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Biological diagnosis of meningococcal meningitis in the African meningitis belt: Current epidemic strategy and new perspectives

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Abstract

Laboratory diagnosis is an essential component in surveillance of meningococcal epidemics, as it can inform decision-makers of the *Neisseria meningitidis* serogroup(s) involved and the most appropriate vaccine to be selected for mass vaccination. However, countries most affected face real limitations in laboratory diagnostics, due to lack of resources. We describe current diagnostic tools and examine their cost-effectiveness for use in an epidemic context. The conclusion is that current WHO recommendations to use only the latex agglutination assay (Pastorex) at epidemic onset is cost-effective, but recently developed rapid diagnostic tests for the major epidemic-causing meningococcal serogroups may prove a breakthrough for the future.

Keywords: Meningococcal meningitis; Diagnosis; Africa

1. Introduction

Meningococcal epidemics occur worldwide but more than 50% of cases are reported from sub-Saharan Africa's "meningitis belt" [1,2]. Large epidemics are attributed predominantly to *Neisseria meningitidis* (Nm) serogroup A [3,4]. However serogroups C and X meningococci have been isolated during some epidemics [5–7] and, more of concern, serogroup W135 (NmW135) was identified in 2002–2003 as the main pathogen during outbreaks in Burkina Faso [8]. This serogroup is responsible for sporadic cases in many countries [9]. With the possibility of epidemics like those in Burkina Faso, and in the context of the limited availability of polysaccharide vaccines which include NmW135, decision-makers at country level in the meningitis belt need the best epidemiological and laboratory evidence in order to make the most appropriate vaccine choice. A diagnostic tool, either a single test or a test combination, which both would identify the serogroup implicated and would be rapid, cheap and simple enough to be performed in local health structures with limited resources, could result in earlier outbreak identification and better preparation for mass vaccination.

Such a tool would also prove vital at patient level, as early and correct antibacterial therapy is essential for a good outcome. The length of time needed for laboratory results to reach peripheral health structures not equipped with either laboratory or technical staff precludes the immediate benefit of such results for the individual patient. Thus, after initial declaration of a meningococcal epidemic in countries in the meningitis belt, treatment at peripheral level is often based on clinical diagnosis. Although signs and symptoms of non-meningococcal disease are indistinguishable from meningococcal disease, the treatment may not be the same, and this could have serious consequences for the patient.

Culture and PCR are both currently considered to be standard diagnostic methods. However, due to numerous obstacles that will not be solved in the near future in the countries of the meningitis belt (including cost, a need for trained staff and sophisticated equipment), these reference

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tests are rarely available, except in some capital cities or other large urban centres. Recent evaluations of the latex agglutination kit (Pastorex®, Biorad, Marne la Coquette, France), conducted in Niger and Burkina Faso under reference laboratory conditions [10], and in a Niger district laboratory in an epidemic context [11], have given promising results.

Here we provide a brief overview of current meningococcal diagnostics available at both national and district levels and provide new perspectives in light of a recently validated rapid diagnostic test.

2. Non-culture methods: latex agglutination test (LAT), Gram stain and white blood cell count (WBC)

2.1. The LAT

LATs for serogroup-specific polysaccharide (PS) have been in use for more than two decades. Although they provide more rapid results than culture or PCR, and can give positive results even after a few days of treatment, while culture or PCR cannot, much controversy has arisen over their proper use and variable performance [12–14]. More recently, the changing epidemiology of meningococcal disease compelled recommendation of kits capable of identifying meningococcal serogroup W135. The Pastorex® test (Biorad) can detect soluble meningococcal PS for NmA, B, C and W135/Y (i.e. it does not differentiate between NmW135 and NmY) in the cerebrospinal fluid (CSF) of the patient. The sensitivity and specificity of the test for the diagnosis of NmA and NmW135, evaluated both under reference laboratory conditions [10] and in a district laboratory in Niger [11] ranged from 84.9 to 88.0% and from 93.0 to 97.4%, respectively. The Pastorex® test, used at district level under laboratory conditions, has acceptable accuracy, and ideally could be used to test all CSF samples taken, regardless of the season. However, its cost is high and once the kit has been opened, its reagents have a limited shelf-life. In addition, each kit provides enough reagents for 20–25 tests; thus having this kit pre-positioned in every district-level laboratory for sporadic cases outside of the meningitis season or even for routine outbreak surveillance is too costly for most countries inside the belt. A more cost-effective option is to pre-position LAT kits at regional or central level so that they can be subsequently sent to the district laboratory in case of a suspected outbreak for preliminary identification of the responsible serogroup(s).

