




Validation of a New Rapid Detection Test for Detection of *Neisseria meningitidis* A/C/W/X/Y Antigens in Cerebrospinal Fluid

Cyrille H. Haddar,^{a,b} Aude Terrade,^c Paul Verhoeven,^{a,d} Berthe-Marie Njanpop-Lafourcade,^e Mireille Dosso,^f Fati Sidikou,^g Ali Elhaj Mahamane,^g Jean-Pierre Lombart,^h Aziza Razki,ⁱ Eva Hong,^c Alain Agnmemel,^c Evelyne Begaud,^b Yves Germani,^b Bruno Pozzetto,^{a,d}  Muhamed-Kheir Taha^c

^aGroupe Immunité des Muqueuses et Agents Pathogènes (GIMAP EA 3064), University of Lyon, Saint-Etienne, France

^bBioSpeedia, Institut Pasteur, Paris, France

^cInstitut Pasteur, Invasive Bacterial Infections Unit and National Reference Centre for Meningococci and *Haemophilus influenzae*, Paris, France

^dLaboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, Saint-Etienne, France

^eAgence de Médecine Préventive, Paris, France

^fBacteriology and Virology Department, Institut Pasteur, Abidjan, Côte d'Ivoire

^gCentre de Recherche Médicale et Sanitaire (CERMES), Niamey, Niger

^hInstitut Pasteur de Bangui, Bangui, Central African Republic

ⁱInstitut Pasteur du Maroc, Casablanca, Morocco

ABSTRACT Meningococcal meningitis remains a life-threatening disease worldwide, with high prevalence in the sub-Saharan meningitis belt. A rapid diagnosis is crucial for implementing adapted antimicrobial treatment. We describe the performances of a new immunochromatographic test (MeningoSpeed, BioSpeedia, France) for detecting and grouping *Neisseria meningitidis*. Cerebrospinal fluids (CSFs) were collected from 5 African countries and France. For the rapid diagnostic test (RDT), the CSF sample was deposited on each of the 3 cassettes for a total volume of 90 μ l. The results of the RDT were compared to those of a reference multiplex PCR assay detecting the major serogroups of *N. meningitidis* on 560 CSF specimens. Five specimens were found uninterpretable by RDT (0.9%). The results of interpretable specimens were as follows: 305 positive and 212 negative samples by both techniques, 14 positive by PCR only, and 24 positive by RDT only (sensitivity, specificity, and positive and negative predictive values of 92.7%, 93.8%, 95.6%, and 89.8%, respectively, with an accuracy of 93.2% and a kappa test of 0.89; $P < 0.05$). From 319 samples positive by PCR for serogroups A, C, W, X, or Y, the grouping results were concordant for 299 specimens (sensitivity of 93.0%, 74.4%, 98.1%, 100%, and 83.3% for serogroups A, C, W, X, and Y, respectively). The MeningoSpeed RDT exhibited excellent performances for the rapid detection of *N. meningitidis* antigens. It can be stored at room temperature, requires a minimal amount of CSF, is performed in 15 minutes or less, and is easy to use at bedside.

KEYWORDS cerebrospinal fluid, *Neisseria meningitidis*, rapid diagnostic test, validation

Neisseria meningitidis is a Gram-negative bacterium that infects only humans; it is one of the major agents of septic meningitis, with the potential to cause either outbreaks or sporadic cases worldwide. The “meningitis belt” of sub-Saharan Africa, involving 26 countries from Senegal in the west to Ethiopia in the east, exhibits the highest rates of the disease (1). However, sporadic and epidemic cases also occur everywhere in the world (2). Meningitis due to *N. meningitidis* is known to exhibit a severe prognosis, including frequent sequelae and life-threatening issues (3). There are

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Address correspondence to Bruno Pozzetto, bruno.pozzetto@univ-st-etienne.fr.

