

Evaluation of Pastorex meningitis kit performance for the rapid identification of *Neisseria meningitidis* serogroup C in Nigeria

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Background: *Neisseria meningitidis* serogroup C (NmC) has caused outbreaks in Nigeria of increasing size in three consecutive years since 2013. Rapid diagnostic tests (RDTs) for meningitis can facilitate quick identification of the causative pathogen; Pastorex can detect *N. meningitidis* serogroups A, C (NmC), Y/W135, *N. meningitidis* serogroup B/*Escherichia coli* K1, *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae*, and group B *Streptococcus*. There is no published field evaluation of Pastorex in the identification of NmC. We report our experience with Pastorex in detecting NmC in field conditions.

Methods: During sequential outbreaks of NmC in Nigeria in 2013, 2014 and 2015, cerebrospinal fluid (CSF) was collected from suspected cases of meningitis that met the case definition. Pastorex latex agglutination rapid test was done in the field and trans-isolate media were inoculated with CSF for culture and/or PCR, which was used as the reference standard for 63 paired samples.

Results: The sensitivity of Pastorex for NmC was 80.0% (95% CI 65.4–90.4%) and the specificity was 94.4% (95% CI 72.7–99.9%). The positive likelihood ratio (LR) was 14.4 (95% CI 2.1–97.3) and negative LR was 0.2 (95% CI 0.1–0.4). The positive and negative predictive values (PPV and NPV) were 97.3% (95% CI 85.8–99.9) and 65.4% (95% CI 44.3–82.8), respectively, with a prevalence estimate of 71.4% (95% CI 58.6–82.1).

Conclusion: Pastorex showed good performance in detecting NmC under field conditions. Prepositioning Pastorex at peripheral health facilities during non-epidemic periods is constrained by a short shelf-life of 1 month after the kit is opened. There is need for development of RDTs that are cheaper and with less challenging requirements for storage and usage.

Keywords: Cerebrospinal fluid, *Neisseria meningitidis*, Nigeria, Rapid diagnostic test, Sensitivity, Specificity

Background

Meningococcal meningitis is a major burden on public health systems across the meningitis belt in sub-Saharan Africa, which stretches from Senegal in the west to Ethiopia in the east.^{1–4} In the past three decades, most meningitis epidemics in sub-Saharan Africa have been caused by *Neisseria meningitidis* serogroup A (NmA), with a small number caused by serogroups W (NmW) and X (NmX).^{2,4} There has been an epidemiological shift following the roll-out of MenAfriVac conjugate A vaccine in 2010 leading to elimination of NmA epidemics. An outbreak in northern Nigeria in 2013 was due to serogroup C (NmC), a serogroup rarely identified in the African meningitis belt in the past

three decades, with subsequent NmC outbreaks in 2014 and of markedly greater scale in 2015.^{2,3,5–7} This epidemiological shift has stimulated a need to rapidly confirm the causative serogroup and determine the type of vaccine required for effective reactive mass vaccination campaigns.^{3,4}

WHO recommends that rapid diagnostic test (RDT) kits for meningitis should be made available at peripheral health facilities to facilitate quick identification of the causative pathogen and inform the response to the epidemic.^{3,4} An RDT has been defined as any test that yields a result in the same clinic visit as diagnosis⁸ or which can be used in health-care settings with little infrastructure or trained personnel, preferably without electricity.⁹ *N. meningitidis* serogroups can be identified using antisera on culture, or

directly on cerebrospinal fluid (CSF) by PCR and by latex agglutination test, such as Pastorex (Bio-Rad Laboratories Inc., Marne-La-Coquette, France).^{10–12} Pastorex can detect *N. meningitidis* serogroups, A, C, Y/W135 and *N. meningitidis* serogroup B/*Escherichia coli* K1. It can also detect *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae* and group B *Streptococcus*. Culture and PCR are generally considered as the reference standards for identification of *N. meningitidis* serogroups, since they are highly specific.^{4,10–14} Pastorex has been evaluated in two studies for NmC, yielding a sensitivity of >80% and specificity of >90%.^{15,16}

In a recent WHO systematic literature review of RDTs for the diagnosis of meningitis in field conditions in sub-Saharan Africa, no study was found evaluating Pastorex in the identification of NmC.⁵ We report the performance of the Pastorex test kit in field conditions during three sequential NmC outbreaks in northern Nigeria in 2013–2015, comparing Pastorex results with those from culture and PCR conducted by the WHO Collaborating Centre for Reference and Research on Meningococci, Norwegian Institute of Public Health (NIPH), Oslo, Norway.

