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ORIGINAL ARTICLE

Rapid identification of *Streptococcus pneumoniae* in blood cultures by using the ImmuLex, Slidex and Wellcogen latex agglutination tests and the BinaxNOW antigen test

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Abstract Rapid identification of *Streptococcus pneumoniae* in blood culture (BC) bottles is important for early directed antimicrobial therapy in pneumococcal bacteraemia. We evaluated a new latex agglutination (LA) test on BC bottles, the ImmuLex™ *S. pneumoniae* Omni (Statens Serum Institut, Denmark), and compared the performance with the Slidex® pneumo-Kit (bioMérieux, France) and the Wellcogen™ *S. pneumoniae* (Remel, UK) LA tests, as well as the BinaxNOW® *S. pneumoniae* (Alere, USA) antigen test. The four tests were directly applied on 358 positive BC bottles with Gram-positive cocci in pairs or chains and on 15 negative bottles. Valid test results were recorded in all cases for ImmuLex and BinaxNOW and in 88.5 % (330/373) and 94.1 % (351/373) of cases for Slidex and Wellcogen, respectively. Based on bottles positive for *S. pneumoniae* by conventional methods, the sensitivity of ImmuLex was 99.6 %, similar to the other tests (range, 99.6–100 %). Based on bottles positive for non-pneumococcal pathogens, the specificity of ImmuLex was 82.6 %, in comparison to 97.6 % for Slidex ($p < 0.01$) and 85.4 % for Wellcogen (p = ns). The BinaxNOW test had a lower specificity (64.1 %) than any LA test ($p < 0.01$). On BC bottles positive for α-haemolytic streptococci, ImmuLex was positive in 12/67 (17.9 %) cases, Slidex in 2/59 (3.4 %) cases, Wellcogen in 11/64 (17.2 %) cases and BinaxNOW in 25/67 (37.3 %) cases. In conclusion, the ImmuLex test provides a valid and sensitive technique for the rapid detection of *S. pneumoniae* in BC bottles, similar to the other compared methods. However, the specificity was sub-optimal, since the test may cross-react with other Gram-positive bacteria.

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Introduction

In today's routine diagnostics, blood culture (BC) is the standard method for the detection of pneumococcal bacteraemia. Identification is based on sub-culturing the positive BC bottle, with results available usually 24 h after BC positivity [1, 2]. *Streptococcus pneumoniae* produces autolysin, a cell wall enzyme responsible for its own lysis, during the stationary growth phase. Autolysis may prevent growth of the bacteria on sub-culture, which, in turn, may hinder the identification of *S. pneumoniae* by culture-based methods [2]. In addition, differentiating *S. pneumoniae* from other viridans group streptococci is diagnostically challenging due to both phenotypic and genotypic similarities [3]. Therefore, studies on rapid and reliable methods for the identification of *S. pneumoniae* directly from BC bottles are in demand.

We and others have previously demonstrated that nucleic acid-based methods applied directly on BC bottles could identify *S. pneumoniae* within 1–3 h [4, 5]. The methods provide rapid detection of resistant genes but may cross-react with pathogens that harbour genes encoding for pneumococcal virulence factors, e.g. autolysin and pneumolysin [5–7]. Matrix-assisted laser desorption/ionisation time-of-flight mass

spectrometry (MALDI-TOF MS) may identify microorganisms in BC bottles with high specificity [8, 9] but with limited sensitivity for *S. pneumoniae* [10, 11]. In addition, the BinaxNOW® *S. pneumoniae* (Alere, USA) antigen test, validated for the detection of cell-wall polysaccharides (CWPS) in urine and cerebrospinal fluid, may detect *S. pneumoniae* in BC bottles with high sensitivity [2, 12, 13]. However, the method has been demonstrated to cross-react with α-haemolytic streptococci both in BC bottles [2] and in bacterial isolates [14], which calls for additional studies with respect to the test specificity.