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12 known serogroups of *N. meningitidis*, differentiated by the composition of the structure of their polysaccharide capsule, but only six of them (A, B, C, W, X, and Y) are commonly involved in human neurological infections. Five of these serogroups (A, B, C, W, and Y) are targets in currently licensed meningococcal vaccines (4).

Despite vaccination programs in some African countries, outbreaks due to *N. meningitidis* are still observed in Africa. Since the introduction of the MenAfriVac immunization program using a vaccine directed against *N. meningitidis* serogroup A, major epidemics involving this serogroup have nearly disappeared in Africa (5). However, other serogroups are emerging in the meningitis belt, and outbreaks involving C, W, or X serogroups (depending on the country) are now reported (2, 6). Ongoing surveillance for early detection of *N. meningitidis* epidemics is important, as illustrated by a recent study from the World Health Organization (WHO) Collaborating Centers for Reference Meningitis that showed that new strains of *N. meningitidis* can cause an epidemic at any time and everywhere (7).

WHO guidelines recommend the use of point-of-care tests, also named rapid diagnostic tests (RDTs), for the early etiological diagnosis of meningitis, notably in resource-limited countries (8). RDTs have been defined as tests able to give a result in the same visit as diagnosis (9) or that can be used in health care settings with limited infrastructure and nontrained personnel (10). Current RDTs aimed to detect *N. meningitidis* in resource-limited countries are mainly Gram staining, latex agglutination kits (11–13), immunochromatographic dipstick diagnostic test (14–18), and nucleic acid amplification tests (NAATs), notably those based on loop-mediated isothermal amplification methods due to high sensitivity, a rapid availability of results, and minimal specialized equipment and laboratory expertise (19–21).

Dipsticks using immunochromatography have been recently developed at the Institut Pasteur de Paris (IPP) in France to detect meningococci of serogroups A, C, W/Y, and X (14, 16, 18) that were subsequently transferred to BioSpeedia, which developed an improved prototype for industrial use. This report describes an innovative, rapid, and easy-to-use immunochromatographic RDT called MeningoSpeed (BioSpeedia, Paris, France). This test uses monoclonal antibodies directed against capsular polysaccharides belonging to A, C, W, X, and Y antigens of *N. meningitidis*. This study was aimed at evaluating this new test on many cerebrospinal fluid (CSF) specimens originating, in large part, from countries of the meningitis belt.

(These results were presented, in part, as a poster at the 28th European Congress of Clinical Microbiology and Infectious Diseases, Madrid, Spain, 21 to 24 April 2018 [22].)

MATERIALS AND METHODS

Population. The studied population included patients consulting for clinical symptoms suspect of meningitis. The study was performed on 560 CSF specimens collected between 2006 and 2017 from 7 different centers located in 5 African countries and France, including 70 samples from the Centre de Recherche Médicale et Sanitaire, Niamey, Niger; 77 from the Pasteur Institute of Abidjan, Côte d'Ivoire; 29 from the Pasteur Institute of Bangui, République Centre-Africaine; 6 from the Pasteur Institute of Casablanca, Morocco; 90 from the Agence de Médecine Préventive-Togo of Dapaong, Togo; 72 from the University-Hospital of Saint-Etienne, France; and 216 from the IPP, France.

Study design. The diagnosis strategy to perform the initial etiological diagnosis of meningitis in each country was left to the initiative of each center and is not described herein. The CSF samples that were evaluated in this study were remaining clinical specimens that were kept frozen at -80°C . The MeningoSpeed cassettes were distributed by the IPP to the other centers, in line with the mission of these centers for the surveillance of meningococcal diseases in the corresponding countries under approvals from the internal board of the IPP. The samples were anonymized before testing. In this study, the performances of the MeningoSpeed RDT were evaluated retrospectively by comparison to the results of an in-house multiplex PCR assay described below, also performed in each center, used as a reference standard.

Microbiological cultures. Bacterial cultures were performed for some specimens (at least 266) by plating a $10\text{-}\mu\text{l}$ volume of CSF on appropriate media; the plates were incubated at 37°C under 5% CO_2 .