Materials and methods

This study analyses data collected during three sequential outbreaks of NmC in northern Nigeria in 2013 (9 February to 23 June; 856 clinically suspected patients treated), 2014 (14 March to 3 May; 333 cases),⁵ and 2015 (10 February to 8 June; 6394 suspected cases¹⁷). During these NmC outbreaks, Médecins Sans Frontières (MSF) managed treatment centres within existing Ministry of Health facilities in areas affected by the outbreaks: three sites in Sokoto state in 2013; one in Kebbi state in 2014; and two in Sokoto and five in Kebbi in 2015 (Supplementary Table 1). The suspected case definition used was a modification of the WHO case definition with the addition of petechial rash. Thus, the case definition used for

suspected meningitis for patients over 1 year of age was sudden onset fever and either neck stiffness or petechial rash. For infants, the case definition was sudden onset fever and either bulging fontanelle or petechial rash. CSF samples were obtained from suspected cases of meningitis at the beginning of the outbreak to confirm the causative pathogen, as well as suspected patients presenting from new geographical areas in the course of the outbreak. CSF samples were not taken if a patient had antibiotic treatment at home or another health facility prior to presentation at the MSF treatment centre. Most patients were treated based on clinical presentation within the context of an identified outbreak. Thus, after initial confirmation of the causative organism using Pastorex, CSF was not routinely obtained for testing.

A combination of culture and/or PCR was used as the gold standard to identify NmC. Gold standard positives were all samples with positive result for NmC from either culture or PCR or both. Gold standard negatives were samples with negative results for both culture and PCR. Samples positive for meningococci but with serogroup inconclusive by culture/PCR, as well as uninterpretable Pastorex results, were excluded from this analysis (Figure 1).

Using samples that had both a Pastorex test and a gold standard test, the positive and negative results were compared to determine the performance of Pastorex for NmC in terms of sensitivity, specificity, likelihood ratios (LRs), positive predictive value (PPV) and negative predictive value (NPV).

Field laboratory procedure

Approximately 4 mL of CSF was collected by a doctor from each patient who met the case definition. A 0.5 mL fraction of each CSF sample was inoculated into trans-isolate media (TI) within 1 hour of collection¹⁸ and sent for confirmatory testing to the

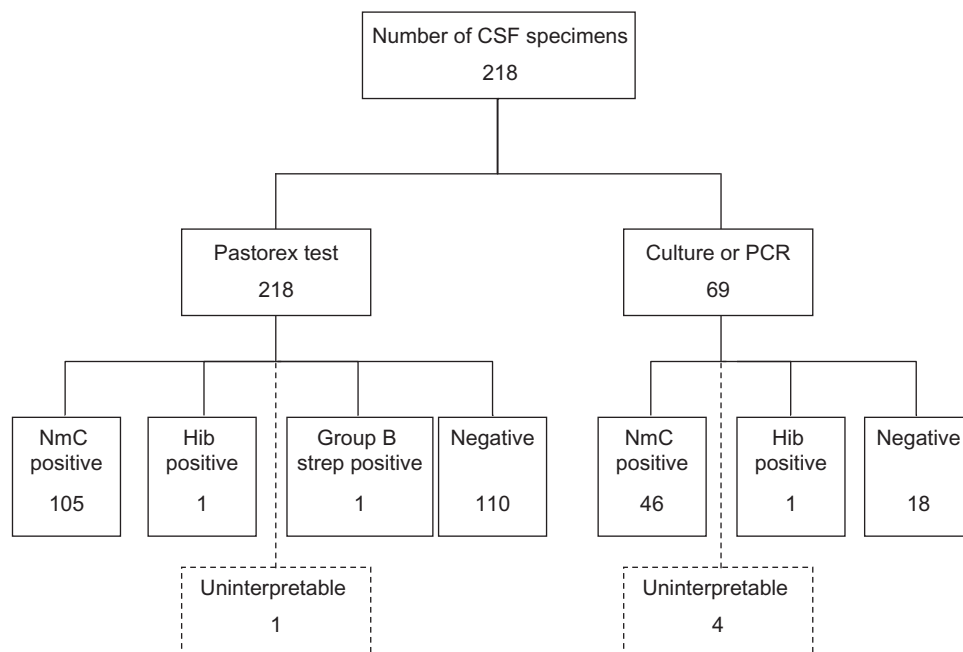


Figure 1. Schematic of all Pastorex latex agglutination and confirmatory diagnostic tests for *Neisseria meningitidis* conducted on cerebrospinal fluid (CSF) samples, showing results obtained for serogroup C. Uninterpretable Pastorex due to bloody CSF and uninterpretable PCR are samples positive for *Neisseria meningitidis* but with no serogroup identified. Hib: *Haemophilus influenzae* type B; NmC: *Neisseria meningitidis* serogroup C; Strep: *Streptococcus*.

