Evaluation of the Pastorex® meningitis kit for the rapid identification of Neisseria meningitidis serogroups A and W135


Centre de Recherche Médicale et Sanitaire (CERMES), Réseau International des Instituts Pasteur, BP 10887, Niamey, Niger
Association pour l’Aide à la Médecine Préventive (AMP), 25 rue du Dr Roux, 75724 Paris Cedex 15, France
Centre Muraz, BP 153, Bobo Dioulasso, Burkina Faso
Centre Hospitalier Universitaire Souro Sanou, 01 BP 676, Bobo Dioulasso, Burkina Faso

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Neisseria meningitidis; Emergency vaccination; Cerebrospinal fluid; Latex agglutination test; PCR

Summary
The recent emergence of Neisseria meningitidis W135 as a cause of epidemic bacterial meningitis and the availability of a trivalent ACW135 vaccine have created a need for accurate and timely meningococcal serogroup determination for organization of epidemic vaccine response. The sensitivity and specificity of the Pastorex® meningitis kit (Bio-Rad) to identify serogroups A and W135 in the African meningitis belt was assessed using PCR testing as the gold standard. The sensitivity and specificity for serogroups A and W135 were 87 and 85%, respectively, while the specificities were 93 and 97%, respectively. The positive and negative likelihood ratios for A were 12 and 0.14 and for W135 were 33 and 0.16. The positive and negative predictive values, computed to simulate an epidemic of meningococcal meningitis with an estimated 70% prevalence of N. meningitidis among suspected cases, were 97% and 75% for A and 99% and 73% for W135. In remote locations of the African meningitis belt, latex agglutination is the only currently available test that can rapidly determine meningococcal serogroup. This study showed that latex agglutination performs well and could be used during the epidemic season to determine appropriate vaccine response.

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Corresponding author. Present address: Unité d’Épidémiologie, CERMES, BP 10887, Niamey, Niger. Fax: +227 753180. E-mail address: pascal.boisier@cermes.ne (P. Boisier).

1. Introduction

During the last decades, epidemics within the meningitis belt were due primarily to Neisseria meningitidis serogroup A. In 2002, and again in 2003, Burkina Faso experienced
the first large epidemics ever identified in Africa, due to

**N. meningitidis** serogroup W135 of the clonal complex ET-37, ST-11 (WHO, 2002). Additional works have documented the wide geographic spread of **N. meningitidis** serogroup W135 within the meningitis belt (Dennis et al., 1982; Kwara et al., 1998; Matar et al., 2000). Current meningococcal polysaccharide vaccine available in large amounts for epidemic control target only serogroups A and C, while the trivalent vaccine ACW135 remains rationed. The decision of which vaccine to use for emergency immunization depends on accurate and rapid serogroup determination.

Within the meningitis belt, with the exception of some reference centres, few laboratories have the capacity to determine meningococcal serogroup using gold standard testing such as immune serum or PCR. For the majority of the African meningitis belt, latex agglutination tests (LATs) represent the only available alternative. These tests do not require expensive equipment or highly skilled technicians and could be implemented at the district level.

The LAT uses latex particles sensitized with monoclonal or polyclonal antibodies specific for a given antigen. In the presence of the soluble antigen released by bacteria, the latex particles agglutinate to form clumps that are visible to the naked eye. The tests can be performed on cerebrospinal fluid (CSF), urine or serum. Several commercial LAT kits are already available to identify meningococcal serogroups A and Y/W135: Wellcogen® (Abbott-Murex), Pastorex® (Bio-Rad) and Directigen® (Becton Dickinson) differ by the reagents used for serogroup identification. Previous studies of antigen detection kits have found great variation in sensitivity (32–96%) with relatively consistent specificity (96–100%) (Camargos et al., 1995; Coovadia and Taha, 2000). The Pastorex kits used in the study were recent production sent directly by the manufacturer. They were transported and delivered according to cold chain requirements and were employed within a short time of receipt. The technicians who performed the LAT were blinded to the results of the PCR assay.

The Pastorex kits were used according to the manufacturer’s recommendations. Briefly, the CSF specimens were heated for 3 min at 100 °C using a water bath and then centrifuged for 5 min at 3000 g. The disposable card was rotated slightly for 3 min using a mechanical system after calibrated drops of the supernatant had been placed on the specific locations with their corresponding reagent. The appearance of agglutination within 10 min was observed with the naked eye.

### 2.3. Data analysis

We calculated the sensitivity (Se) and specificity (Sp) of LAT versus PCR. In addition, we calculated the positive (LR+) and negative (LR−) likelihood ratio of the LAT for **N. meningitidis** A and W135 as (Se/(1−Sp)) and LR− as ((1−Se)/Sp), respectively.

Positive (PPV) and negative (NPV) predictive values were calculated as follows:

\[
PPV = \frac{(Prev \times Se)}{(Prev \times Se) + (1 - Prev) \times (1 - Sp))} \\
NPV = \frac{(1 - Prev) \times Sp)}{(1 - Prev) \times Sp) + (Prev \times (1 - Se))}
\]

where Prev represents the prevalence of the disease in the population to which the test is applied.

As PPV and NPV depend on the epidemiological context in which a test is used, and to make their interpretation more concrete, the predictive values were calculated using two hypothetical situations: (1) a **N. meningitidis** A or W135 epidemic managed by skilled health staff complying well with cases definition of a suspected case of ABM, so that the prevalence of **N. meningitidis** equals 70% among clinically suspected cases; and (2) an absence of **N. meningitidis** epidemic, with a prevalence of **N. meningitidis** of 20% among clinically suspected cases. We also provide a graphic representation of PPV and NPV for values of prevalence ranging from 0 to 100%.

### 2.4. Ethical aspects

All specimens were collected during routine clinical management of patients and were used as part of clinical management. Consequently, informed consent was not sought and an ethical review committee did not review the study.