MeningoSpeed rapid diagnostic test. The MeningoSpeed RDT (BioSpeedia, Paris, France) is a lateral flow immunochromatographic test composed of 3 cassettes. The first one detects A and W serogroups, the second one detects Y and C serogroups, and the third one detects the X serogroup. Each cassette comprises a control line for verifying that the sample was deposited correctly. One drop (approximately $30\ \mu\text{l}$) of CSF specimen was deposited in the sample well of each cassette with the help of a disposable

TABLE 1 Comparison of the results obtained by the rapid diagnostic test (RDT) and the IPP RT-PCR assay taken as reference standard on cerebrospinal fluids for the diagnosis of *Neisseria meningitidis* infection

RDT result	No. of samples with PCR result:		Total no. of samples
	+	–	
+	305	14	319
–	24	212	236
Total	329	226	555

pipette present in the kit. The result was read as soon as the bands appeared, for a maximum time of 15 min. In the absence of positivity of the control line, the test was declared uninterpretable.

PCR assays. An in-house real-time PCR (RT-PCR) multiplex assay developed by the IPP (referenced as IPP RT-PCR assay further in the text) was used in each center for the detection of *N. meningitidis* DNA targeting the meningococcal-specific gene *crgA* coding for a LysR-like transcriptional regulator and targeting six serogroup-specific (A, B, C, W, X, and Y) *N. meningitidis* capsular genes. A Qiagen column-based method was used for the optimal extraction of DNA from CSF. Internal quality controls were included to monitor extraction of DNA and PCR inhibition, as previously described (23).

Additional PCR assays were performed for the detection of *Haemophilus influenzae* and *Streptococcus pneumoniae* DNA targeting *hpd* and *lytA* genes, respectively, according to formerly described techniques (24).

Statistical analysis. Descriptive variables and sensitivity, specificity, and predictive values were reported with their 95% confidence interval (CI). These data, together with the Cohen's kappa concordance coefficient, were calculated by using GraphPad Prism 5 software (CA, USA). *P* values under 5% were considered to be statistically significant.

RESULTS

Results combining culture and PCR techniques. Five hundred and sixty CSF samples were included in the study. By combining conventional culture (performed on at least 288 samples) and the IPP RT-PCR assay (performed on all the samples), the following results were observed: 334 samples were positive for *N. meningitidis* (including 44 serogroup A, 10 serogroup B, 47 serogroup C, 161 serogroup W, 60 serogroup X, and 12 serogroup Y), 83 samples were positive for *Streptococcus pneumoniae*, 16 samples were positive for *Haemophilus influenzae*, 5 samples were positive for *Streptococcus suis*, and 122 samples were negative.

Performances of the MeningoSpeed RDT by comparison to the IPP RT-PCR assay. Five of the 560 specimens were found to be uninterpretable by RDT (0.9%). Table 1 depicts the comparative results between the MeningoSpeed RDT and the IPP RT-PCR assay taken as reference standard (23) on the 555 interpretable CSF specimens. The accuracy between the two tests was 93.2% (Cohen's kappa test of 0.89, $P < 0.05$). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of RDT were 0.927 (95% confidence interval [CI], 0.892 to 0.952), 0.938 (95% CI, 0.896 to 0.964), 0.956 (95% CI, 0.926 to 0.974), and 0.898 (95% CI, 0.851 to 0.932), respectively, by comparison to PCR. The variations of NPV and PPV for the diagnosis of *N. meningitidis* (serogroups A/C/W/Y/X) according to prevalence 0% to 100% are shown in Fig. 1.

Analysis of discrepant results. Table 2 gives the details of the 43 specimens that gave discrepant results by PCR and RDT (5 uninterpretable by RDT, 24 positive by PCR and negative by RDT, and 14 exhibiting the opposite pattern). Concerning false-positive results, they were observed in negative specimens as well as in specimens positive for another bacterium; in 8 of these 14 samples, the cross reactivity was observed with the W valence. Concerning false-negative results, it is worthwhile to note that the MeningoSpeed RDT does not include antigens targeting the B serogroup, which explains why the 10 samples found positive for this serogroup, all coming from CSF sampled in France, were all missed by the RDT.

