

Development and Evaluation of a Dipstick Diagnostic Test for *Neisseria meningitidis* Serogroup X.

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1 **Development and evaluation of a dipstick diagnostic test for *Neisseria meningitidis***
2 **serogroup X**

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23

24 **Abstract**

25 The emergence of *Neisseria meningitidis* serogroup X (NmX) in the African meningitis belt,
26 urged the development of diagnostic tools and vaccines for this serogroup, especially following
27 the introduction of a conjugate vaccine against meningococci serogroup A (NmA). We have
28 developed and evaluated a new rapid diagnostic test (RDT) for detecting the capsular
29 polysaccharide (cps) antigen of this emerging serogroup. Whole inactivated NmX bacteria were
30 used to immunize rabbits. Following purification by affinity chromatography, the cpsX-specific
31 IgG antibodies, were utilized to develop a NmX-specific immunochromatography dipstick RDT.
32 The test was validated against purified cpsX and meningococcal strains of different serogroups.
33 Its performance was evaluated against PCR on a collection of 369 cerebrospinal fluid (CSF)
34 samples obtained from patients living in countries within the meningitis belt (Cameroon, Côte
35 d'Ivoire and Niger) or in France. The RDT was highly specific for NmX strains. A cut-off of 10^5
36 CFU/mL and 1 ng/mL was observed for the reference NmX strain and purified cpsX,
37 respectively. Sensitivity and specificity were 100% and 94%, respectively. A high agreement
38 between PCR and RDT (Kappa coefficient of 0.98) was observed. The RDT test gave a high
39 positive likelihood ratio and a low negative likelihood (0.07) indicating almost 100% probability
40 to declare disease or not when the test is positive or negative, respectively. This unique NmX-
41 specific test could be added to the available set RDT tests for the detection of meningococcal
42 meningitis in Africa as a major tool to reinforce epidemiological surveillance after the
43 introduction of the NmA conjugate vaccine.

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48 INTRODUCTION

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50 *Neisseria meningitidis* (Nm) is an exclusively human capsulated bacterium that can provoke
51 severe invasive infections such as meningitis and septicaemia (1). Meningococcal disease is still
52 a major public health concern due to potential epidemic spread. While the disease occurs
53 sporadically in Europe and North America, it is responsible for major recurrent epidemics within
54 the African meningitis belt (2). The bacterial capsular polysaccharide determines the 12 Nm
55 serogroups currently described. Six serogroups (A, B, C, Y, W and X) are responsible for the vast
56 majority of cases of meningococcal disease worldwide. However, they differ in their global
57 frequencies and geographical distribution (3). This distribution impacts on vaccination strategies,
58 which for the most part involve the established polysaccharide-based vaccines against serogroups
59 A, C, Y and W. Besides, an innovative recombinant protein-based vaccine was recently licensed
60 in Europe and Australia against meningococci of serogroup B (4). This multicomponent vaccine
61 targets conserved proteins among meningococci regardless of their serogroup. Therefore, it has
62 the potential to cover non-serogroup B isolates such as those of serogroup X (5). In the
63 meningitis belt, *N. meningitidis* serogroup A (NmA) predominated prior to the introduction of the
64 NmA polysaccharide-protein conjugate vaccine (MenAfriVac™) (6), while other serogroups
65 (mainly serogroups W (NmW) and X (NmX)), were also detected and still are. Of particular
66 concern, outbreaks due to isolates of NmW and NmX were recently reported in Africa (7-9).
67 Surveillance of the distribution of meningococcal serogroups is therefore important and its
68 comprehensiveness will benefit from diagnosis tools that can be widely used at bedside. We
69 have, in recent years, contributed to the development and validation of immunochromatography
70 dipstick rapid diagnostic tests (RDT) for the identification of *N. meningitidis* serogroups A, C, Y
71 and W (10-11). This major achievement was a first step to improve bedside diagnosis of

72 meningococcal infection in Niger, a country within the meningitis belt (10,12). While NmX is
73 still rare in Europe (13), its increasing importance in the meningitis belt supports the licensing of
74 an efficient diagnosis device against NmX infection as well as ongoing studies toward a NmX
75 polysaccharide-based vaccine (14). Herein, we report the design, development, and validation in
76 the field of a new RDT for the detection of NmX isolates. Hence, this work contributes to
77 complete the available tools for the diagnosis and surveillance of meningococcal meningitis in
78 the meningitis belt.