### 3. Results

Overall, 599 CSF specimens were tested, including 333 at CERMES and 266 at Centre Muraz. The distribution of specimens according to the laboratory is presented in Table 1.
Pastorex meningitis kit to guide emergency immunization

Table 1  Distribution of tested CSF specimens according to the centre and to the PCR result; Burkina Faso and Niger, 2002

<table>
<thead>
<tr>
<th></th>
<th>NmA</th>
<th>Nm W135</th>
<th>Nm C</th>
<th>Nm Y</th>
<th>Nm ND</th>
<th>S. pneumoniae</th>
<th>Hibd</th>
<th>Tested negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CERMES</td>
<td>211</td>
<td>29</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>76</td>
<td>333</td>
</tr>
<tr>
<td>MURAZ</td>
<td>13</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>79</td>
<td>266</td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
<td>179</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>17</td>
<td>8</td>
<td>155</td>
<td>599</td>
</tr>
</tbody>
</table>

a  Neisseria meningitidis.

b  N. meningitidis for which the PCR assay could not predict the capsular antigen (i.e. N. meningitidis not belonging to serogroups A, B, C, Y or W135).

c  Streptococcus pneumoniae.

d  Haemophilus influenzae b.

Table 2  Cross-tabulation of latex agglutination versus PCR for N. meningitidis A; Burkina Faso and Niger, 2002

<table>
<thead>
<tr>
<th></th>
<th>PCR positive for N. meningitidis A</th>
<th>PCR negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex agglutination positive</td>
<td>194</td>
<td>11</td>
<td>205</td>
</tr>
<tr>
<td>for N. meningitidis serogroup A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latex agglutination negative</td>
<td>30</td>
<td>144</td>
<td>174</td>
</tr>
<tr>
<td>for N. meningitidis serogroup A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
<td>155</td>
<td>379</td>
</tr>
</tbody>
</table>

3.1. N. meningitidis serogroup A (Table 2)

For serogroup A, the sensitivity of LAT = 86.6% (95% CI 81.3—90.6%) and the specificity = 92.9% (95% CI 87.3—96.2%). The LR+ = 12.2 and the LR− = 0.14.

3.2. N. meningitidis serogroup Y/W135 (Table 3)

For serogroup Y/W135, the sensitivity = 84.9% (95% CI 78.6—89.7%) and the specificity = 97.4% (95% CI 93.1—99.2%). The LR+ = 32.7 while the LR− = 0.16.

Table 3  Cross-tabulation of latex agglutination versus PCR for N. meningitidis W135 (latex agglutination tested for W135/Y); Burkina Faso and Niger, 2002

<table>
<thead>
<tr>
<th></th>
<th>PCR positive for N. meningitidis W135</th>
<th>PCR negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex agglutination positive</td>
<td>152</td>
<td>4</td>
<td>156</td>
</tr>
<tr>
<td>for N. meningitidis serogroup Y/W135</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latex agglutination negative</td>
<td>27</td>
<td>151</td>
<td>178</td>
</tr>
<tr>
<td>for N. meningitidis serogroup Y/W135</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>155</td>
<td>334</td>
</tr>
</tbody>
</table>

Table 4  Cross-reactions between latex agglutination reagents for N. meningitidis A and N. meningitidis Y/W135; Burkina Faso and Niger, 2002

<table>
<thead>
<tr>
<th>Characterization by PCR assay</th>
<th>No. tested</th>
<th>Positive with reagent NmA</th>
<th>Positive with reagent Nm Y/W135</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. meningitidis A</td>
<td>224</td>
<td>na</td>
<td>0</td>
</tr>
<tr>
<td>N. meningitidis W135</td>
<td>179</td>
<td>1</td>
<td>na</td>
</tr>
<tr>
<td>N. meningitidis C</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N. meningitidis Y</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N. meningitidis ND</td>
<td>13</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>H. influenzae b</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>17</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

na: not applicable.
3.3. Cross-reactions

Cross-reactions were evaluated on 444 CSF specimens (Table 4). No cross-reaction was observed for \(N.\) meningitidis A specimens; one was observed for W135.

3.4. Predictive values

3.4.1. Situation 1: well-managed \(N.\) meningitidis A or W135 epidemic

Assuming that the true proportion of meningococcus of a given serogroup would be about 70\% among the suspected cases, the PPV and NPV would be 97 and 75\%, respectively, for \(N.\) meningitidis serogroup A and 99 and 73\%, respectively, for \(N.\) meningitidis serogroup W135.

3.4.2. Situation 2: absence of \(N.\) meningitidis A or W135 epidemic

Assuming that the true proportion of meningococcus of a given serogroup would be about 20\% among the suspected cases, the PPV and NPV would be 75 and 97\%, respectively, for \(N.\) meningitidis A and 89 and 96\%, respectively, for \(N.\) meningitidis W135.

The range of PPV and NPV according to the proportion of \(N.\) meningitidis cases of serogroup A, or serogroup W135, in the group of clinically suspected cases are presented in Figures 1 and 2.

4. Discussion

In countries within the African meningitis belt, biological specimens sent to the laboratory are frequently subjected to delivery times that are incompatible with the poor viability of some bacteria. The simple way of storing and dispatching CSF specimens meant that PCR was very suitable for this work, carried out in regions where only very few laboratories can perform classical bacteriological diagnosis, including culture. The choice of PCR as a reference in this study does not question the key importance of bacteriological methods such as Gram staining, culture and biochemical identification, but it was the only means to collect an adequate sample size within a reasonable time. The PCR has proven high performance for the diagnosis of meningococcal meningitis and for serogrouping (Issa et al., 2003; Kaczmarski et al., 1998; Parent du Châtelet et al., 2005; Sidikou et al., 2003; Tzanakaki et al., 2003). Furthermore, it was established that PCR has indisputably a higher confirmation rate than culture when the two methods are applied concurrently on fresh specimens (Guiver et al., 2000). While PCR should remain confined to country-level reference centres, our results indicate that LAs such as Pastorex are a reasonable alternative at district or health centre levels, assuming that the test kits are properly transported, stored and used.