WHO Collaborating Centre for Reference and Research on Meningococci, NIPH.

The remainder of the CSF sample was tested in the field by a trained doctor or nurse using Pastorex latex agglutination test kit to determine the causative organism.

Pastorex kits were kept in controlled, refrigerated storage. Temperature monitoring tools such as 3M card and freeze tags were used to ensure that the temperature was maintained at 2–8°C. Cold chain was maintained while transporting test kits daily to the field in Gio'Style boxes with ice packs. The field teams conducted quality control tests on newly opened kits using positive and negative controls provided as part of the test kit. The controls were repeated when in doubt of the test result. Test kits were valid for use only within 1 month of opening the kits and were used within this timeframe.

The field mobile laboratories were equipped with lumbar puncture kits, safety boxes for needle disposal, trays, test tube holders, a kerosene powered burner, a hand operated centrifuge, 5 mL normal saline ampoules, 2 mL syringes, sterile pipettes, gloves, an aluminium kettle for water, TI bottles and Pastorex Kits in cool boxes containing icepacks. These items were taken to the field every day, and Pastorex tests were done in a side room in the treatment centres. Pastorex kits were used according to the manufacturer's instructions. Briefly, CSF samples were heated for 3 min at 100°C in a water bath then centrifuged for 5 min. A drop of the supernatant was placed in each circle on the disposable card from the kit using a pipette and the corresponding kit reagents were added. The card was rotated by hand and observed for agglutination with the naked eye within 10 min. This was the only diagnostic test done in the field.

TI media inoculated with CSF were sent to the MSF base where they were kept ventilated at room temperature by inserting a 19 G needle into the TI bottle without touching the CSF and placing a piece of cotton wool at the plastic base of the needle to prevent contamination. The ventilated TI bottles were sent in batches approximately fortnightly by airfreight in protective quadruple-packaging to NIPH along with CSF sample information forms on which Pastorex test results were usually indicated.

Reference laboratory procedure

Details for specimens received by NIPH were compiled into a single list, excluding the Pastorex field result. The list was forwarded with the samples to the team carrying out culture and PCR. Bacterial identification was performed using standard biochemical tests. *N. meningitidis* strains were serogrouped by slide agglutination with commercial antisera (Remel, Lenexa, KS, USA). PCR analysis of the genes coding for the polysaccharide capsule was performed for genogroup determination of non-serogroupable isolates as described.¹⁴ From culture-negative TI, DNA was purified from the supernatant using QiAmp DNA mini kit (Qiagen, Valencia, CA, USA) and analysed by real-time PCR for species identification. Identification of *N. meningitidis* was followed by genogrouping¹⁸ and determination of the PorA variant by sequencing of the *porA* gene using a nested *porA*-PCR.¹³

Data analysis

Data were entered into Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). The evaluation was reported according

to STARD (Standard for Reporting Diagnostic Accuracy) guidelines.^{19,20}

The sensitivity (Sn), specificity (Sp), PPV and NPV of Pastorex^{21–23} and LRs²¹ were calculated for NmC, with 95% CIs.¹⁸ The positive LR (LR+ = Sn/[1–Sp]) indicates how many times a positive result is likely to be observed in samples with NmC compared to those without NmC. The negative LR (LR– = [1–Sn]/Sp) indicates how many times a negative result is likely to be observed in samples with NmC compared to those without NmC. LR+ above 10 and LR– below 0.2 are considered convincing diagnostic evidence.²⁰ PPV and NPV were calculated as follows:

$$PPV = \frac{(\text{Prev} \times \text{Sn})}{(\text{Prev} \times \text{Sn}) + [(1 - \text{Prev}) \times (1 - \text{Sp})]}$$

and

$$NPV = \frac{(1 - \text{Prev}) \times \text{Sp}}{[(1 - \text{Prev}) \times \text{Sp}] + (\text{Prev} \times (1 - \text{Sn}))}$$

Where Prev is the prevalence of the disease in the population tested. PPV and NPV were calculated for the prevalence of the included samples, and also for prevalence in 5% increments from 0–100% to support interpretation in a range of epidemiological contexts.