Latex agglutination (LA) tests are routinely used in clinical microbiological laboratories for the rapid detection of *S. pneumoniae* in BC bottles. The method is easy to perform and provides a result within minutes after BC positivity. Several LA tests have been evaluated, with sensitivities of 88–100 % and specificities of 83–100 %, respectively [15]. In 2013, the new ImmuLex™ *S. pneumoniae* Omni (Statens Serum Institut, Denmark) LA test was introduced to the market. According to the manufacturer, the test detected 91 of 92 pneumococcal serotypes in spiked BC bottles in a previous study [16], and the sensitivity and specificity rates were 98 and 96 %, respectively (ImmuLex package insert), but no independent study on the test performance has yet been performed.

In this study, we compared the performance of the ImmuLex LA test with the Slidex® pneumo-Kit (bioMérieux, France) and the Wellcogen™ *S. pneumoniae* (Remel, UK) LA tests in the detection of *S. pneumoniae* from BC bottles. The results were also compared with those of the BinaxNOW *S. pneumoniae* antigen test, which was tested simultaneously on the BC bottles.

Materials and methods

Study design

The study was conducted at the Department of Clinical Microbiology, Karolinska University Hospital Huddinge, Stockholm, Sweden. BC bottles collected from patients at our hospital were analysed from August 2013 to December 2014. The BC bottles used were BacT/ALERT® FA, FA Plus, FN, FN Plus, PF, PF Plus and SN (bioMérieux, USA). All bottles were incubated in a BacT/ALERT® 3D (bioMérieux, USA) system until signalling positive, or for a maximum incubation time of 5 days. All culture-positive BC bottles were Gram stained and bottles with Gram-positive cocci in pairs or chains were included in the study. If both culture-positive and culture-negative BC bottles were collected simultaneously from a patient, the negative BC bottles were included in the study as well. Also, three negative BC bottles from patients with no positive BC bottle were included. All included bottles were stored at +4 °C and analysed with three LA tests within 24 h of culture positivity; ImmuLex™ *S. pneumoniae* Omni, Slidex® pneumo-Kit (bioMérieux, France) and Wellcogen™ *S. pneumoniae* (Remel, UK). For comparison, the BinaxNOW® *S. pneumoniae* antigen test was applied on the same BC bottles simultaneously.

Conventional methods for the identification of pathogens

After Gram stain was performed, all included positive BC bottles were subsequently sub-cultured on blood, chocolate and cystine lactose electrolyte deficient (in-house production, Karolinska University Hospital Huddinge, Stockholm, Sweden) agar plates. Positive anaerobic BC bottles were also sub-cultured on anaerobic agar plates incubated in anaerobic atmosphere. All isolated bacteria were identified to the species level using standard methods, including VITEK® 2 XL (bioMérieux, France), MALDI-TOF MS (Bruker Daltonik, Germany) and agglutination tests for A, B, C, D and G streptococci and *S. pneumoniae* (Oxoid Ltd., UK), as previously described [10]. Strains of Gram-positive cocci in pairs or chains were sub-cultured on bile esculin agar plates (in-house production, Karolinska University Hospital Huddinge, Stockholm, Sweden) and tested for optochin susceptibility. In order to differentiate α-haemolytic streptococci between species, isolates were sent to Bruker Daltonik, Leipzig, Germany for DNA isolation, amplification and sequencing of 16S rRNA and/or recA genes by polymerase chain reaction (PCR), as described previously [17].

Antigen tests

The three LA tests were performed as recommended by the manufacturers. Briefly, to perform the ImmuLex test, one drop of BC broth was mixed with one drop of anti-*S. pneumoniae* latex suspension in one circle on the reaction card. In another circle, one drop from the negative control bottle was mixed with one drop of latex suspension. Each combination was mixed individually for a maximum of 10 s while any agglutination was observed. A positive result was recorded when agglutination was observed within 5 s in the test circle and no agglutination was observed in the negative control circle. No agglutination in either circle on the reaction card was recorded as a negative result. If agglutination appeared in the negative control circle, the test was considered invalid.

To perform the Slidex test, 1 ml of BC broth was centrifuged at 2000 rpm for 10 min. One drop of the supernatant was mixed with one drop of anti-*S. pneumoniae* latex suspension on a glass slide. On another glass slide, one drop of supernatant was mixed with one drop of control latex suspension. Both slides were rotated for a maximum of 2 min while any agglutination was observed. A positive result was recorded when agglutination was observed on the test slide within

2 min and no agglutination was observed on the control slide. No agglutination on either slide was recorded as a negative result. If agglutination appeared on the control slide, the test was considered invalid.