A positive likelihood ratio above 10 can be considered as providing ‘convincing’ diagnostic evidence, while a negative likelihood ratio between 0.1 and 0.2 can be considered as giving ‘strong’ diagnostic evidence (Jaeschke et al., 1994). For both serogroup A and W135, the negative and positive likelihood ratios indicate that the Pastorex kit has good discriminatory capability in identifying meningococcal serogroup.

The 70\% prevalence of \(N.\) meningitidis that was used for calculations, although arbitrary, can be considered realistic (Spiegel et al., 1990) and allowed an assessment of the predictive values of LAT during epidemics. The high PPV calculated suggests that this test is appropriate to guide the choice between bivalent AC or trivalent ACW vaccine for epidemic response.

By contrast, the lower value for an NPV signifies that about 25\% of negative results would be false negatives. Consequently, at the onset of the epidemic, a sufficient number of specimens should be tested to guarantee that if an epidemic due to A or W135 was occurring, it would be detected.
The recommendations of a WHO expert committee (WHO, 2003) are that 20–30 CSF specimens be collected from an area affected by the onset of an epidemic to ensure that at least 10 positive results for N. meningitidis are obtained; examining 30 specimens with the Pastorex kit would give a 99% probability of obtaining at least 12 positive results, while testing 20 would give a 99% probability of obtaining at least seven positive results. PPV of LAT could be increased by screening with preliminary tests such as Gram stain and cell count of CSF.

The Pastorex kit has several limitations. It does not distinguish between serogroups Y and W135. Although currently few cases due to serogroup Y occur in Africa, this situation may change over time and it will be essential to obtain secondary confirmation from laboratory methods able to differentiate between serogroups Y and W135. Additionally, the current study was done under relatively ideal laboratory conditions. It will be useful to assess the performance when operated by less skilled staff and in remote locations, where prolonged exposure of the kit to high ambient temperatures may decrease its performance. Finally, the cost of the kit is still relatively high for sub-Saharan African countries.

5. Conclusion

Our study demonstrated the potential usefulness of latex agglutination tests for the identification of serogroups A and W135 of N. meningitidis. The test additionally permits the identification of Streptococcus pneumoniae and Haemophilus influenzae, which are responsible for a substantial amount of meningitis cases in the African meningitis belt.

The study was performed under relatively ideal conditions. We recommend widening the evaluation to determine whether similar results could be obtained at district health centre level under field conditions.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgements

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References


High sensitivity and specificity of the Pastorex® latex agglutination test for Neisseria meningitidis serogroup A during a clinical trial in Niger


a Epicentre, 8 rue St Sabin, 75011 Paris, France
b Médecins sans Frontières, 8 rue St Sabin, 75011 Paris, France
c Centre de Recherche Médicale et Sanitaire (CERMES), B.P. 10887, Niamey, Niger
d Ministère de la santé publique et de la lutte contre les endémies, B.P. 623, Niamey, Niger

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Summary There is a great need for a rapid diagnostic test to guide vaccine choice during outbreaks of meningococcal meningitis in resource-poor countries. During a randomised clinical trial conducted during an epidemic of Neisseria meningitidis serogroup A in Niger in 2003, the sensitivity and specificity of the Pastorex® latex agglutination test for this serogroup under optimal field conditions were assessed, using culture and/or PCR as the gold standard. Results from 484 samples showed a sensitivity of 88% (95% CI 85–91%) and a specificity of 93% (95% CI 90–95%). Pastorex® could be a good alternative to current methods, as it can be performed in a local laboratory with rapid results and is highly specific. Sensitivity can be improved with prior microscopy where feasible. A study specifically to evaluate the Pastorex® test under epidemic conditions, using laboratories with limited resources, is recommended.

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1. Introduction

Almost every year sub-Saharan Africa faces an outbreak of meningitis, usually caused by Neisseria meningitidis. In order to diagnose, treat and possibly to perform a timely mass vaccination campaign to contain an outbreak, both timeliness and accuracy of epidemic detection are crucial (Lewis et al., 2001). A rapid diagnostic tool, which is also accurate and affordable for resource-poor countries, is therefore essential.

Diagnosis of bacterial meningitis is based on identification of the pathogen in the cerebrospinal fluid (CSF), obtained by lumbar puncture, using a combination of macroscopic, microscopic and culture or PCR techniques. For a macroscopic examination, samples are checked for clarity,
cloudiness and the presence of blood. Microscopy includes white blood cell (WBC) count and Gram stain. Intracellular Gram-negative diplococci on Gram stain indicate the presence of meningococci, but this technique cannot confirm the serogroup(s) involved.

To date, the traditional gold standard technique for laboratory confirmation of a CSF sample for *N. meningitidis* has been culture (García-de-Lomas and Navarro, 1997). In addition, PCR is often performed to enhance confirmatory results (Abdel-Salam, 1999; Carrol et al., 2000; Taha, 2000) or for epidemiological/surveillance purposes (Boisier et al., 2005; Sidikou et al., 2003). PCR takes a shorter time to process than culture, although a single PCR assay can take 2–3 h. The PCR technique is more efficient if several assays are run simultaneously (e.g. 80 samples can take just 5 h (Gray et al., 1999)). However, in the resource-poor settings where these epidemics often occur, both culture and PCR are expensive and difficult to perform.