2.2. Gram stain

This method can be highly specific for indicating absence of epidemic-causing meningococci, if performed by a well-trained technician, when a clinical case definition is stringently applied and disease prevalence is very high, such as during an epidemic. If CSF samples can reach the laboratory quickly enough, presence of intracellular Gram-negative diplococci can only indicate epidemic-causing meningococcus. However, sensitivity is often compromised by the fragility of the bacteria and the ease of sample contamination.

We analysed laboratory data from the CSF of 412 patients obtained during a randomized non-inferiority trial conducted during an epidemic of NmA in Niger in 2003 [15]. The sensitivity and specificity of the Gram stain for indicating presence or absence of intracellular diplococci versus a gold standard of culture and/or PCR was 66.3 and 96.4%, respectively. As the Gram stain cannot indicate serogroup, any positive specimen should subsequently be tested by a LAT.

2.3. White blood cell count (WBC)

Numerous studies have reported the value of granulocyte cell counts using microscopy, to detect presumptive bacterial meningitis. We calculated the diagnostic value of the WBC (cut-off ≥ 50 cells/mm^3) using data from the same study described above, and repeated the analysis to determine the value of using WBC as an initial screening test, prior to the LAT.

Using culture and/or PCR as the gold standard for meningococcal detection, the sensitivity and specificity of the WBC ($n = 412$ CSF samples) were, respectively, 95.4 and 42.7%. Table 1 shows that, among the WBC positive CSF samples, the proportion of specimens positive by Gram stain was 59.6%, by LAT 79.1% and according to the gold standard 85.8%. Thus, in an epidemic context, a positive WBC was well correlated with the gold standard. Similar results have been reported previously [16]. Table 1 shows that the WBC threshold that we used will over-estimate the number of positive specimens by about 11% (36/323), while the LAT under-estimates the number of positive specimens by a similar proportion (34/323).

In order to find an affordable and feasible strategy to be used at peripheral level that may allow initial suspicion of meningococcal meningitis and possibly reduce the number of unnecessary LATs, we examined the performance of Gram

Table 1
Results of Gram stain, Pastorex agglutination test and the gold standard assays according to the results of white blood cell count in cerebrospinal fluids from clinical meningitis suspect patients

White blood cell count (threshold 50 cells/mm^3)	Number cerebrospinal fluid samples	Gram positive	Pastorex positive	Gold standard positive (culture and/or PCR)
Negative	53	4 (7.5%)	5 (9.4%)	15 (28.3%)
Positive	359	214 (59.6%)	284 (79.1%)	308 (85.8%)
Total	412	218 (52.9%)	289 (70.1%)	323 (78.4%)

Table 2
Current biological tests for the diagnosis and serogrouping of *N. meningitidis* in Niger: cost in year 2005[a]

WBCC[b]	Gram staining	Pastorex® agglutination	Culture, identification	Serogrouping[c]	Multiplex PCR
€0.10	€1.95	€11	€5.5 (negative) €15.5 (positive)	€19.7 (4 serogr.) €37.9 (7 serogr.)	€5 (negative) €10 (positive)

[a] Includes reagents, disposables and air freight, but excludes human resources and general wear and tear of equipment.
[b] White blood cell count.
[c] Co-agglutination of bacteria using polyclonal rabbit anti-sera from Biorad (Marne la Coquette, France) or Difco (Fisher Scientific, Paris, France).

stain or WBC alone versus Gram stain or WBC followed by LAT, versus LAT alone, in order to develop a logical and cost-effective sequence of tests for early outbreak confirmation. The cost in 2005 for each test in Niger was used in these calculations (Table 2). Prices include reagents and disposables but exclude the cost of human resources and general wear and tear of equipment.