To perform the Wellcogen test, 1–2 ml of positive BC broth was mixed with one drop of anti-*S. pneumoniae* latex suspension in one circle on the reaction card. In another circle, one drop of supernatant was mixed with one drop of control latex. The card was rotated for a maximum of 2 min while any agglutination was observed. A positive result was recorded when agglutination was observed in the test circle within 3 min and no agglutination was observed in the control circle. No agglutination in either circle was recorded as a negative result. If a positive reaction appeared in the control circle, the test was considered invalid.

All LA tests were read visually under normal light conditions. Positive and negative controls for each LA test were performed according to the manufacturer's instructions. Any invalid test was re-analysed by a second laboratory technician.

The BinaxNOW test was performed on BC broth as recommended by the manufacturer for usage on urine specimens. Briefly, the BC broth was sampled with the sample swab, whereafter the swab was inserted into the test device. The test results were recorded by visual reading after 15 min. A visible test line of any intensity was considered a positive test result.

Statistical methods

McNemar's test for paired samples was used for the comparison of proportions. A confidence interval (CI) of 95 % was used for statistical precision. A two-tailed p-value of <0.01 was considered statistically significant to adjust for multiple comparisons. To evaluate the contribution of invalid test results, a sensitivity analysis of the study results was performed, by calculating the sensitivity and specificity with and without invalid test results included. The statistical analyses were performed with a statistical software package (GraphPad Prism 6).

Results

A total of 373 BC bottles obtained from 159 patients were included in the study. Of these, 358 BC bottles were positive for Gram-positive cocci in pairs or chains and 15 bottles were negative by Gram stain. Based on standard microbiological methods, 266/358 (74.3 %) bottles were positive for *S. pneumoniae* and 92/358 (25.7 %) bottles were positive for non-pneumococcal pathogens, including α-haemolytic streptococci ($n=67$) ± other species, *Enterococcus* species ($n=23$), *Enterobacter cloacae* ($n=1$) and *Klebsiella pneumoniae* ($n=1$); see Table 2. A total of 53/67 (79.1 %) α-haemolytic streptococci isolates were analysed by sequencing of 16S rRNA and/or recA genes in order to differentiate between species. Of 15 negative BC bottles, 12 were identified as companion bottles collected from patients with another bottle positive for *S. pneumoniae* and three bottles were collected from patients with only negative bottles. Of the 373 included BC bottles, the ImmuLex test was valid in all cases ($n=373$; 100 %), in comparison with the Slidex ($n=330$; 88.5 %; $p<0.001$) and Wellcogen ($n=351$; 94.1 %; $p<0.001$) tests (Tables 1 and 2). Only valid test results were included in the performance analyses of the four tests.

The three LA tests and the BinaxNOW test showed similar sensitivities, ranging from 99.6 to 100 % on BC bottles positive for *S. pneumoniae*, with negative test results in only two cases (Table 1). One bottle was negative only with the ImmuLex and Wellcogen tests and one bottle was negative only with the Slidex test. The BinaxNOW test was positive on all BC bottles. If invalid test results were included in the performance analysis, the positivity rates of the Slidex and Wellcogen tests yielded 86.5 % (CI, 81.8–90.1 %) and

Table 1 Sensitivity of the ImmuLex LA test in comparison with the Slidex and Wellcogen LA tests and with the BinaxNOW antigen test when applied on BC bottles ($n=266$) positive for *Streptococcus pneumoniae*

	ImmuLex	Slidex	Wellcogen	BinaxNOW
Positive test result, *n*	265	230	246	266
Negative test result, *n*	1	1	1	0
Invalid test result, *n*	0	35	19	0
Sensitivity, invalid results not included, % (95 % CI)[a]	99.6 (97.9–99.9)	99.6 (97.6–99.9)	99.6 (97.8–99.9)	100 (98.6–100)
Sensitivity, invalid results included, % (95 % CI)[b]	99.6 (97.9–99.9)	86.5 (81.8–90.1)	92.5 (88.7–95.1)	100 (98.6–100)