The Pastorex® latex agglutination test (Bio-Rad Laboratories, Inc., Marnes-la-Coquette, France) can differentiate between *N. meningitidis* serogroups A, C and Y/W135, *N. meningitidis* serogroup B/Esherichia coli, *Haemophilus influenzae*, *Streptococcus pneumoniae* and Group B *Streptococcus*. The test relies on soluble antigen detection in CSF, serum or urine samples (but for confirmation of meningococcal meningitis, antigen must be identified in CSF). Pretreated latex beads agglutinate in the presence of the same antigen, giving a result visible to the naked eye. In contrast to the hours spent on either culture or PCR, the Pastorex® test can be completed in approximately 30 min (including the time taken to heat and centrifuge the CSF sample). A recent evaluation of the Pastorex® kit for detection of *N. meningitidis* serogroups A and W135 was conducted by the Centre de Recherche Médicale et Sanitaire (CERMES; the national meningitis surveillance laboratory in Niger) in collaboration with the WHO and the Association pour l'Aide à la Mèdecine Préventive (AMP) under favourable laboratory conditions on CSF samples taken during 2002–2004. Using PCR as the gold standard, sensitivity and specificity for detection of *N. meningitidis* A during an epidemic were high (87% and 93%, respectively (Djibo et al., 2006)).

In mid February 2003, in the Zinder region of Niger, a meningitis outbreak was declared. Two weeks later it had reached the neighbouring region of Maradi. Both regions are in the south of the country, bordering Nigeria. By the end of the epidemic (mid June) in Zinder, there had been 4192 cases reported to the WHO, with 250 deaths, giving a case fatality ratio (CFR) of 6.0% and an attack rate (AR) of 180/100 000 population for the epidemic period. In Maradi there had been 1750 cases and 99 deaths reported (CFR 5.7%), giving an AR of 70/100 000 population (W. Perea, personal communication).

During this epidemic, a multicentre, randomised, non-inferiority trial comparing the efficacy of a short course of ceftriaxone with oily chloramphenicol was carried out on all suspect cases presenting to health structures in the Zinder and Maradi regions between 24 March and 27 April 2003. Here we report on the performance of the Pastorex® latex agglutination test as a method for rapid diagnosis of *N. meningitidis* A under optimal field conditions during an epidemic. Details of case selection, informed consent and treatment provided during the clinical trial have been published elsewhere (Nathan et al., 2005).

2. Materials and methods

Eligible suspect cases of meningococcal meningitis underwent a lumbar puncture to obtain a CSF sample. Most samples underwent direct macroscopic and microscopic examinations with WBC count followed by centrifugation, Gram stain and Pastorex® agglutination test.

Samples were classified as negative by direct microscopy if no bacteria were seen or if the presence of bacteria was reported as rare with a WBC count <10/mm³. Samples were considered positive if Gram-negative diplococci were seen and the WBC count was >50/mm³. All samples with direct microscopy results not fulfilling the criteria for either positive or negative were classified as doubtful.

Culture was performed for all samples that were positive or doubtful on direct microscopy. PCR was performed for all CSF samples that were bloody, or that did not have culture performed, or that had negative or contaminated culture results, or discordant results for Pastorex® and culture.

Although 100% specific, culture is easily contaminated in field conditions and *Neisseria*, a fragile bacterium, must be cultured within 2 h of CSF collection. Similarly, microscopy becomes difficult after more than 2 h delay. The effectiveness of the PCR technique, however, is not dependent on the length of time since sample collection.

The Pastorex® test was performed following the manufacturer’s instructions. Conventional culture (blood agar and chocolate agar, incubated at 37°C with 5% CO₂), biochemical identification (Api-NH system; bioMérieux, La Bâmeles-Grottes, France) and serogrouping with specific antisera (Difco, Detroit, MI, USA) were performed. Direct microscopy, Pastorex® and culture were carried out at the national hospital in Zinder, in a laboratory designed specially for the trial.

An aliquot of each CSF sample was frozen at −20°C and transported in an ice box to the CERMES laboratory in Niamey, where the multiplex PCR assays for the determination of *N. meningitidis* serogroups were performed following a previously described protocol (Taha, 2000).

A ‘gold standard’ combination of culture and/or PCR was used to identify cases of *N. meningitidis* serogroup A. The gold standard positives were selected as all samples with a positive result for serogroup A from either culture or PCR, or both. Gold standard negatives were only those samples with negative results for both culture and PCR. Samples with contaminated or uninterpretable culture and negative or uninterpretable PCR were excluded from this analysis, as well as those with uninterpretable Pastorex® results (see Figure 1).

Using samples that had had both a Pastorex® test and a gold standard test (culture and/or PCR), the positive and negative results were compared to determine the performance of the Pastorex® test in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for determining cases due to serogroup A. The proportion of results misclassified by the Pastorex® test was calculated.

To determine whether sensitivity could be improved by performing a microscopic examination prior to the Pastorex®
test, a supplementary analysis was conducted. Only samples with clear positive or negative results for direct microscopy (defined as above) followed by a Pastorex® test were included. The samples in this analysis were considered to be positive only if the results for both Pastorex® and direct microscopy tests had been positive, and negative if results for both Pastorex® and direct microscopy tests had been negative. These combined results were then compared with the gold standard results as before. Samples with discordant results for direct microscopy and Pastorex® were excluded from this analysis (see Figure 1).

Statistical analyses were performed using Stata™ 8.2 for Windows (StataCorp, College Station, TX, USA) statistical software. Calculation of misclassification was performed using the DAG_Stat Excel spreadsheet (Mackinnon, 2000).

3. Results

During the study period, 510 suspect meningitis cases examined on arrival at health structures in the two regions were included in the clinical trial and randomised for treatment, 495 (97.1%) of whom had a successful lumbar puncture. Of these 495 CSF samples, 494 (99.8%) had either PCR or culture, 491 (99.2%) had direct microscopy (WBC count and Gram stain) and 488 (98.6%) were tested using the Pastorex®
kit (Table 1). Of the 494 samples tested by PCR and/or culture, 352 were positive for \textit{N. meningitidis} (of which 350 were serogroup A; data not shown). Figure 1 gives the schematic diagram for the initial microscopy tests followed by the tests performed for serogroup confirmation.