Correlation between MeningoSpeed RDT and IPP RT-PCR assay at the serogroup level. After exclusion of the 10 samples found positive for serogroup B that was absent from the RDT, 319 CSF samples remained positive by PCR. From these 319

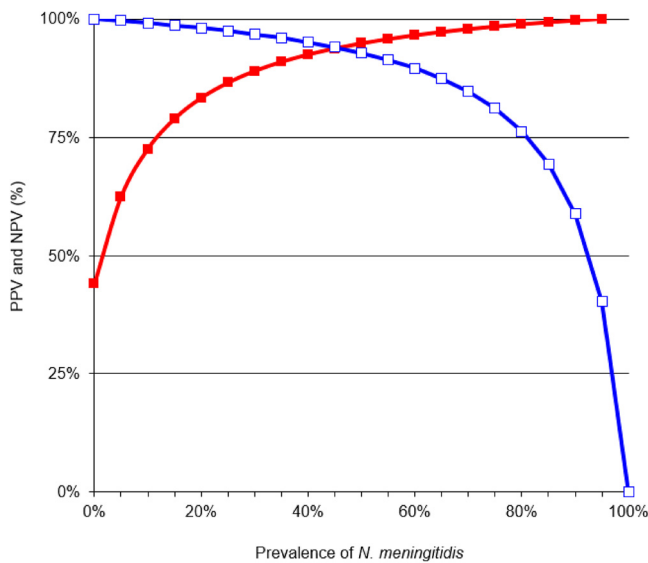


FIG 1 Positive (red line with filled squares) and negative predictive values (blue line with open squares) of the MeningoSpeed rapid diagnostic test for the detection of *N. meningitidis* in cerebrospinal fluid according to prevalence varying from 0% to 100%.

samples, 299 gave concordant results at the serogroup level by both techniques with the following distribution: 40 serogroup A, 35 C, 156 W, 58 X, and 10 Y. For 6 samples, the RDT detected *N. meningitidis* infection but with a wrong serogroup; specifically, 3 samples were identified as A by RDT but were typed C by PCR, and 3 other samples identified as X by RDT were typed A, C, and Y, respectively, by PCR. The 14 remaining samples positive by PCR were found negative by RDT. Table 3 illustrates the performances of the RDT for each serogroup; sensitivity was excellent for serogroups A, W, and X; acceptable for serogroup Y; and weak for serogroup C. The specificity and NPV were excellent for all the 5 serogroups (Table 3).

DISCUSSION

The identification and serogrouping of *N. meningitidis* infection are important for disease control and vaccination strategies (25, 26). The availability of RDTs is important for the early diagnosis of meningitis due to this bacterium, notably in the case of outbreaks that frequently occur in the meningitis belt of sub-Saharan Africa (27). The MeningoSpeed RDT evaluated in this study answers to most of the diagnostic criteria required by WHO for limited-resource areas because it is easy to store (room temperature with stability of 2 years between +4°C and 30°C), perform, and read, without needing sophisticated equipment nor electricity; small amounts of CSF are required (less than 100 µl for the 3 cassettes); the result is available within 15 minutes; 5 of the 6 most commonly identified serogroups can be detected, which permits simultaneous identification and serogrouping with a good confidence; and, finally, the overall performances of the test are very satisfactory compared to a sensitive PCR assay taken as reference standard when tested on a large panel of CSF belonging to various

TABLE 2 Analysis of discrepant results between the PCR assays taken as reference standard and the MeningoSpeed rapid diagnostic test

RDT result	Total no. of specimens	PCR assays results (no. of specimens) for:							No. of specimens with negative ^a PCR result	
		A	B	C	W	X	Y	<i>Streptococcus pneumoniae</i>		<i>Haemophilus influenzae</i>
Uninterpretable	5	1	0	0	2	2	0	0	0	0
False negative	24	2	10 ^b	8	3	0	1	0	0	0
False positive	14	0	0	0	0	0	0	2	3	9

^aNegative result by the PCR tests independently targeting *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*.