LA latex agglutination; *BC* blood culture; *CI* confidence interval

[a] Sensitivity was calculated on BC bottles positive for *S. pneumoniae* ($n=266$) by dividing the sum of positive test results by the number of valid results

[b] In a sensitivity analysis of the study result, sensitivity was calculated on BC bottles positive for *S. pneumoniae* ($n=266$) by dividing the sum of positive test results by the total number of results including the invalid results

Table 2 Specificity of the ImmuLex LA test in comparison with the Slidex and Wellcogen LA tests and with the BinaxNOW antigen test when applied on BC bottles with respect to identified pathogen

	n	ImmuLex	Slidex	Wellcogen	BinaxNOW
α-Haemolytic streptococci[a]					
Streptococcus oralis	11	5	1	1	10
Streptococcus mitis	7	1	0	0	4
Streptococcus anginosus	6	0	0	1	0
Streptococcus sanguinis	5	0	1	1	1
Streptococcus tigurinus	5	2	0	3	1
Streptococcus dentisani	4	1	0	1	1
Streptococcus salivarius	3	0	0	0	0
Streptococcus gallolyticus	2	1	0	0	1
Streptococcus constellatus	1	1	0	0	0
Streptococcus intermedius	1	0	0	0	0
Streptococcus lutetiensis	1	0	0	0	1
α-Haemolytic streptococci	10	1	0	2	5
α-Haemolytic streptococci[a] + other species					
S. mitis + CoNS	2	0	0	1	0
S. mitis + *Pseudomonas aeruginosa*	2	0	0	1	0
Streptococcus parasanguinis + CoNS	1	0	0	0	0
S. salivarius + *Streptococcus pyogenes*	1	0	0	0	0
S. sanguinis + CoNS	1	0	0	0	1
α-Haemolytic streptococci + *Staphylococcus aureus*	2	0	0	0	0
α-Haemolytic streptococci + *Haemophilus influenzae*	2	0	0	0	0
Other Gram-positive cocci					
Enterococcus species	23	2	0	2	8
Gram-negative pathogens[b]					
Enterobacter cloacae	1	1	0	0	0
Klebsiella pneumoniae	1	1	0	0	0
Total	92	16	2	13	33
Invalid test results		0	8[e]	3[f]	0
Specificity, invalid results not included, % (95 % CI)[c]		82.6 (73.6–89.0)	97.6 (91.7–99.4)	85.4 (76.6–91.3)	64.1 (53.9–73.2)
Specificity, invalid results included, % (95 % CI)[d]		82.6 (73.6–89.0)	89.1 (81.1–94.0)	82.6 (73.6–89.0)	64.1 (53.9–73.2)

LA latex agglutination; *BC* blood culture; *CoNS* coagulase-negative staphylococci; *CI* confidence interval

[a] A total of 53/67 (79.1 %) α-haemolytic streptococci isolates were analysed by sequencing of 16S rRNA and/or recA genes in order to differentiate between species

[b] Identified by MALDI-TOF MS

[c] Specificity was calculated on BC bottles positive for non-pneumococcal pathogens ($n = 92$) by dividing the sum of negative test results by the number of valid results

[d] In a sensitivity analysis of the study result, specificity was calculated on BC bottles positive for non-pneumococcal pathogens ($n = 92$) by dividing the sum of negative test results by the total number of results including invalid results

[e] Eight BC bottles positive for *S. mitis* ($n = 2$), *S. tigurinus* ($n = 2$), *S. oralis* ($n = 1$), *S. sanguinis* ($n = 1$), *S. dentisani* ($n = 1$) and *S. mitis* + CoNS ($n = 1$) not shown in the table elsewhere

[f] Three BC bottles positive for *S. oralis* ($n = 1$), *S. anginosus* ($n = 1$) and *S. mitis* ($n = 1$) not shown in the table elsewhere

92.5 % (CI, 88.7–95.1 %), respectively, which were significantly lower than the sensitivity rates of the other two methods ($p < 0.01$ for all comparisons; Table 1).