After exclusions due to contamination, or uninterpretable or inconclusive results, the total number of samples with positive or negative results for serogroup A, for both the Pastorex® test and ‘gold standard’ (culture and/or PCR), was 484 (Table 1). For direct microscopy followed by Pastorex®, after excluding 207 samples with doubtful microscopy results and 19 with discordant microscopy and Pastorex® results, the number of samples remaining for supplementary analysis was 263 (Figure 1).

Table 2 shows the results for the sensitivity, specificity, PPV and NPV for the Pastorex® direct agglutination test alone and with initial direct microscopy compared with PCR and/or culture as gold standard (95% CI in parentheses).

<table>
<thead>
<tr>
<th>Test used</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Misclassification rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pastorex® alone (N = 484)</td>
<td>88% (85–91)</td>
<td>93% (90–95)</td>
<td>97% (95–98)</td>
<td>75% (71–79)</td>
<td>11% (8–14)</td>
</tr>
<tr>
<td>Direct microscopy and Pastorex® (N = 263)</td>
<td>97% (94–99)</td>
<td>91% (88–95)</td>
<td>98% (96–99)</td>
<td>88% (85–92)</td>
<td>5% (2–8)</td>
</tr>
</tbody>
</table>

Table 2 shows the results for the sensitivity, specificity, PPV and NPV for the Pastorex® test alone and the Pastorex® test with prior direct microscopy compared with the gold standard. The sensitivity for the Pastorex® test alone was 88% (95% CI 85–91%). With prior microscopy, although results were available for fewer samples, sensitivity improved to 97% (95% CI 94–99%). Our data suggest an excellent PPV for the Pastorex® test alone (97%; 95% CI 95–98%). The NPV for Pastorex® alone was 75% (95% CI 71–79%), which improved to 88% (95% CI 85–92%) with prior direct microscopy.

The rate of misclassification (positive and negative) for the Pastorex® test alone was 11% (95% CI 8–14%), falling to 5% (95% CI 2–8%) for samples with prior direct microscopy.

4. Discussion

The benefits of early and accurate diagnosis of true cases of meningococcal meningitis are clear, as a diagnostic test that is highly sensitive will leave fewer false negatives untreated in the community potentially continuing to spread the epidemic. One that is also specific, however, will allow true negatives to be identified and receive appropriate treatment for their condition.

Our results suggest that the Pastorex® test has high specificity and PPV for detection of \textit{N. meningitidis} serogroup A as well as being sensitive compared with the gold standard of culture and/or PCR. In addition, it is a rapid alternative, requiring relatively short training, and can be performed in non-specialised laboratories (Djibo et al., 2006). For example, only one sample tested by Pastorex® was excluded from our analysis for uninterpretable results (less than 0.5%). In contrast, 9% of samples that had been cultured were contaminated. This proportion may be higher in ‘true’ field conditions owing to the fragility of meningococci and the feasibility of performing culture before deterioration or contamination of the sample (e.g. due to transport difficulties).

Although cost may still be an issue in resource-poor settings (relative costs for Pastorex® lie between those for microscopy and PCR), simplification of the kit for use in epidemic situations could be considered by the manufacturers.

Although the direct microscopy technique can indicate the presence of meningococci, this test cannot differentiate the serogroup, therefore it cannot be used on its own to provide information on which vaccine should be utilised. In further analysis we investigated whether direct microscopy followed by the Pastorex® test could improve on the use of the Pastorex® test alone for detection of serogroup A. Although this combination did indicate better sensitivity and NPV than the Pastorex® test alone, the analysis was conducted on a smaller number of samples owing to the high proportion of ‘doubtful’ direct microscopy results (42%) not included in the combination analysis. It is most likely that these were a result of delays in transporting samples from further away to the study laboratory.

We recommend following diagnostic algorithms such as those suggested in Figure 2, but only in settings where microscopy is feasible (and bearing in mind the potential...
Figure 2  Two suggested alternative algorithms for optimal use of the Pastorex® test in the field for determining the epidemic serogroup (feasible if capacity for microscopy exists). (a) If resources (human and financial) permit, perform a microscopic examination followed by a Pastorex® test to confirm the epidemic strain. Often, field conditions preclude the option of microscopy as there may be no laboratory available. In this case, the Pastorex® test alone should be used. For this algorithm, excluding the 19 discordant results for Pastorex® and direct microscopy, and using Pastorex® results where microscopy results were doubtful, the calculated sensitivity (N=465) was 90% (95% CI 86–93%), specificity 92% (95% CI 87–96%), positive predictive value (PPV) 97% (95% CI 94–98%) and negative predictive value (NPV) 79% (95% CI 71–85%). (b) For a more economical option, perform microscopic examination followed by a Pastorex® test only on those that are positive. For this algorithm, calculated sensitivity (N=282 after 2 exclusions) was 91% (95% CI 86–94%), specificity 92% (95% CI 83–97%), PPV 98% (95% CI 94–99%) and NPV 75% (95% CI 64–84%).

\(^{a}\) Positive: Gram-negative diplococci seen and white blood cell count >50/mm³.

\(^{b}\) Negative: no bacteria present or presence of bacteria rare; white blood cell count <10/mm³.

\(^{c}\) Doubtful: any other result not covered by positive or negative definitions.
impact of delayed testing on microscopy results). In practice, especially during an epidemic affecting remote areas of a resource-poor country, there may be limited or no capacity for direct microscopy, in which case for this serogroup the Pastorex® test alone provides the best solution.

Our study was designed to measure non-inferiority of treatment rather than specifically to evaluate the Pastorex® test under epidemic conditions. We also utilised a specialised laboratory, which would not be the norm for a resource-poor area during an epidemic. Thus, the field conditions during our study were exceptionally good, which may influence our results. We conducted this evaluation during an epidemic of N. meningitidis serogroup A. Therefore, our results cannot be used to predict the performance of the Pastorex® test for other bacteria or N. meningitidis serogroups.

