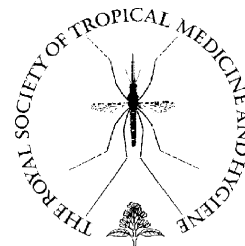




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High sensitivity and specificity of the Pastorex® latex agglutination test for *Neisseria meningitidis* serogroup A during a clinical trial in Niger

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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,

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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 (Garcia-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/*Escherichia 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). Pre-treated 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 Médecine 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 Balmeles-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[®]

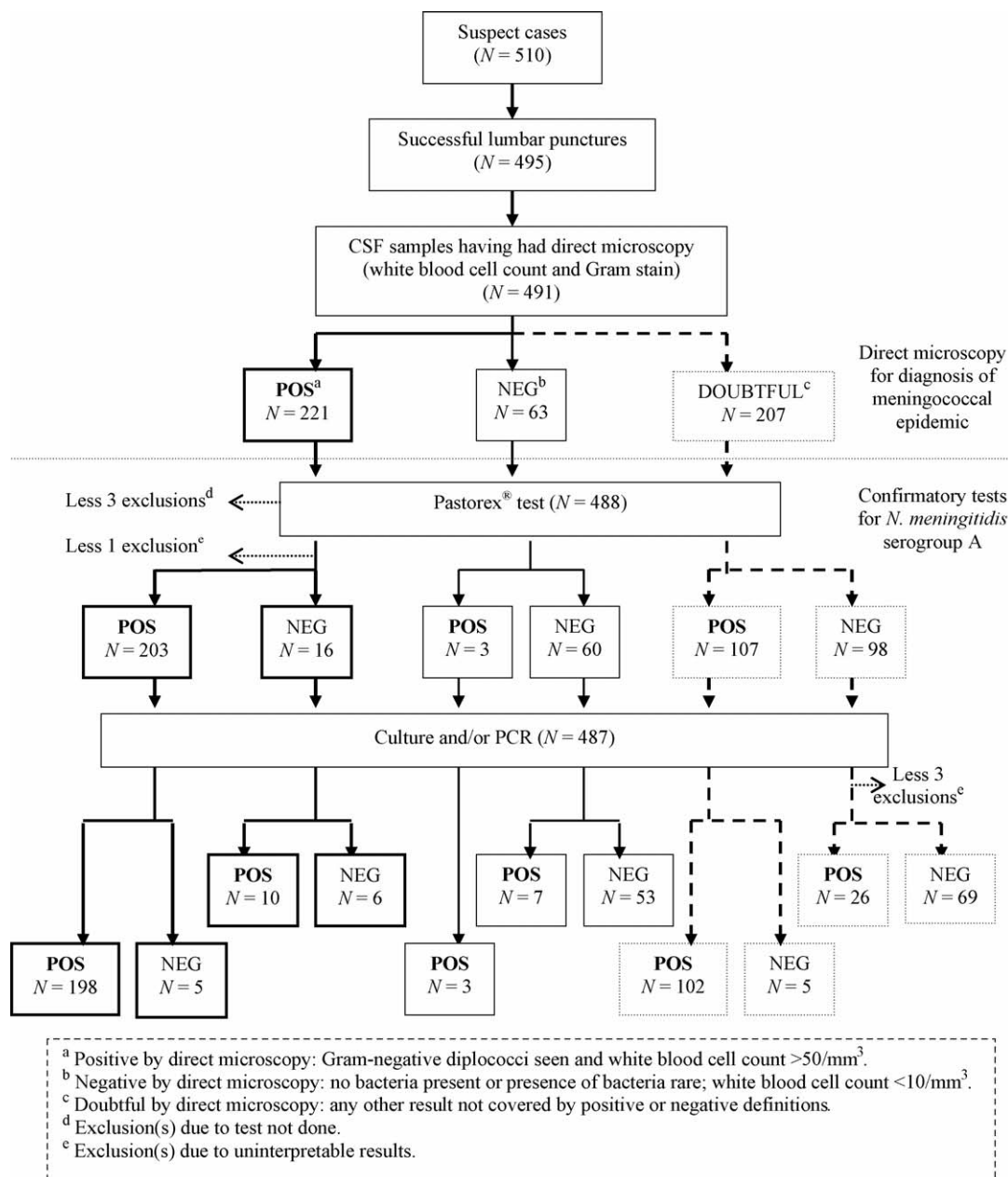


Figure 1 Schematic diagram showing diagnostic and confirmatory tests and results. The total number of positive results after culture and/or PCR ($N=346$) differs from the overall total stated in the text ($N=350$) as the latter included samples that had not had prior microscopy.

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) statisti-

cal 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[®]

Table 1 Description of tests carried out on 495 successful lumbar puncture samples during an epidemic of *Neisseria meningitidis* A in Niger, 2003

Test	No. tested (%)	No. excluded (%) ^a	Total (%)	No. also having positive or negative gold standard result (%)
PCR and/or culture	494 (99.8)	3 (0.6)	491 (99.4)	—
Direct microscopy ^{b,c}	491 (99.2)	207 (42.2)	284 (57.8)	284 (100.0)
Pastorex [®]	488 (98.6)	1 (0.2)	487 (99.8)	484 (99.4)

^a Exclusions due to contamination, or uninterpretable or inconclusive results.