There were significant differences between the specificity rates among the four methods, calculated on BC bottles positive for non-pneumococcal pathogens (Table 2). The ImmuLex test was less specific than the Slidex test (82.6 % vs. 97.6 %, $p < 0.01$) but performed similarly to the Wellcogen test (85.4 %; $p =$ ns). In comparison, the ImmuLex test was more specific

than the BinaxNOW test (64.1 %; $p<0.01$). If invalid test results were included, the negative rates of the Slidex and Wellcogen tests yielded 89.1 % (CI, 81.1–94.0 %) and 82.6 % (CI, 73.6–89.0 %), respectively, which were similar to the negative rates of the ImmuLex test ($p=$ns for both comparisons) but still higher than the BinaxNOW test ($p<0.01$; Table 2). On BC bottles positive for α-haemolytic streptococci, the ImmuLex test was false-positive in 12/67 (17.9 %) valid cases, which was a higher rate compared to the Slidex test (2/59; 3.4 %; $p<0.01$) but a similar rate compared to the Wellcogen test (11/64; 17.2 %; $p=$ns). The BinaxNOW test was positive in 25/67 (37.3 %) valid cases, which was a significantly higher rate compared to any of the LA tests ($p<0.01$ for all comparisons). Of 23 BC bottles positive for *Enterococcus* species, two bottles (8.7 %) were positive with the ImmuLex and the Wellcogen tests only, and eight (34.8 %) bottles were positive with the BinaxNOW test (Table 2). Two BC bottles with primarily identified Gram-positive cocci by microscope were identified as *K. pneumoniae* and *E. cloacae* by MALDI-TOF MS, both of which were positive with the ImmuLex test alone. The four methods were negative in 15/15 (100 %) negative BC bottles.

Discussion

In this study, we evaluated the ImmuLex *S. pneumoniae* Omni test on BC bottles and compared the performance with two other LA tests and the BinaxNOW antigen test. When compared with the other LA tests, the sensitivity of the ImmuLex test was high (99.6 %) and similar to the Slidex and Wellcogen tests (99.6 and 100 %, respectively; Table 1). However, the specificity was inferior to the Slidex test (82.6 % vs. 97.6 %; $p<0.01$) but similar to the Wellcogen test (85.4 %; $p=$ns; Table 2). In addition, while both the ImmuLex and BinaxNOW tests yielded valid test results in all cases, the Slidex and Wellcogen tests were invalid in 11.5 and 5.9 % of all cases, respectively, which may have influenced the overall performance.

In a previous comparison study made by the manufacturer, the sensitivity of the ImmuLex test was 87 % when applied on BC bottles positive for *S. pneumoniae* ($n=31$) and based on valid test results, while the sensitivities of the Slidex, Wellcogen and DrySpot™ Pneumo (Oxoid Ltd., UK) tests were 100, 65 and 25 % of cases, respectively [18]. If invalid test results were included in the performance analysis, the three competing tests were positive in only 42, 48 and 10 % of cases, respectively, while the ImmuLex test was valid in all cases. Thus, the high proportions of invalid test results among the competing tests, in addition to the low number of included BC bottles, complicated a fair comparison between the tests. In the same study, the specificity of the ImmuLex test was 95 % on BC bottles with non-pneumococcal pathogens ($n=59$), similar to the Slidex, Wellcogen and DrySpot tests (100, 96 and 98 %, respectively). Here, the low proportions of invalid test results (none for ImmuLex and 5 % each for the competing tests) had limited impact on the false-positivity rates. Furthermore, 12 negative BC bottles were all negative with the four tests [18]. Altogether, our findings support the estimation of the ImmuLex test as a sensitive method for the detection of *S. pneumoniae* in BC bottles, but the specificity rate was lower than expected in the light of results reported by the manufacturer.

False-positive test results of the ImmuLex test were previously described only on BC bottles positive for *Streptococcus* group C and *Pseudomonas aeruginosa* within the recommended observation time [18], and for *Enterococcus* species, *Staphylococcus aureus* and Gram-negative bacteria after 10 to 30 s (ImmuLex package insert). For other LA tests, cross-reactions with *Streptococcus* group C were reported in previous studies, including studies on the Wellcogen test [19, 20], and is suggested to be due to common antigen structures [19, 21]. The Wellcogen test was also reported by the manufacturer to cross-react with viridans streptococci, *S. sanguinis*, *S. epidermidis*/*Enterococcus* species (in combination) and *Pseudomonas* species on BC bottles (Wellcogen package insert). According to the manufacturer of the Slidex test, the assay may cross-react with *Streptococcus* group C, *Enterococcus* species and α-haemolytic streptococci (Slidex package insert). However, no cross-reaction was observed when the test was applied on spiked BC bottles, as well as on BC bottles collected from clinical routine in previous studies [15, 22].