Other studies have estimated the sensitivity of rapid tests for meningococcal antigen detection with varying results. Although recent studies in the UK have found improved sensitivities using ultrasound-enhanced latex agglutination with the Pastorex® (Sanofi Diagnostics Pasteur), Slidex (bioMérieux) and Wellcogen™ (Abbott Murex) kits for N. meningitidis A/C/Y/W135 detection (Gray et al., 1999; Sobanski et al., 2002), another in Australia (Porritt et al., 2003) found that this technique had a lower sensitivity than PCR and, as it requires special ultrasonic equipment, it may not be a good alternative to culture and PCR under epidemic conditions, especially in resource-poor settings. An earlier study on a conventional test card latex agglutination technique gave a much lower sensitivity than the latex test evaluated in our study (Gray et al., 1999). However, the most recent study evaluating the Pastorex® test under ideal laboratory conditions and using a gold standard of PCR alone found almost identical results to ours (Djibo et al., 2006).

Our study clearly suggests the potential for the use of the Pastorex® test in the field during an epidemic. Further studies are needed that are designed to assess the test’s performance under normal field conditions, i.e., outside of a clinical trial setting and in a country with limited laboratory resources. If similar results are found, then this test could prove to be an invaluable tool for early N. meningitidis serogroup detection in meningitis outbreaks, permitting more timely selection of the correct vaccine to prevent further spread of the disease and the inevitable deaths this could cause.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgements
We dedicate this work to the memory of our friend and colleague Nicolas Nathan who left us prematurely in May 2004. We thank Amina Abdoulaye and Danielle Bonneville for all their work with us in the laboratory in Niger, and Saacou Djibo and Pascal Boisier for their biological and epidemiological support (CERMES). We are grateful for the support of Laurence Flévaud (MSF Paris) and David Evans, study co-ordinator for the clinical trial. Thanks also to Laurence Bonte (MSF Paris), Kate Alberti and Rebecca Grais (Epicentre) for advice and for reading and commenting on early drafts. This study was supported financially by Médecins sans Frontières.

References
Porritt, R.J., Mercer, J.L., Munro, R., 2003. Ultrasound-enhanced latex immunoagglutination test (USELAT) for detection of capsular polysaccharide antigen of Neisseria meningitidis from CSF and plasma. Pathology 35, 61—64.
1. 사용 원리
특이적인 상동 항체를 코팅한 라텍스 입자와 해당 항원과의 반응을 검사합니다. 상동 항원이 존재할 때 라텍스 입자와 응집이 일어납니다. 항원이 존재하지 않으면, 그대로 균질의 현탁액 상태로 존재합니다.

2. 시약 구성
1. 시약 구성
1) Reagent 1(R1) : *N.meningitidis* B/E.coli K1
   - 쥐의 *N.meningitidis* B/E.coli K1에 특이적인 단클론 항체가 코팅되어 있는 빨간 라텍스 0.4ml 1병
2) Reagent 2(R2) : *N.meningitidis* B/E.coli K1 negative control
   - 쥐의 파상풍 특소이드에 특이적인 단클론 항체로 코팅되어 있는 빨간 라텍스 0.4ml 1병
3) Reagent 3(R3) : *H.influenzae* b
   - 토끼의 *H.influenzae* b에 특이적인 항체로 코팅되어 있는 홍색 라텍스 0.4ml 1병
4) Reagent 4(R4) : *S.pneumoniae*
   - 토끼의 *S.pneumoniae*에 특이적인 항체로 코팅되어 있는 녹색 라텍스 0.4ml 1병
5) Reagent 5(R5) : *Streptococcus* B
   - 토끼의 *Streptococcus* B에 특이적인 항체로 코팅되어 있는 노란색 라텍스 0.4ml 1병
6) Reagent 6(R6) : *N.meningitidis* A
   - 토끼의 *N.meningitidis* A에 특이적인 항체로 코팅되어 있는 흰색 라텍스 0.4ml 1병
7) Reagent 7(R7) : *N.meningitidis* C
   - 토끼의 *N.meningitidis* C에 특이적인 항체로 코팅되어 있는 파란색 라텍스 0.4ml 1병
8) Reagent 8(R8) : *N.meningitidis* Y/W 135
   - 토끼의 *N.meningitidis* Y/W135에 특이적인 항체로 코팅되어 있는 핑크색 라텍스 0.4ml 1병
9) Reagent 9(R9) : 다가 음성 대조
   - 비 면역화된 토끼의 IgG 면역항체(글로불린)로 코팅된 보라색 라텍스 0.4ml 1병
10) Reagent 10(R10) : 다가 양성 대조
모든 시약은 0.02 % 치오메살을 함유합니다.
11) 반응카드 1팩
12) 혼합막대 3팩
13) 사용설명서 1부

2. PASTOREX™ MENINGITIS 희석액 (별도구매)
혈청 희석액 40ml code 61717

3. 보관
모든 시약은 라벨에 표시된 유효기간까지 2-8℃에서 보관합니다.
다가 양성 대조 시약인 R10은 재현탁 후 2-8℃에서1달간 보관이 가능하며, 장기간 보관을 위해서 -20℃에서 보관합니다.

4. 제공되지 않는 기구
* 검체를 40-50㎕ 분주할 수 있는 파이펫
* 혈청 용 튜브
* 배양기 혹은 100℃에서 사용가능한 water bath
* 원심분리용 튜브
* 살균기
* 증류수, sterile saline, 혹은 희석액(code 61717)

5. 주의 사항
전문의약품입니다.
* 모든 시약과 샘플은 사용 전에 실온 18-25℃에 방치합니다.
* 반응이 진행중인 시약과 접촉하지 않습니다.
* 각 샘플 테스트에 각각의 파이펫과 펑트를 사용합니다.
* 라텍스 시약은 사용 전에 흔들어 놓습니다.
* 시약의 점적기의 펑트 부분은 정량의 점적되도록 깨끗이 사용합니다.
* 라텍스 시약은 수직으로 잡고 점적합니다.
* 각 반응에 각각의 혼합막대를 사용합니다.
* 모든 일회용 기구는 고압살균이나 살균 처리한 후 폐기합니다.
* 다가 양성 대조 시약은 오염을 최소화하여 멸균된 증류수로 재현탁합니다.

