiella</i> spp	8
<i>Hafnia</i> sp	1
<i>Serratia</i> sp	1
<i>Salmonella indiana</i>	1
<i>Salmonella typhi</i>	2
<i>Pseudomonas aeruginosa</i>	2
<i>Acinetobacter</i> spp	2
<i>Candida albicans</i>	1
<i>Bacteroides fragilis</i>	1
Viridans streptococci	11
<i>Streptococcus pyogenes</i>	1
<i>Streptococcus agalactiae</i>	2
<i>Streptococcus faecalis</i>	1
<i>Staphylococcus aureus</i>	16
<i>Staphylococcus epidermidis</i>	34
<i>Corynebacterium</i> sp	1
<i>Propionibacterium acnes</i>	3
<i>Bacillus</i> sp	2
<i>S aureus</i> + <i>S epidermidis</i>	5
<i>S epidermidis</i> + <i>Serratia</i> sp	1
<i>S aureus</i> + <i>S faecalis</i>	2
Total	106

Table 1 Detection of bacteria in blood culture and cerebrospinal fluid* by LPA

Isolate	No of specimens examined	No positive with appropriate latex suspension	No positive with inappropriate latex suspension	No negative with all latex suspension
<i>N meningitidis</i> group B	14 (8)	13 (7)	1 (1)	0 (0)
<i>N meningitidis</i> groups A, C, Y, W135	5 (3)	5 (3)	0 (0)	0 (0)
<i>H influenzae</i> type b	17 (2)	17 (2)	0 (0)	0 (0)
<i>Streptococcus pneumoniae</i>	28 (0)	28 (0)	0 (0)	0 (0)
"Other"	106 (9)	0 (0)	10 (0)	96 (9)
Nil	187 (63)	0 (0)	0 (0)	187 (63)
Total	357 (85)	63 (12)	11 (1)	283 (72)

*Cerebrospinal fluid examinations and results are shown in parentheses.

Table 3 Percentage performance characteristics of LPA test for *N meningitidis* group B, *N meningitidis* groups A, C, Y, W135, *H influenzae* type b, and *S pneumoniae* (polyvalent)

Performance characteristic	<i>N meningitidis</i>			
	Group B	Groups A, C, Y, W135	<i>H influenzae</i> type b	<i>S pneumoniae</i> polyvalent
Sensitivity	100	100	100	100
Specificity	99	99	99	98
Predictive value for positive result	87	63	85	87
Predictive value for negative result	100	100	100	100

Rapid bacterial antigen identification

The blood culture sets inoculated with the Cowan strain of *S aureus* all give false positive reactions and some were stable to boiling. The protein A suspension behaved similarly.

The series of 26 blood culture sets stored at room temperature after initial testing remained negative when retested in the same way at seven days. When retested at 21 days six agglutinated with all antibody coated and control latex particles but became negative after boiling.

Table 3 shows the performance characteristics of the latex particle agglutination test for four antibody coated latex particle suspensions.

Discussion

The results indicate that the latex particle agglutination technique is sensitive and specific (with high positive and negative predictive values) for *S pneumoniae*, *H influenzae* type b, *N meningitidis* groups, A, C, Y, W135, and *N meningitidis* group B/*E coli* K1 ("community" acquired oropharyngeal flora). Moreover, the technique is cheap, simple to perform, and rapid to carry out. The main problems occur with *S aureus*, which accounts for 70% of the false positive results from blood cultures. A Gram stain was found to be useful under these conditions. Protein A, which is carried by some *S aureus* strains, is an Fc receptor which may bind to Fc components exposed on the surface of antibody coated latex particles. This was not a major disadvantage in our hands.

Human cells have been shown to share epitopes with bacteria, especially *N meningitidis* group B.¹⁴ It is therefore possible that false reactions may occur with methods based on antigen detection, including latex particle agglutination, particularly following the breakdown of blood cells¹⁰ during the prolonged incubation or storage of clinical specimens. We found such false reactions to be rare (none of 26 at seven days and six of 26 at 21 days) and they became negative after the specimen had been heated in a boiling water bath for five minutes.

Bacteria causing meningitis generally belong to the "community" acquired oropharyngeal flora. The positive predictive values for these organisms ranged from 63% to 87% and would be higher if a Gram stain was used. The predictive values for negative results—that is, sterile or negative for "community" bacteria—was 100%. An important group of bacteria for which antibody coated latex particles are not available includes Enterobacteriaceae/Pseudomonadaceae/*Acinetobacter* sp ("hospital" acquired flora). While this group is associated with infections such as septicæmia, it is rarely responsible for acute meningitis after the neonatal period. Rather, it is associated with chronic disease—for example, colonisation of shunts used to drain cerebrospinal fluid in children with

hydrocephaly.

Cross reactions between *N meningitidis* and *E coli* occurred in two cases. This represents a major limitation of the latex particle agglutination test when used to guide antimicrobial treatment: *N meningitidis* is sensitive to penicillin G, *E coli* is not; a Gram stain would readily resolve this difficulty.

Based on our experience, antibody coated latex particle agglutination provides valuable support to the routine bacteriological examination of blood culture and cerebrospinal fluid in paediatric meningitis and septicæmia.

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