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96 **Materials and Methods**

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98 **Bacterial strains and samples**

99 *N. meningitidis* isolates used in this study were isolates from cases of meningococcal disease (see
100 Table 1 for details). Bacteria were cultured on GCB medium (GC Agar Base, Difco, Detroit, MI,
101 USA) supplemented with Kellogg supplements (15). The serogroup was determined by
102 agglutination with serogroup-specific antisera according to the standard procedure (16). Further
103 phenotyping (serotyping and serosubtyping) was performed using monoclonal antibodies against
104 the meningococcal proteins PorA and PorB as previously described (17). The cerebrospinal fluid
105 (CSF) samples tested in this study corresponded to suspected bacterial meningitis cases. They
106 were obtained from the National Reference Laboratories for Meningococci located at the Institut
107 Pasteur of Côte d'Ivoire and at the Institut Pasteur, Paris, France, as well as from the Centre de
108 Recherche Médicale et Sanitaire (CERMES) in Niamey, Niger, and from the Centre Pasteur of
109 Garoua, Cameroon. These samples were received in the frame of these centres' mission for the
110 surveillance of meningococcal diseases in the corresponding countries under approvals from the
111 internal board of the Institut Pasteur to collect, characterize and use these samples that are all
112 anonymized.

113 The PCR analysis of these samples was used as a reference method to detect *N. meningitidis*,
114 *Streptococcus pneumoniae* and *Haemophilus influenzae*, as well as to genogroup meningococcus-
115 positive specimens. PCR conditions and primers were as previously described (8). Culture was
116 not used as it has been constantly shown to be less sensitive than PCR (18). Culture data were
117 available only for 26 of the 369 tested CSF samples.

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119 **Purification of the capsular polysaccharide from NmX**

120 The capsular polysaccharide of serogroup X (cpsX) was purified from the NmX strain 19504
121 (that gave the highest yield when cultured on GCB medium with Kellogg supplements) by the
122 Cetavlon extraction method as previously described (19). Briefly, bacteria (1 L) at late-
123 logarithmic phase of growth were formaldehyde-inactivated (1% v/v) and then treated with
124 Cetavlon (0.1% w/v) (Sigma Aldrich, France). After centrifugation, the pellet was dissolved in
125 cold aqueous CaCl₂ (0.9M). The solubilised materials were cleared by precipitation in 25%
126 aqueous ethanol and the remaining supernatant was precipitated by 80% aqueous ethanol. The
127 pellet was dissolved in phosphate buffer (Na₂HPO₄, NaH₂PO₄, 0.2 M) and treated with Dnase
128 and Rnase followed by proteinase K treatment (Sigma Aldrich, France) and cold phenol
129 extraction. The extract was extensively dialyzed against distilled water and lyophilized to obtain
130 the crude capsular polysaccharide. Ten mg of the preparation were dissolved in 2 mL of
131 phosphate buffer K₂HPO₄, KH₂PO₄ (0.05 M), pH 7, and purified by gel filtration on a Biosep-
132 SEC-S3000 column (300 x 21.2 cm, Phenomenex, France) that was equilibrated with the same
133 buffer. Elution was carried out with the same phosphate buffer at 5 mL/min, and monitored at
134 214 nm and 280 nm. The void volume fractions containing cpsX in the high molecular-weight
135 range were pooled and dialyzed against distilled water at 4°C, using a dialysis membrane with a
136 cut-off size of 10K-15K, and the residue was lyophilized. The yield was about 20 mg/L of
137 culture. The profile of the purified cpsX was checked by proton nuclear magnetic resonance (¹H
138 NMR) (data not shown) as previously described (20). CpsA, cpsB, cpsC, cpsY and cpsW were
139 similarly purified from five strains of serogroups A, B, C, Y and W (strains 21524, 21721, 22639,
140 16366 and 19995 respectively, Table 1).

141 **Rabbit immunization and purification of specific anti-cpsX IgG antibodies**

142 Two New Zealand White female rabbits (3 kg) were immunised intravenously three times with
143 doses of 1mL of a suspension of 10⁹ colony forming units, CFU, of freshly heat-inactivated

144 NmX strain 19504 (30 min at 56°C), at day 0, 7 and 21. Sera were taken before immunization
145 and at day 28 after the first injection to evaluate the immune response by ELISA (see below). Dot
146 blotting with rabbit sera (1:1000 serum dilution) was performed using Amersham ECL kits (GE
147 Healthcare Life Sciences Velizy-Villacoublay, France) as previously described (21). Rabbit
148 immunisation was performed according to the European Union Directive 2010/63/EU (and its
149 revision 86/609/EEC) on the protection of animals used for scientific purposes. Our laboratory
150 has the administrative authorization for animal experimentation (Permit Number 75–1554) and
151 the protocol was approved by the Institut Pasteur Review Board that is part of in the Regional
152 Committee of Ethics of Animal Experiments of the Paris region (CETEA 2013-0190).