Results

Overall, 218 CSF specimens were tested using Pastorex, of which 69 specimens were sent for culture/PCR (Figure 1). Of the 218 Pastorex results: 48.2% (105/218) were positive for NmC; 0.5% (1/218) for *H. influenzae* type b; 0.5% (1/218) for group B *Streptococcus*; and 0.5% (1/218) were uninterpretable as the specimen was bloody, but were positive for NmC by PCR. Of the 69 culture specimens, 38% (26/69) were NmC positive and 62% (43/69) negative. Of the 43 negative culture specimens for which PCR was done, 47% (20/43) were positive for NmC, giving a total of 46 specimens positive for NmC on either culture or PCR. PCR identified an additional 2% (1/43) positive for *H. influenzae* type b and 9% (4/43) PorA positive for *N. meningitidis* with no serogroup identified, although the sequence of the *porA* gene suggested they were NmC (these four are categorised in Figure 1 as 'uninterpretable'). The remaining 42% (18/43) were negative. One Pastorex positive specimen was negative by culture and PCR. All culture-positive NmC were PorA 21-15,16, and all but one PCR-positive sample harboured *porA* DNA that was sequenced as 21-15,16. The exception was an NmC sample harbouring a minor variant of the same *porA* gene, 21-15,16-50.

The uninterpretable test results by Pastorex and PCR and positive results for other pathogens were excluded from the sensitivity and specificity calculation for Pastorex for NmC (n = 6 excluded). The sensitivity of Pastorex was 80.0% (95% CI 65.4–90.4%) and the specificity 94.4% (95% CI 72.7–99.9%). The LR+ for NmC was 14.4 (95% CI 2.1–97.3) and LR– 0.2 (95% CI 0.1–0.4) (Table 1). The PPV and NPV for NmC were 97.3% (95% CI 85.8–99.9) and 65.4% (44.3–82.8), respectively, with a prevalence estimate of 71.4% (95% CI 58.6–82.1). PPV and NPV for NmC for prevalence 0–100% are shown in Figure 2.

Table 1. Comparative results of Pastorex latex agglutination test and culture or PCR for *Neisseria meningitidis* serogroup C detection in suspected meningitis patients

	Culture or PCR positive, n (%)	Culture or PCR negative, n (%)	Total
Pastorex positive	36 (80)	1 (6)	37
Pastorex negative	9 (20)	17 (94)	26
Total	45	18	63

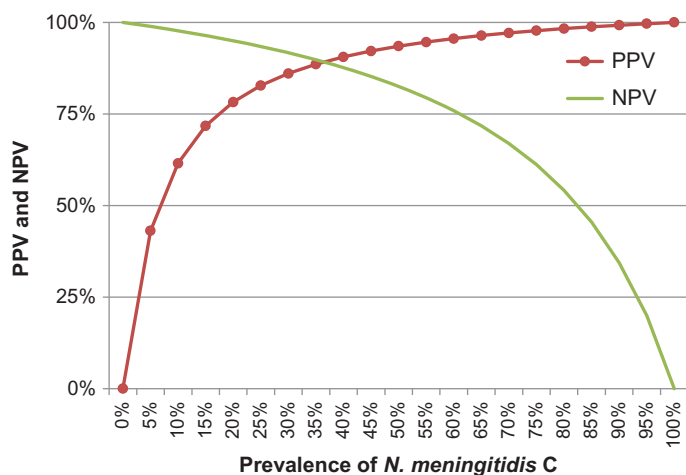


Figure 2. The positive and negative predictive values (PPV and NPV) of Pastorex test for *Neisseria meningitidis* serogroup C (NmC) according to prevalence of NmC among tested cerebrospinal fluid samples.

Discussion

Pastorex showed a moderate sensitivity of 80.0% (95% CI 65.4–90.4%) and high specificity of 94.4% (95% CI 72.7–99.9%) for diagnosing NmC under field conditions. The likelihood ratios for Pastorex demonstrated good diagnostic evidence, with LR+ above 10 and LR- equal to 0.2. The prevalence of NmC among our samples was similar to a hypothetical 70% estimate in an evaluation of Pastorex for identifying NmA and NmW135 under ideal laboratory conditions among clinically suspected cases diagnosed by skilled health staff complying with cases definitions in an epidemic.¹⁵ The high PPV of Pastorex for NmC suggests it will be a useful guide in the choice of vaccines during epidemics. In contrast to the high PPV, the low NPV indicates that a negative Pastorex result should not prevent treatment based on clinical diagnosis.