6. 검체 준비 : CSF, serum, urine
샘플은 2-8℃에서 보관하며 장기간 보관을 위해서 -20℃에서 보관합니다. (이때, 원심분리 후 -20℃에서 상층액만 보관합니다.) 최소 검체량은 0.5ml입니다.
A) 검체 준비

a) CSF (cerebrospinal fluid)
혼탁하거나 혈액이 혼합된 샘플은 5분간 350g에 원심분리한 후 상층액을 재취합니다.
* 샘플은 100℃에서 3분간 가열합니다. 실온에서 식힌 후, 5분간 3000g에서 원심분리 하거나 0.45㎛ 여과지로 정제합니다.

b) Serum
* 화학액(code 61717) 1.5ml에 샘플 0.5ml로 3배 희석합니다.
* 100℃에서 3분간 가열합니다.
* 5분간 3000g에서 원심분리합니다.
주의 : 혈장샘플은 사용하지 않습니다. 알부민, 지방질, 헤모글로빈과 빌리루빈(bilirubin)은 테스트에 적합하지 않습니다. 최적의 결과를 위해선 신선한 혈청을 사용합니다.

c) Urine
* 샘플을 100℃에서 3분간 가열합니다.
* 5분간 3000g에서 원심분리합니다.

B) 실험방법
* 전체리한 검체의 상층액을 반응카드에 한 방울 (40㎕-50㎕) 점적합니다.
* 라텍스 시약을 천천히 흔듭니다.
* 각 라텍스 시약을 수직으로 잡고 반응 카드에 상층액 주변에 한 방울씩 점적합니다.
* 혼합막대를 각각 따로 사용합니다.
* 반응 카드를 (~120RPM) 5분간 로테이터에 둘러주면서 10분 내에 응집을 관찰합니다.

혈액 배양물은 비특이적 결과가 나타나거나 결과 해석에 어려움이 있을 수 있습니다. 응성 대조로 멸균된 혈액을 접종한 혈액 배양물과 다른 시약으로 실험합니다.

8. 결과 해석
양성 결과
양성 결과는 육안으로 관찰하며, 응성 대조와는 달리 응집이 나타납니다.
혈액의 항원 농도와 시간에 따라 응집의 강도가 달라집니다.
양성 결과가 나타나지만 배양에서 응성일 때, 배양 샘플에선(샘플을 체취하기 전에 항균제를 처리하였거나, 검체 운반 중에 세균이 배양될 수 없는 경우) 세균이 사멸될 가능성이 있습니다.
대부분 N.meningitidis B/E.coli K1 라텍스 시약에서 보이는 양성 결과는 신생아나 조산아의 경우 E.coli K로 결과 보고합니다. 성인은 Meningococcus B 로 결과 보고하며 배양결과로 확인합니다.

음성결과
응집이 없는 균질의 현탁액

비특이적 결과
검체가 응성 대조군(R2나 R9)과 반응하여 응집이 있거나 키트내 한가지 이상의 시약과 반응하는 것은 비특이적 반응입니다. 이 경우, 다른 검체를 테스트 해보거나 배양 결과로 확인합니다. (매우 드물게, 두 종류의 세균에 감염된 경우도 있습니다.)

9. 플레이트 배양 절차
배지에서 세균 중의 그룹 분리 (순수 배양 절차)
라텍스 Y/W135는 두 그룹을 분리할 수 없습니다. 이 그룹의 분리는 전통적인 방법으로 항혈청(Y/W135, 29E; Ref #58704, 3x1ml)을 권장합니다. 이 검사를 시행하기 전에:
* 성상과 그룹 염색 결과를 확인합니다.
* 그룹 응성 세균이면 염색이 차이가 있는 Meningococcus B를 실험합니다.
* 그람 양성 세균이면 카탈라아제 테스트(카탈라아제에 양성인 검체는 테스트 하지 않습니다.)를 실시합니다. S.pneumoniae와 Haemophilus influenzae균 종은 이 절차에 적합하지 않습니다.

a) N.meningitidis (A,B,C만 해당) , H.influenzae, E.coli, S.pneumoniae의 분리
* 멸균된 saline을 반응원 안에 30㎕을 점적합니다.
* 샘플 준비:
  - N.meningitidis, H.influenzae, E.coli 배양물에서 1㎕ 루프로 최대한 2-3 콜로니를 채취합니다.
  - S.pneumoniae 배양물에서 5㎕ 루프로 최대한 10-12 콜로니를 채취합니다.
* 샘플 콜로니를 에멀젼화합니다.
* 해당 라텍스 시약을 가볍게 흔들어줍니다; 수직으로 잡아 세균 현탁액 외부에 한 방울 점적합니다.
* 혼합막대를 사용해 라텍스와 세균 현탁액을 혼합합니다.
* ~120rpm에서 반응카드를 흔들어줍니다.
* 2분 이내에 응집을 관찰합니다.
* 전통적인 생화학 테스트로 균종의 분리를 확인합니다.

b) Streptococcus B의 분리(β-용혈의 집락)
* Todd Hewitt broth 2ml에서 5 콜로니를 채취합니다.
* 37℃ water bath에서 2-3 시간 배양합니다.
* 3000g에서 5분간 원심분리합니다.
* 반응원 안에 상층액 한 방울 (40-50㎕)을 점적합니다.
* 해당 라텍스 시약을 가볍게 혼들어줍니다; 수직으로 잡아 세균 현탁액 외부에 한 방울 점적합니다.
* 혼합막대를 사용해 라텍스와 세균 현탁액을 혼합합니다.
* ~120rpm에서 반응카드를 흔들어줍니다.
* 2분 이내에 응집을 관찰합니다.
* 전통적인 생화학 테스트로 균종의 분리를 확인합니다. 분리 배양된 콜로니의 직접 검사는 PASTOREX™STREP(code 62721)로 검사합니다.