153 IgG antibody purification was performed by affinity chromatography in two steps. First, the
154 rabbit's sera were passed through a HiTrap Protein G HP column (GE Healthcare, France) and
155 eluted with glycine-HCl 0.1 M pH 2.7. Fractions of 1 mL were recovered in 50 µL of Tris-HCl
156 buffer (1 M, pH 9). Fractions were tested for protein content by measuring their absorbance at
157 280 nm. Pooled fractions were passed through a cpsX affinity column obtained by chemical
158 coupling of the amine functions of the CarboxyLink resin and the phosphate functions from
159 cpsX, according to manufacturer recommendations (Thermo Scientific, Rockford, IL. USA). The
160 eluted fractions were tested by ELISA against purified cpsX and whole inactivated NmX
161 bacteria. To do so, ELISA wells were coated overnight with 100 µL of a solution containing 2
162 µg/mL of purified cpsX or 100 µL of a bacterial suspension of 3×10^8 CFU/mL (NmX strain
163 19504). The purified antibodies (at a 500 pg/ml concentration) were tested against serial dilutions
164 of bacteria from serogroup A, B, C, Y, W and X in a dot blot experiment, and serial dilutions of
165 the antibodies were then tested in ELISA on counterpart coated cps at 2 µg/mL concentration.

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167 **Production and validation of a RDT against NmX**

168 A one-step vertical flow immune-chromatography dipstick was set up using purified cpsX-pAbs
169 that were conjugated to gold particles (British Biocell International, Cardiff, UK) as previously
170 described (22). Unconjugated cpsX-pAbs were used as capture antibodies and goat anti-rabbit
171 IgG (ICN Biomedicals, Aurora, Ohio, United States) were used as control antibodies. Both types
172 of antibodies were sprayed onto nitrocellulose (Schleicher & Schuell Bioscience, Ecquevilly,
173 France) at 2 µg and 1 µg per line centimeter respectively. For the test evaluation, dipsticks were
174 dipped, for a 10-15 min period at room temperature, in 100 µL of PBS containing bacterial
175 suspensions or CSF samples.

176

177 **Data analysis**

178 Sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value
179 (NPV) were calculated using a 2 x 2 contingency table. The positive likelihood ratios LR ($LR^+ =$
180 $Se/[1-Sp]$) and the negative LR ($LR^- = [1-Se]/Sp$), were also calculated (23). These values give
181 an indication of the likelihood that the sample is positive or negative prior to testing. The
182 diagnostic odds ratio (DOR), defined as the ratio of the odds of positive test results in specimens
183 with NmX on the odds of positive test results in specimens negative for NmX, was calculated as
184 follows $DOR = (Se/[1 - Se])/([1 - Sp]/Sp)$ (24). Finally, the Cohen's kappa (j) statistic was
185 calculated to measure concordance between PCR and RDT (25).

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192 **RESULTS**

193 **Characterization of rabbit anti-meningococcal serogroup X rabbit serum**

194 Following the three dose-immunization regimen with whole NmX bacteria, the rabbit sera
195 were tested in dot blot analysis against spotted bacteria. While no bacteria detection was obtained
196 with control pre-immune sera, a strong detection was obtained with the sera from immunized
197 rabbits (Fig.1). Sera from the two responding rabbits were pooled and anti-cpsX-specific IgG
198 were purified by affinity chromatography on a NmX cps activated column. Dot plot analysis of
199 the purified IgG response against decreasing numbers of bacteria (from 5×10^5 to 5×10^3 cells per
200 spot) from serogroups A, B, C, Y, W and X showed that antibodies only recognized serogroup X
201 strain (Fig. 2A). The absence of recognition of the other serogroups (A, B, C, Y and W) was
202 further confirmed independently by ELISA analysis of the antibody response against coated (1
203 $\mu\text{g/mL}$) purified cps corresponding to the six serogroups (Fig. 2B).

204 A dipstick rapid diagnostic test for NmX was produced (see Material and Methods), and its
205 detection limits were established. For the purified cpsX, this limit was 1 ng/mL (Fig. 2C) and
206 was 10^5 CFU/mL (data not shown) for NmX bacteria (strain 19504) The cut-off analysis was
207 repeated 3 times with identical findings that were not affected by dipstick storage for 3 weeks at
208 25°C . We also tested the RDT on a collection of bacterial suspension (Table 1) at 10^6 CFU/mL.
209 Only the serogroup X isolates were detectable (data not shown). As different concentrations of
210 antibodies were used in these assays, the data suggest that the concentrations of antibodies do not
211 preclude detectable reactivity with other serogroups.