2.4. Strategy 1: Gram stain versus Gram stain followed by LAT versus LAT alone

In terms of case management using Gram stain alone, as shown in Table 1, 32.5% (105/323) of actual patients will be missed, which is not acceptable.

Further calculations based on an algorithm of Gram stain followed by LAT on those samples that were Gram positive, and applying the sensitivity and specificity of the Pastorex® LAT determined in reference laboratories for NmA and NmW135 [10] resulted in a low sensitivity (57.7%) but a high specificity (99%). Results were very similar for NmW135 (sensitivity 56.4%; specificity 99.9%).

Regarding the WHO advocated strategy for serogroup identification [17], the low sensitivity of this algorithm means that a large number of CSF samples would need to be collected before the required number of positive samples (at least 10) could be reached. Applying this algorithm to serogroup A and assuming a prevalence of 73% (found during the above cited clinical trial), 40 CSF samples would be needed in order to obtain 15 g positive results from which 10 LAT positives would be obtained with a probability of 99% (total cost €243). If, instead, one used only the LAT, only 26 specimens would be necessary (for a total cost of €286). Although there is an economic advantage (of €43) to the strategy of Gram stain followed by confirmatory LAT, this must be weighed against the effort needed to take 14 additional samples, as well as the extra cost in terms of time and human resources for conducting 40 Gram stains and microscopic examinations.

In an emergency context, therefore, initially using the LAT to declare an outbreak does seem more feasible than having an initial screen with either WBC or Gram stain.

2.5. Strategy 2: WBC versus WBC followed by LAT versus LAT alone

In terms of appropriate management for individual cases, in those areas where laboratory technicians and basic laboratory supplies are available, one strategy could be to treat all those found positive on initial WBC screen and to use a LAT on those found to be negative by WBC. Our calculations, using the data in Table 1, showed that this would result in an additional five cases being identified for treatment and would be much cheaper than using LAT alone (€624 versus €4532). An alternative would be to test all suspect cases using only WBC (€41). Although the latter may be the cheapest option, it must be weighed against the fact that treating all those found positive by WBC alone would mean that 5% of true cases (15/323) would not be receiving the necessary treatment for meningococcal meningitis, while 57% of true negatives (51/89) would be receiving unnecessary meningococcal meningitis treatment. Taken together, 16% of suspect cases would not be treated appropriately.

For the aetiological diagnosis of meningococcal outbreaks, assuming an outbreak prevalence of 73%, and applying the sensitivity for WBC found from the data in Table 1 (95%), in order to find 11 LAT positive cases at a probability of 99%, it would be necessary to screen 28 suspect cases, which would yield 19 WBC positive patients. The financial cost of using WBC as a first-line assay followed by LAT only on the positive CSF will cost €212 compared with €286 for the strategy of using LAT alone as the initial screening test.

As there is neither significant money nor time saved by using WBC as an initial screen prior to LAT the WHO-recommended strategy of using LAT as a first-line assay [17] remains the most appropriate in the context of outbreak determination.

In the study cited above, many CSF samples were classified as "doubtful" after overall microscopic examination (WBC and Gram stain), even though the examination was performed by trained technicians. This was probably due to the delays (up to 16 h) in transporting the samples from remote healthcare centres to the district laboratory. Indeed, in conditions in the field, the diagnostic value of microscopy may be totally variable. Dramatic differences can be observed between laboratories, between technicians of the same laboratory and even over time for the same technician. The quality of microscopes, their servicing in dusty countries and the shelf-life of reagents at high ambient temperatures (up to 45 °C) may explain some of the differences. In addition, the Gram stain should be done on the pellet residue after centrifugation of 0.5 ml of CSF, and this is not always feasible. Reading a large series of slides is time-consuming and can be demotivating. Permanent quality control is necessary but requires additional resources which may not be available in

many countries. As a result, although microscopy is relatively cheap and apparently easy to perform, inconsistent results are observed. Despite years of efforts to improve overall standards, the current situation in countries in the meningitis belt remains far from satisfactory.