To our knowledge, cross-reactions with α-haemolytic streptococci have not been demonstrated by any independent research group for any of the three LA tests evaluated in this study, but was previously described by Browne et al. for a different LA assay [23]. In this study, the ImmuLex test yielded false-positive results in 12/67 (17.9 %) cases of α-haemolytic streptococci, similar to the Wellcogen test, while the Slidex test was positive in only a few cases (3.4 %). Bottles with *S. oralis* and *S. tigurinus* were positive in more cases than others (Table 2). Furthermore, we were able to demonstrate that the ImmuLex test yielded false-positive results on two BC bottles positive for *K. pneumoniae* and *E. cloacae*, respectively, which has not been described previously.

The BinaxNOW test showed similar sensitivity (100 %) to the LA tests, but the specificity was significantly lower (64.1 %; $p<0.01$). The test was false-positive in 25/67 (37.3 %) BC bottles positive for α-haemolytic streptococci and 8/23 (34.8 %) positive for *Enterococcus* species. Bottles with *S. oralis* ($n=10$) and *S. mitis* ($n=4$) were positive in more cases than others (Table 2). Cross-reactions between *S. pneumoniae* and viridans group streptococci species have

previously been reported for the test on urine samples [24] and isolates [14], and is explained by common CWPS antigens of the bacteria [25, 26]. On BC bottles positive with α-haemolytic streptococci, false-positive tests were previously observed in 3/12 (25 %) cases by Petti et al. [2] and in 13/49 (26.5 %) cases by Baggett et al. [12], similar to our results. Interestingly, we noted that the all LA tests and the BinaxNOW test was negative in all 15 culture-negative BC bottles, 12 of which were collected from patients with companion bottles positive for *S. pneumoniae*, which could be explained by the absence of pneumococcal polysaccharide antigens in those bottles. Similarly, Petti et al. observed that only 1/7 (14.3 %) of culture-negative BC bottles tested positive with the BinaxNOW test [2]. In contrast, Saha et al. were able to identify the *lytA* genome by PCR in eight culture-negative BC bottles, which were positive with the BinaxNOW test [13]. By this procedure, they enhanced the sensitivity rate for *S. pneumoniae* in BC bottles with 17 % (8/48) and were able to identify the capsular serotype by using sequential multiplex PCR [13, 27].

In this study, we used BacT/ALERT® BC bottles and did not compare test performance using BC bottles from different manufacturers, since the ImmuLex is validated on BACTEC™ (Becton Dickinson and Co., USA) and BacT/ALERT® bottles only, and the Slidex test is validated on BacT/ALERT® bottles only. This may have influenced the proportions of positive, negative and invalid results between the tests, since differences between BC systems have been observed [2]. Also, the four methods were only evaluated on BC bottles with no clinical data available. For example, the effect of antibiotic treatment before the BC collection was not analysed. Therefore, the performance of the tests should be further evaluated using other BC systems and with clinical data including the prior use of antibiotics collected from patients.

In conclusion, this study demonstrated that the ImmuLex LA test detected *S. pneumoniae* with high sensitivity directly from BC bottles, similar to the sensitivities of the Slidex and Wellcogen tests. However, due to a high rate of false-positive test results on BC bottles positive for α-haemolytic streptococci, the test was less specific than the Slidex test but similar to the Wellcogen test. In comparison, the BinaxNOW antigen test had similar sensitivity, but inferior specificity, as the LA tests. In the present study, we analysed the analytical performance of the four rapid assays and, therefore, we recommend further studies on the clinical impact of these methods.

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Compliance with ethical standards This study was performed in accordance with the Declaration of Helsinki and with the ethical standards of the research committee in Stockholm, Sweden.

Conflict of interest The authors declare no conflicts of interest.

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