212

213 **Use of the NmX dipsticks on clinical samples**

214 The NmX dipstick was tested on a panel of 369 CSF selected from historical collections kept in
215 National Reference Centre/Laboratory from four different countries, differing in terms of

216 meningitis incidence (Cameroon, Côte d'Ivoire, France and Niger). Noticeably, three out of the
217 four laboratories are located in countries within the meningitis belt. The CSF samples
218 corresponded to suspected cases of acute bacterial meningitis. They were characterized by PCR
219 for etiological diagnosis (Table 2). Culture results were only available for 26 samples (8 samples
220 positive for *S. pneumoniae*, 4 positive for *N. meningitidis* (2 serogroup B and 2 serogroup W), 1
221 positive for *H. influenzae*, 1 positive for *S. agalactiae* and 12 CSF samples were sterile by
222 culture).

223 Among these isolates, 52% (n = 191) were positive for Nm, 8% (n = 28) were positive for other
224 bacterial species, namely *S. pneumoniae*, *H. influenzae* and *S. agalactiae*, and 40% (n = 150)
225 were negative by PCR for these species. Among the Nm positive CSF, the six meningococcal
226 capsular groups involved in invasive meningococcal infections were represented: group A (n =
227 27), group B (n = 8), group C (n = 7), group Y (n = 2), group W (n = 38) and group X (n = 92). In
228 addition, 17 CSF samples were positive for Nm by PCR although they were negative for groups
229 A, B, C, Y, W and X. All samples that were negative for NmX by PCR were also negative for
230 this group by the new NmX-specific RDT. Among the 92 CSF positive for NmX by PCR, 86
231 were also positive by RDT. All the 26 CSF samples with culture data were tested negative by
232 NmX-specific RDT (data not shown).

233 We also conducted a limited prospective analysis during the epidemic season 2014 in the three
234 laboratories located in countries of the meningitis belt. We tested all the 153 CSF samples that
235 were received in the three laboratories in Cameroon, Côte d'Ivoire and Niger between 1 January
236 2014 and 15 March 2014. No NmX was detected by PCR or by RDT in any of the samples. In
237 contrast, several samples were positive by PCR for *S. pneumoniae* (14%), NmW (7%) and *H.*
238 *influenzae* (3%).

239

240 **Performance of the NmX-specific RDT: sensitivity, specificity, likelihood ratios, and**
241 **predictive values**

242 RDT data showed a good correlation with PCR data, indicating a Kappa correlation coefficient of
243 98%. The sensitivity, specificity and 95% CI (confident interval) data of the RDT obtained for
244 the documented 369 CSF samples are summarized in Table 3. The specificity of RDT for CSF
245 infected by NmX was 100%, while the sensitivity reached 94%. Calculating the positive
246 likelihood LR^+ and DOR was not feasible due to a Sp value of 100%. LR^+ and DOR values were
247 therefore calculated using a value for the specificity that corresponded to the lower 95%
248 confidence interval for specificity (0.99) (Table 3).

249 The prevalence of NmX among the 369 tested CSF was 25%. Therefore, the NPV and PPV are
250 given in Table 3 under this prevalence value. However, the tested samples were selected from the
251 collections of the participating laboratories and may not reflect the real prevalence of the disease.
252 Moreover, the frequency of NmX meningitis may also vary across time and countries within the
253 meningitis belt and elsewhere. We therefore calculated the negative and positive predictive
254 values (NPV and PPV) according to a prevalence varying from 0 to 100%, using the Se and Sp
255 obtained from the CSF samples in this study (Fig. 3).

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264 **DISCUSSION**

265 Reliable tests for the identification of cases of meningococcal meningitis and serogroup-
266 determination are crucial to ensure proper individual (case-by-case) as well as collective
267 management of cases and epidemiological surveillance. Culturing *N. meningitidis* may frequently
268 fail due to early antibiotic treatment and fragility of this bacterial species (26). During the last
269 two decades, PCR-based nonculture methods have been developed, enabling a significant
270 improvement of the management and surveillance of bacterial meningitis (18). PCR-based
271 methods require specific laboratory equipment and trained staff and can not be used as a bedside
272 method. Nevertheless, the PCR technology was implemented in several reference laboratories
273 located in countries within the African meningitis belt (18). However, PCR may not be
274 sufficiently set to ensure country-wide surveillance, especially in populations living in remote
275 areas. Other tests, such as the currently available latex agglutination kits, require trained staff and
276 an unbroken cold chain for storage and distribution of the kits.