3. Gold standard methods: culture and polymerase chain reaction (PCR)

In most countries in the African meningitis belt, neither culture nor PCR is available, unless in regional or reference centres. Culture can be performed only on freshly collected CSF samples that have been correctly transported, as the meningococci are very fragile. Alternatively, they can be inoculated in Trans Isolate (TI) media (public price of €1.40 per bottle) and stored at adequate room temperature (around 37 °C) until their reception at the nearest laboratory. In this way they can survive several weeks, although contamination of the CSF during collection and TI inoculation dramatically reduces the possibility of meningococcal isolation.

Culture requires skilled technicians, a carbon dioxide atmosphere incubator, biosafety equipment, procurement of perishable reagents and fresh blood–agar media. In addition to cold chain for reagents, an air-conditioned laboratory is necessary to maintain the temperature stability of the incubators during the meningitis season, when the weather is extremely hot and dry. Results will be available 2–3 days after culture inoculation. Culture costs are €5.50 for a negative (sterile) CSF and €15.50 for a Nm-positive CSF. Further determination of serogroup is performed by co-agglutination using specific anti-sera, giving an additional cost per isolate of €20 for four serogroups and €38 for seven serogroups. Despite the high cost of the process, obtaining clinical isolates of Nm is invaluable for the monitoring of drug susceptibility and genetic studies to trace epidemic clone expansion.

With the advent of the PCR, which amplifies specific DNA, the need to have fresh CSF and viable meningococci has been circumvented. Multiplex PCR is now in use for the main virulent or epidemic-prone meningococcal serogroups A, B, C, W135, X and Y [18,7], as well as for *Streptococcus pneumoniae* [19] and *Haemophilus influenzae* b [20]. With the advent of this molecular technique in the region, epidemiological surveillance has been strengthened, particularly in Burkina Faso and Niger [21,22]. Reliable microbiological data are available for 39 out of 42 districts in Niger. Between 2003 and 2005, more than 5000 CSF samples were tested, of which 64.2% came from outside the capital. PCR performs as well on CSF inoculated in bi-phasic TI media, regardless of contamination [23]. In 2005, this technique was transferred successfully to Ivory Coast and Central Africa. Although PCR was proved to be a valuable alternative confirmation tool for the epidemiological surveillance of bacterial meningitis, its implementation poses significant technical limitations outside reference or central laboratories and thus the test is still inadequate as an urgent and early warning diagnostic tool. The financial cost of using PCR is reasonable (€5 for a negative CSF and €10 for a positive CSF sample) in view of its considerable benefits.

4. The new rapid diagnostic tests (RDTs)

In view of the current situation described above, new diagnostic tools tailored to the developing world are a high priority. Improving laboratory tests adapted to the realities of the meningitis belt region is vital for the support of epidemiological meningitis surveillance. Toward this end, Institut Pasteur (Paris, France) and CERMES (Niamey, Niger) have developed easy-to-perform, rapid and robust one-step assays called RDT1 and RDT2 to enable identification of the four serogroups of Nm [24]. These are based on the principle of vertical flow immunochromatography, in which gold particles and nitrocellulose membrane are coated with monoclonal antibodies to capture serogroup-specific Nm PS.

The two new RDTs have been evaluated according to standard rules for reporting diagnostic accuracy for new assays [25]. The sensitivity and specificity was 100% for clinical isolates of serogroups A, W135, C and Y. Those for CSF infected with NmA, NmW135 or NmY collected from Nigerien patients ranged from 93.8 to 100% (Table 3) [24]. The positive and negative predictive values (PPV, NPV) of the new RDTs for NmA and NmW135, compared with the Pastorex LAT [10] are higher for the RDTs (see Fig. 1).