Although Pastorex is the most common RDT used in the African meningitis belt, some constraints limit its use in peripheral health facilities in regions with shortages of staff and power supply. In this study, the need to keep the kit in cold chain (2–8°C) required overnight storage in a monitored refrigerator and daily transport in cool boxes to the treatment sites. Once a kit of 25 tests has been opened, all the tests must be used within 1 month. This means that prepositioning kits in peripheral health facilities during non-epidemic periods where there

are only few cases each month is not economically viable. This study reports on an epidemic situation when kits were fully used in less than a month. The need to heat and centrifuge CSF samples prior to testing was a challenge in areas lacking power supply, but was addressed through the use of kerosene powered burners and hand-operated centrifuges. Training staff in the proper use of the Pastorex kit was essential for ensuring that testing was done correctly. While Pastorex identifies a number of causes of bacterial meningitis, it does not differentiate meningococcal serogroups W135 and Y, although this may be of limited importance since vaccines often include both serogroups. Pastorex also does not identify serogroup X, and thus would not be useful in a serogroup X outbreak. Despite these constraints, Pastorex can provide a result within 20 min and was useful in confirming a clinical diagnosis and informing outbreak response by determining the appropriate vaccines to use.

Limitations

Our sample size is small, and hence the point estimates of sensitivity and specificity of Pastorex for NmC have relatively wide confidence intervals. Samples were obtained to inform clinical care and outbreak response rather than systematically collected for research purposes. Thus they may not be representative of all suspected cases in the three outbreaks, limiting the representativeness of the prevalence. We therefore provided PPV and NPV estimates for the full spectrum of possible prevalence. Due to the lack of cold chain facilities in the hospitals where MSF established treatment centres it was not possible to keep TI bottles overnight. Therefore, CSF samples taken at night had Pastorex test done only in the morning when the teams from the MSF base arrived.

Conclusions

Pastorex performed well in the identification of NmC under field conditions and can be used during epidemics for establishing diagnosis and deciding appropriate vaccine response. Its prepositioning in peripheral health facilities remains a challenge in low income countries, primarily due to temperature storage requirements and short expiry after opening. Prepositioning Pastorex test kits in state or central public health laboratories may be more feasible, as this would reduce the cost of storage and wastage from short expiry times. There is a need for RDTs that are stable under the high ambient temperatures (40–44°C) common in the African meningitis belt and viable for use in health facilities during epidemic and non-epidemic periods.

Supplementary data

Supplementary data are available at Transactions online (<http://trstmh.oxfordjournals.org/>).

Authors' contributions: KU and JG contributed to study design, and KU and AB implemented the study. KU, DAC, JG analysed and interpreted the data. KU, AB, CK, DAC, JG contributed to writing the manuscript and approved the final version. KU had access to the complete data and is guarantor of the paper.

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Competing interests: None declared.

Ethical approval: All specimens were collected as part of clinical management of patients. Lumbar puncture procedure was explained to every patient and/or care giver after which it was done if verbal consent was given. The data analysed were collected as part of routine activities which MSF has approval to conduct from the Ministries of Health. This retrospective analysis of routinely collected programme data met the criteria of the MSF Ethics Review Board for exemption from ethics review.