10. 정도 관리
라텍스 시약은 혼들어 준 후 균질화되어야 합니다. 양성 대조 시약은 A,B,C, E.coli, K1, S.pneumoniae는 각 라텍스의 연역학적 반응을 확인합니다.
* 정도 관리를 시행하기 위해, 양성대조(R10)를 한 방울 (40㎕)를 반응원 안에 점적합니다.
* 라텍스 시약을 가볍게 혼들어 줍니다.
* 병을 수직으로 잡아 각 라텍스 시약과의 패턴을 확인합니다. R9,R6,R7,R1,R2는 흰색 반응원 안에, R8,R3,R4,R5는 검은색 반응원 안에 점적합니다.
* 라텍스 시약과 양성 대조 시약을 혼합 막대로 혼합하여, 라텍스 시약마다 각각의 혼합막대를 사용합니다.
* ~120rpm에서 10분간 반응카드를 흔들어 줍니다. 10분 이내에 응집 현상을 관찰합니다. (음성 대조 시약과 라텍스의 반응을 비교합니다.)

11. 제품 성능 시험
* CSF(cerebrospinal fluid)검체를 전통적인 방법(배양, 현미경 관찰)으로 준비합니다.
* Mueller Hinton 배지, chocolate +PVS 배지, Columvia +5% sheep blood 배지에서 분리 배양합니다.

<table>
<thead>
<tr>
<th>검체 또는 배양한 집락</th>
<th>희석한 항원 (Nacl,9%)</th>
<th>CSF</th>
<th>균주</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>검출한계 농도</td>
<td>생물 결과</td>
<td>양성 대조</td>
</tr>
<tr>
<td>N.meningitidis A</td>
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<td>12</td>
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<tr>
<td>N.meningitidis B</td>
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<td>ND</td>
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<tr>
<td>E.coli K1</td>
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<tr>
<td>N.meningitidis C</td>
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<td>3</td>
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<tr>
<td>N.meningitidis Y</td>
<td>5ng/ML</td>
<td>ND</td>
<td>-</td>
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<td>N.meningitidis W</td>
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<tr>
<td>S.pneumoniae</td>
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<tr>
<td>H.influenzae type b</td>
<td>0.1ng/ML</td>
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<td>33</td>
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</tbody>
</table>
특이도
각 시약의 특이도는 다음 방법으로 실시합니다:
* 멸균된 CSF와 수막염 원인인 균에 감염된 라텍스에 검출되는 CSF를 준비합니다.
* Neisseria, Brangamella, Acinetobacter, Streptococcus, Klebsiella, Haemophilus, Escherichia coli non K1, Pseudomonas의 균주를 준비합니다.

<table>
<thead>
<tr>
<th>라텍스 시약</th>
<th>멸균된 CSF(a)</th>
<th>감염된 CSF(b)</th>
<th>군주(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.meningitidis A</td>
<td>52 52</td>
<td>50 50</td>
<td>122 122</td>
</tr>
<tr>
<td>N.m. B /E.coli K1</td>
<td>12 12</td>
<td>ND</td>
<td>53 53</td>
</tr>
<tr>
<td>N.meningitidis C</td>
<td>52 52</td>
<td>56 56</td>
<td>127 127</td>
</tr>
<tr>
<td>S.pneumoniae</td>
<td>60 60</td>
<td>39 39</td>
<td>33 33</td>
</tr>
<tr>
<td>Streptococcus B</td>
<td>49 49</td>
<td>58 58</td>
<td>17 17</td>
</tr>
<tr>
<td>H.influenzae type b</td>
<td>61 61</td>
<td>32 32</td>
<td>31 31</td>
</tr>
<tr>
<td>비선택적인 CSF</td>
<td>샘플 결과</td>
<td>응성대조</td>
<td></td>
</tr>
<tr>
<td>N.meningitidis W</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

* 1/1 Neisseria flavescens 균주, 2/4 Klebsiella pneumonia 균주, 2/5 비특이 반응을 보인 Acinetobacter 균주

14. 사용상의 제한점
* 면역학적인 라텍스 법은 대부분 미생물의 감별 진단을 목적으로 합니다. 그러나 미생물 항원의 능도가 검출한 계에 이르지 못하면 응성 결과가 나타납니다. 이 경우 재검사합니다.
* 이 검사법은 항균제 감수성 검사를 동반한 세균 배양을 대체하지 못합니다.
* 혈액 배지는 매우 다양하기 때문에, 모든 배지에서의 결과가 해당되지는 않습니다. (Cf.7-D)
* 현재 임상과 논문에서 라텍스 시약을 이용한 혈청과 소변에서의 항원 검출은 제한적으로 보고 되고 있습니다.
* 연관성이 없는 몇몇 세균 배양에서 유사한 항원이 검출이 보고 되고 있습니다. 교차 반응의 가능성을 고려합니다(1,4,5).
* 최종 진단은 한 가지 전단 결과만을 참고 하지 않습니다. 임상에서의 결과와 생화학적, 세포학적, 면역학적 결과를 함께 참고합니다.
* 혈액배양물에서 유해된 항원의 검출과 마찬가지로 배지에서 분리된 군종의 분리는 세균의 종까지 추가적인 동정이 필요합니다.

혈액배지에서의 배양률
PASTOREX™ MENINGITIS는 혈액배지에서의 배양들로도 검사 가능합니다. 성능 시험 결과는 다음과 같습니다:
* 민감도 : 100%(4/4)
(2종의 S.pneumoniae군주와 2종의 Streptococcus B군주에서)
* 특이도 : 100 % (37/37)
    R3, R4, R5, R6, R7, R8 시약
* 특이도 : 97.5 % (39/40) R1 시약
3종의 Klebsiella pneumoniae의 혈액배지에서의 배양률, 1종에서 비특이 반응 보임.