A blind study was undertaken from January 2005 to September 2006 at CERMES (reference laboratory in Niger), comparing results from the new RDTs versus gold standard assay (PCR and/or culture). The results obtained from the study of 847 CSF samples from meningitis suspected patients (Table 4) showed that the two methods are highly concordant: Cohen's kappa coefficient was 0.79 [0.74–0.85]. The meningitis season in 2006 was marked by high prevalence of Nm X meningitis, neither detected by RDT1 nor RDT2.

Table 3
Diagnostic value of duplex rapid diagnostic tests (RDT1 and RDT2) for detection of *N. meningitidis* serogroups A, C, W135 and Y in documented cerebrospinal fluids[a] [24]

	Serogroup A	Serogroup W135	Serogroup C	Serogroup Y
Specificity % [IC]	97.1 [94.3–98.5]	99.4 [98.1–99.8]	99.6 [97.9–99.9]	100 [98.6–100]
Sensitivity % [IC]	93.8 [90–96]	100 [92.6–100]	No CSF tested	100 [51–100]

[a] The gold standard diagnostic test was culture and/or multiplex PCR test.

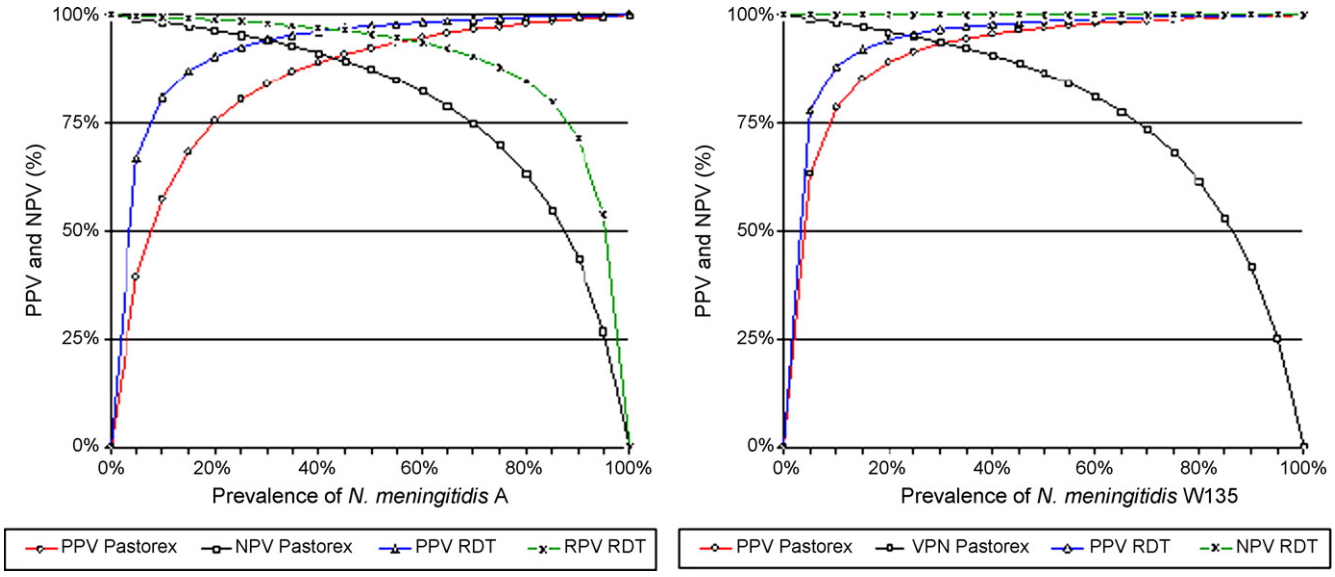

Fig. 1. Comparative positive and negative predictive values of Pastorex agglutination assays and RDTs for the diagnosis of *N. meningitidis* A and W135, according to their prevalence.