^bIt was predicted that these samples would be negative since the RDT assay lacks the B antigen.

TABLE 3 Performances of the MeningoSpeed RDT by comparison to the IPP RT-PCR assay used as reference standard for the serogroup determination of the 319 specimens found positive for *Neisseria meningitidis* by PCR^a

Parameter ^b	Values by serogroup, determined by PCR				
	A	C	W	X	Y
No. of CSF+ samples by PCR	43	47	159	58	12
Sensitivity (95% CI)	0.930 (0.799–0.981)	0.744 (0.594–0.856)	0.981 (0.942–0.945)	1 (0.923–1)	0.883 (0.509–0.970)
Specificity (95% CI)	0.990 (0.976–0.996)	1 (0.990–1)	0.980 (0.960–0.990)	0.986 (0.976–0.994)	1 (0.991–1)
PPV (95% CI)	0.889 (0.751–0.958)	1 (0.877–1)	0.951 (0.903–0.978)	0.892 (0.784–0.952)	1 (0.655–1)
NPV (95% CI)	0.994 (0.982–0.998)	0.977 (0.959–0.988)	0.992 (0.976–0.998)	1 (0.990–1)	0.996 (0.985–0.999)
Cohen's kappa coefficient (95% CI)	0.901 (0.834–0.969)	0.842 (0.755–0.930)	0.952 (0.924–0.980)	0.936 (0.889–0.983)	0.907 (0.780–1)

^aAfter exclusion of the 10 samples serogrouped as NmB, as this antigen was absent from the RDT.

^bPPV, positive predictive value; NPV, negative predictive value.

serogroups (Tables 1 and 3). However, the risk of missing a positive sample (as illustrated by the overall NPV of 89.8% with regard to PCR assay) confirms that a negative result with this technique does not avoid further testing and the start of an empirical antimicrobial treatment if the clinical and biological context is evocative of purulent meningitis.

At the serogroup level, the analytical performances of the MeningoSpeed RDT were in good concordance with those of other rapid antigenic tests, as illustrated in Table 4 for sensitivity and specificity. Even if the comparison of results is not totally pertinent given the size and origin of the panels that were evaluated in these different studies, it is worthwhile to note that the performances of the MeningoSpeed RDT were at least equivalent to and often higher than those of former tests. The only exception concerned serogroup C for which a few samples were missed; the same moderate sensitivity for this serogroup was noticed in the study of Uadiale et al. conducted in Nigeria (13). These observations stressed once more that a negative result for *N. meningitidis* antigens in a CSF sample from a patient suspect with purulent meningitis does not discard the diagnosis of meningococcal infection and must be confirmed by additional microbiological investigations. In the future, an improvement in the quality of the antibodies used in the MeningoSpeed test may contribute to reduce the number of cross-reactions and of false-negative results.

We are aware that the present study has some limitations. First, its retrospective design is not compatible with a correct evaluation of the feasibility of the RDT in field condition. However, the technology of the MeningoSpeed RDT is very close to that already evaluated in points of care by other studies; the study of Collard et al. (17) performed in Niger on a large panel of samples is illustrative of the good concordance between results obtained with a dipstick system in the field and in a reference

TABLE 4 Respective sensitivity and specificity of RDT by comparison to PCR as referenced in different studies of the literature^a