^b Direct microscopy = white blood cell count and Gram stain.

^c 'Doubtful' direct microscopy results excluded from further analysis.

Table 2 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the Pastorex[®] direct agglutination test alone and with initial direct microscopy^a compared with PCR and/or culture as gold standard (95% CI in parentheses)

Test used	Sensitivity	Specificity	PPV	NPV	Misclassification rate ^b
Pastorex [®] alone (N = 484)	88% (85–91)	93% (90–95)	97% (95–98)	75% (71–79)	11% (8–14)
Direct microscopy and Pastorex [®] (N = 263)	97% (94–99)	91% (88–95)	98% (96–99)	88% (85–92)	5% (2–8)

^a Direct microscopy = white blood cell count and Gram stain.

^b Overall proportion of results misclassified for each test used.

kit (Table 1). Of the 494 samples tested by PCR and/or culture, 352 were positive for *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[®] 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 *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

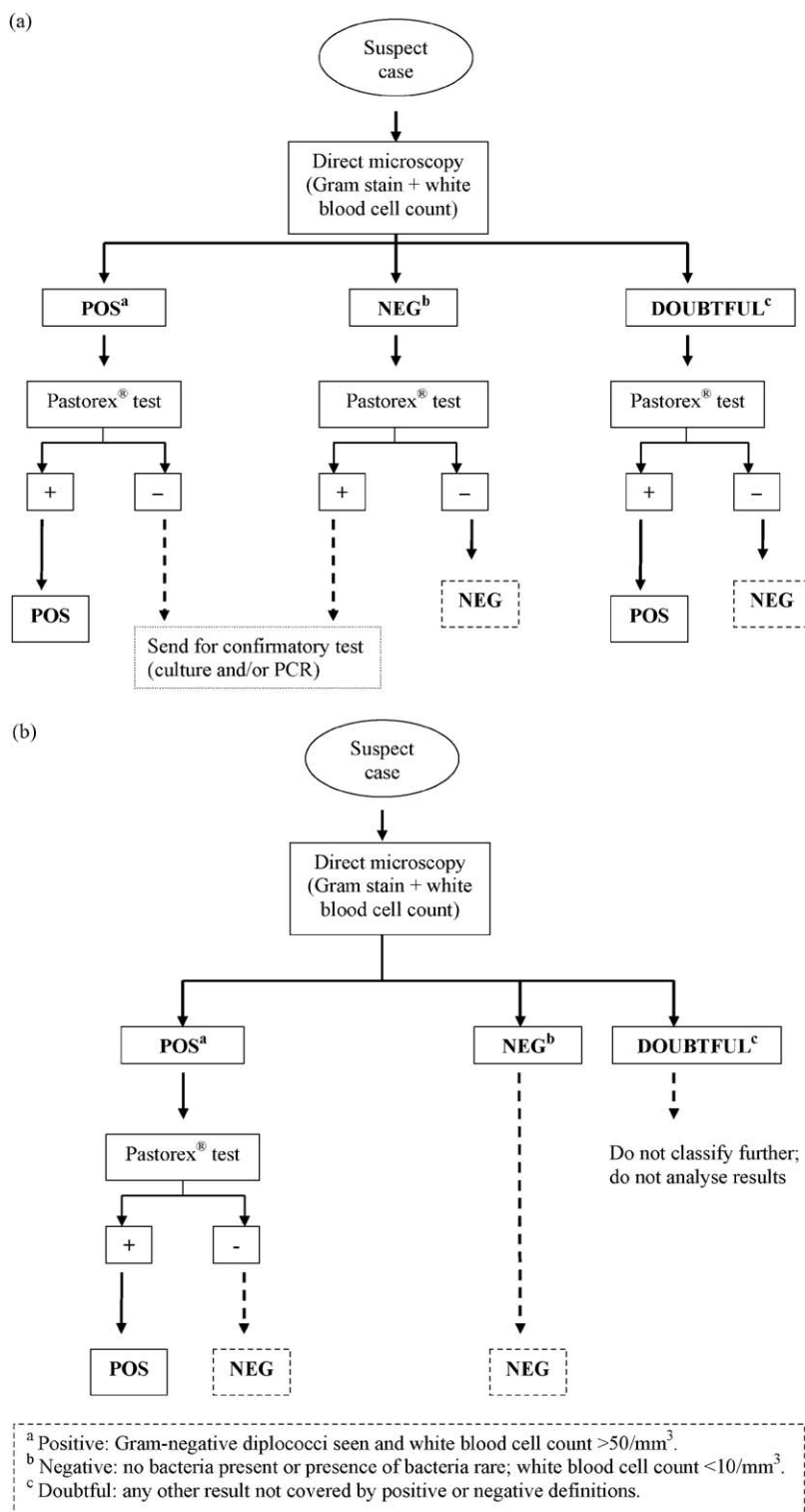


Figure 2 Two suggested alternative algorithms for optimal use of the Pastorex[®] test in the field for determining the epidemic serogroup (feasible if capacity for microscopy exists). (a) If resources (human and financial) permit, perform a microscopic examination followed by a Pastorex[®] test to confirm 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%).

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 have influenced 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.

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