Table 4
Comparison between gold standard diagnostic test and RDTs for the diagnosis of *N. meningitidis* serogroups A and W135 in cerebrospinal fluids of meningitis suspected patients

PCR and/or culture	RDT positive serogroup A	RDT positive serogroup W135	RDT negative	RDT non-interpretable	Total
Serogroup A	155	0	19	0	174
Serogroup W135	0	12	1	1[a]	14
Negative[b]	39[c]	7[d]	613[e]	0	659
Non-interpretable[d]	0	0	0	0	1
Total	194	19	633	1	847

[a] RDT positive to A, W135 and C.
[b] Negative for the targeted NmA, W135, C and Y (so included other aetiological agents).
[c] Included 32 negative, 3 NmX, 2 *S. pneumoniae*, 2 *H. influenzae* b.
[d] Included four negative, two NmX, one *S. pneumoniae*.
[e] Included 425 negative, 145 NmX, 4 Nm indeterminate, 32 *S. pneumoniae*, 4 *H. influenzae* b.

For the new RDTs, CSF samples can be tested without prior heat or centrifugation, as opposed to the LAT. In addition, these tests are equally reliable at 25 and 45 °C ambient temperature, and the RDT performance is resistant to exposure for 3 weeks to 60 °C (simulating 2 years storage at 25 °C) [26,27]. These tests can therefore be used without the need for expensive or sophisticated machinery, they do not require cold chain or electricity, avoid the need for transportation of samples to reference centres and also obviate the need for highly skilled technical staff. In addition, the new RDTs can differentiate serogroup W135 from serogroup Y, unlike the LAT.

The RDTs are not without limitations, however, including time for training and supervision of staff. Results from ongoing field studies in Niger to evaluate the RDTs' performance on fresh CSF specimens in peripheral health structures during an outbreak are encouraging (data not shown). Further evaluation and minor adaptation of the tests to operational conditions may be needed before they can be made available on a large scale to the region.

5. Conclusion

Simplification and decentralization of the diagnosis of Nm cases, including serogroup identification, are essential initial components of a successful strategy for the rapid implementation of reactive mass vaccination to prevent further morbidity and mortality during an epidemic.

The cost of current confirmatory biological assays is prohibitive. In addition, these tests are not easy to perform and require sophisticated techniques and trained staff in well-equipped laboratories. The WHO current recommendation to test CSF samples using only LAT (e.g. the Pastorex kit) for outbreak identification, is at present both relevant and cost-effective, due to a lack of more affordable and/or simpler tools. This can however only be done in healthcare structures with access to cold chain and using trained laboratory staff.

In view of these limitations, the recent development of RDTs permitting rapid diagnosis of Nm serogroups at the most peripheral level is most encouraging. The RDTs could be useful both as a first-line diagnostic tool for epidemio-

logical surveillance of outbreaks and at the patient's bedside for improved case management. Current guidelines for epidemic meningococcal meningitis treatment for countries in the meningitis belt, is a single-dose i.m. oily chloramphenicol injection. This treatment is not appropriate for non-meningococcal meningitis, such as that caused by pneumococci or *Haemophilus*. Use of RDTs to indicate absence or presence of meningococci at this level could thus contribute to a lowering of mortality from meningitis caused by these other bacteria, although development of a specific RDT to identify these non-meningococcal organisms would be even more beneficial.

Analyzing CSF samples is the most adapted for meningitis diagnosis; however, lumbar puncture remains a highly invasive technique and can have serious consequences for the individual patient. Bearing in mind these risks it is also important, therefore, to evaluate the performance of the new RDTs on blood and/or urine samples. In addition, a rapid test including diagnosis of NmX is now a priority for countries within the meningitis belt, as the threat of a recurrence of an outbreak due to this serogroup is currently possible [5,7].

Much progress is being made to further develop and validate the new RDTs. In-house production on a small scale will enable further field evaluation in countries of the meningitis belt other than Niger. Initial results indicate that they could be a promising first-line diagnostic tool for timely identification of epidemic meningococcal serogroup(s). The challenges ahead are to facilitate access to affordable RDTs (at a price of less than €2), to reach sustainable production for the most widespread access and to secure lower-priced RDTs for countries in the developing world.

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