Reference and yr	Type of RDT	No. of CSF samples	Origin of CSF samples	Serogroup	% Sensitivity (95%CI)	% Specificity (95% CI)
(11) 2006	LA	484	Niger 2003	A	88 (85–91)	93 (90–95)
(12) 2009	LA	126	Niger 2006	A	69 (57–79)	81 (68–91)
	Dipstick				89 (80–95)	62 (48–75)
(15) 2010	Dipstick	265	Burkina Faso 2007	A	70.0 (55.4–82.1)	97.0 (93.4–98.7)
(16) 2013	Dipstick	401	France, Burkina Faso, Niger	A	88 (81–92)	99 (98–100)
(17) 2014	Dipstick	1,113 ^b	Niger 2008–2012		87.6 (85.7–89.3)	85.7 (83.1–87.2)
		1,162 ^c	Niger 2008–2012	A/W	92.5 (89.8–92.9)	84.6 (82.0–86.9)
(18) 2015	Dipstick	369	Cameroon, Côte d'Ivoire, Niger, France	X	94 (86–98)	100 (99–100)
(13) 2016	LA	63	Nigeria 2013–2015	C	80.0 (65.4–90.4)	94.4 (72.7–99.9)
This study 2019	Dipstick	560	Niger, Côte d'Ivoire, République Centre-Africaine, Togo, France	A	93.0 (79.9–98.1)	99.0 (97.6–99.6)
				C	74.4 (59.4–85.6)	100 (99.0–100)
				W	98.1 (94.2–99.5)	98.0 (96.0–99.0)
				X	100 (92.3–100)	98.6 (97.6–99.4)
				Y	83.3 (50.9–97.0)	100 (99.1–100)

^aRDT, rapid diagnostic test; CSF, cerebrospinal fluid; LA, latex agglutination.

^bTested in point of care.

^cTested in reference laboratory.

laboratory (see Table 4). A prospective in-field study is currently being conducted in Niger and Burkina Faso to validate the MeningoSpeed assay. Second, despite the very low proportion of uninterpretable results (0.9%), it is important to consider that all of them were found positive for *N. meningitidis* DNA by the IPP RT-PCR assay (Table 2), which means that such samples need imperatively to be tested by an alternative technique. Third, absence of the B serogroup in the MeningoSpeed panel led us to miss 10 samples found positive for this serogroup by the IPP RT-PCR assay. Although the NmB serogroup is less frequently involved than others in meningitis outbreaks occurring in Africa (all the B strains of this study were detected in CSF sampled in France), it was shown to be responsible for 29% of all the outbreaks observed in other continents (25). In order to fill the gap, we have the objective to add this valence to a future version of the MeningoSpeed RDT. Fourth, a few methodological aspects were neglected, including an intersite quality control in order to assess that the results of the different tests were reproducible from one site to another, or a blind reading of each RDT by two independent observers in order to minimize the misinterpretations due to eye reading. These limitations are linked to the difficulty of organizing a ground trial in 7 different centers, including 5 of them in African countries. It is obvious that these limitations may have underestimated the performances of the tested RDT. Finally, the eye reading of the result of the present RDT may introduce subjectivity in the interpretation of the test, notably when nontrained personnel is in charge of it; for the detection of other agents, and notably viruses, automatic readers are available, which improves substantially the traceability and the performances of such “digital immunoassays” (28). It could be interesting to propose this improvement for a future version of the test.

Despite these limitations, the test evaluated in this study brings together most of the qualities of a point-of-care RDT. In most cases, its performances can guarantee an accurate detection of *N. meningitidis* antigens and a correct serogrouping of the agent. The test is now in evaluation in large-scale studies performed in the African meningitis belt.

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E.B. and Y.G. are working at BioSpeedia, a spin-off the Paris Pasteur Institute. The other authors have no conflict of interest to declare in relation with the matter of this study.

C.H.H. did some experiments, analyzed the results, and contributed to the writing of the paper. A.T., P.V., B.-M.N.-L., M.D., F.S., A.E.M., J.-P.L., A.R., E.H., and A.A. provided the results of the different centers involved in the trial and approved the final manuscript. E.B. and Y.G. designed the MeningoSpeed RDT and approved the final manuscript. B.P. contributed to the analysis of the results and wrote the manuscript. M.-K.T. conceived the design of the study, contributed to the analysis of results, and contributed to the writing of the manuscript. All the authors agreed with the revised manuscript.

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