References

- Brown V, Guerin PJ, Legros D et al. Research in complex humanitarian emergencies: The Médecins sans Frontières/Epicentre experience. *PLoS Med* 2008;5:e89. doi:10.1371/journal.pmed.0050089.
- Lingani C, Bergeron-Caron C, Stuart JM et al. Meningococcal meningitis surveillance in the Africa meningitis belt, 2004-2013. *CID* 2015; 61:410-15.
- World Health Organization. Revised guidance on meningitis outbreak response in sub-Saharan Africa. *Wkly Epidemiol Rec* 2014;89: 577-88.
- WHO. Meningitis outbreak response in sub-Saharan Africa. WHO guideline. Geneva: World Health Organization; 2014. <http://www.who.int/csr/resources/publications/meningitis/guidelines2014/en/> [accessed 19 June 2016].
- Waite T. Development proposal for WHO guideline: Meningitis response in sub-Saharan Africa. PICO 2. A systematic review of rapid diagnostic test for the diagnosis of meningitis in field conditions in sub-Saharan Africa. London: Public Health England; 2014.
- Funk A, Uadiale K, Kamau C et al. Sequential outbreaks due to a new strain of *Neisseria Meningitidis Serogroup C* in Northern Nigeria, 2013-14. *PLOS Curr* 2014;6. doi:10.1371/currents.outbreaks.b50c2aaf1032b3ccade0fca0b63ee518.
- WHO. Serogroup C in the Meningitis Belt: Facing the Challenge. Report of meeting held in Geneva, October 2015. Geneva: World Health Organization; [n.d.]. www.who.int/csr/disease/meningococcal/Facing_NmC_Epidemics_Meningitis_Belt.pdf [accessed 3 February 2016].
- Pai NP, Vadnais C, Denkinger C et al. Point-of-care testing for infectious diseases: diversity, complexity and barriers in low and middle income countries. *PLoS Med* 2012;9:e1001306.
- Yansouni CP, Bottieau E, Lutumba P et al. Rapid diagnostic test for neurological infections in central Africa. *Lancet Infect Dis* 2013;13: 546-58.
- Rose AMC, Mueller JE, Gerstl S et al. Meningitis dipstick rapid test: evaluating diagnostic performance during an urban *Neisseria meningitidis* serogroup A outbreak, Burkina Faso, 2007. *PLoS One* 2010;5: e11086.
- Rose AMC, Gerstl S, Mahamane AEH et al. Field evaluation of two rapid diagnostic tests for *Neisseria meningitidis* serogroup A during the 2006 outbreak in Niger. *PLoS One* 2009;4:e7326.
- Nathan N, Rose AMC, Legros D et al. Meningitis serogroup W135 outbreak, Burkina Faso, 2002. *Emerg Inf Dis* 2007;13:920-3.
- Caugant DA, Høiby EA, Frøholm LO, Brandtzaeg P. Polymerase chain reaction for ascertainment of *Meningococcal Meningitis*: application to the cerebrospinal fluids collected in the course of the Norwegian meningococcal serogroup B protection trial. *Scand J Infect Dis* 1996; 28:149-53.
- Taha MK. Simultaneous approach for noncultured PCR-based identification and serogroup prediction of *Neisseria meningitidis*. *J Clin Microbiol* 2000;38:855-7.
- Djibo S, Lafourcade B-MN, Boisier P et al. Evaluation of the Pastorex meningitis kit for the rapid identification of *Neisseria meningitidis* serogroups A and W135. *Trans R Soc Trop Med Hyg* 2006;100: 573-8.
- Borel T, Rose AMC, Guillerm M et al. High sensitivity and specificity of the Pastorex latex agglutination test for *Neisseria meningitidis* serogroup A during a clinical trial in Niger. *Trans R Soc Trop Med Hyg* 2006;100:964-9.
- Chow J, Uadiale K, Bestman A et al. Invasive meningococcal meningitis serogroup C outbreak in northwest Nigeria, 2015 - third consecutive outbreak of a new strain. *PLOS Curr* 2016. doi: 10.1371/currents.outbreaks.06d10b6b4e690917d8b0a04268906143.
- CDC. Laboratory Methods for the Diagnosis of Meningitis Caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. 2nd ed. Atlanta: Centers for Disease Control and Prevention; 2011. [WHO Manual WHO/iVB.11.09] www.cdc.gov/meningitis/lab-manual [accessed 22 December 2015].
- Bossuyt PM, Reitsma JB, Bruns DE et al. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD initiative. *Clin Chem* 2003;49:1-6.
- The TDR Diagnostics Evaluation Expert Panel. Evaluation of diagnostic tests for infectious diseases general principles. *Nature Rev Microbiol* 2010 8(12 Suppl):S17-29. doi:10.1038/nrmicro1570.
- Parikh R, Mathai A, Parikh S et al. Understanding and using sensitivity, specificity and predictive values. *Indian J Ophthalmol* 2008;56: 45-50.
- Jaeschke R, Guyatt GH, Sackett DL. Users' guides to the medical literature. III. How to use an article about a diagnostic test. B. what are the results and will they help me in the caring for my patients? The Evidence-Based Medicine Working Group. *JAMA* 1994; 271:703-7.
- MedCalc easy-to-use statistical software. Version 15.11.14. https://www.medcalc.org/calc/diagnostic_test.php [accessed 27 December 2015].