277 The recent implementation of RDT for meningococci of serogroups A, C, Y and W was a
278 major breakthrough for individual diagnosis and for surveillance of meningococcal diseases in
279 the African meningitis belt (12). These tests are stable at temperature up to 45°C at least. They
280 are easy to use and to interpret in the absence of extensive training, and therefore are adapted for
281 bedside use. The emergence of meningococcal isolates of serogroup X urged the development of
282 a RDT test for this serogroup to complete the current RDT tools. We first analyzed the inherent
283 quality of such a serogroup X specific test. The specificity and sensitivity parameters were
284 evaluated under laboratory conditions using a selected panel of relevant CSF samples. The good
285 quality of the new RDT was reflected by its high sensitivity and specificity for NmX with a very
286 high likelihood ratio for positive test (Table 3).

287 We also evaluated its usefulness that depends not only on the quality of the test but also
288 on the prevalence of the NmX meningitis in the tested population. The prevalence of NmX within
289 the panel of CSF samples that was used to evaluate the RDT specificity and sensitivity was
290 25.7%. It may not properly reflect the real prevalence of NmX in areas at risk. Usefulness is
291 usually evaluated using two parameters, the PPV and NPV. When NmX prevalence was forced to
292 vary between 0 and 100%, the PPV remained stable at 1 indicating that the test remained highly
293 proficient in ruling-in a case. Moreover, the NPV retained high values when the prevalence of
294 NmX was very low. In addition, the test remained proficient (NPV of 0.95 or higher) if this
295 prevalence increased to 50%. These considerations seem realistic and reflect the current
296 epidemiological situation in the meningitis belt after the introduction of MenAfriVac™ that was
297 associated with significant decrease of NmA (9). Indeed, this small scale prospective use of the
298 new RDT in the three centres located in this area (Abidjan, Garoua and Niamey), suggests, on the
299 basis of sensitivities of RDT and PCR (that are less than 100%), that NmX may be present albeit
300 not as a dominating pathogen. In contrast, NmW was the most frequently isolated Nm species,
301 while most cases were associated to *S. pneumoniae*. However, a large-scale multi-site prospective
302 study comparing PCR and all the available RDT (A, C, Y, W, Y and X) is warranted in the
303 future. Moreover, additional work is required to miniaturize the RDT to be applicable on small
304 volume samples (< 100 µL).

305 In summary, this work reports a new reliable and rapid diagnostic test to detect serogroup X that
306 should enhance diagnosis of meningitis due to this serogroup and is expected to improve
307 epidemiological surveillance. Epidemiological changes upon the implementation of
308 MenAfriVac™ can be better monitored. This test would be helpful for the development and the
309 implementation of vaccines with broad serogroup coverage that can target NmX (5) or with
310 NmX-specific vaccines (14).

311 **LEGENDS OF FIGURES**

312
313 Figure 1. Dot blotting analysis of rabbit sera. Sera from two rabbits prior to immunization (day 0)
314 and 7 days after injection of the third dose of NmX strain 19504 (day 28) were used at 1:1000
315 dilutions in immunoblotting. Four meningococcal isolates were spotted at $2 \cdot 10^5$ colony forming
316 units, CFU/mL (1: strain 19404, 2: strain 23557, 3: strain 24196, 4: strain 24287).

317
318 Figure 2. Specific recognition of the purified rabbit anti-cpsX IgG antibodies. (A) Dot blotting
319 analysis against whole bacteria. Serogroups are indicated above the dots and amounts of loaded
320 bacteria in each spot are indicated on the right (in colony forming units, CFU). Antibodies were
321 used at a final concentration of 500 pg/mL. (B) ELISA analysis using coated purified capsular
322 polysaccharide for serogroups A, B, C, Y, W and X (Table 1). Data are expressed as OD 492 nm
323 absorption for each concentration of antibodies (in pg/mL). Data correspond to the means of two
324 independent experiments. The corresponding serogroups are indicated on the right. (C) Detection
325 cut-off value for purified cpsX. The amounts are indicated in ng above each dipstick. A dipstick,
326 before use, is shown on the left. The upper two arrows indicate the capture control line
327 corresponding to the goat anti-rabbit IgG. The lower two arrows indicate the capture line
328 corresponding to the anti-cpsX-specific IgG (cpsX line).

329
330 Figure 3. Predictive values for *N. meningitidis* diagnosis. Positive Predictive Values and Negative
331 Predictive Values (PPV and NPV, respectively) for the diagnosis of NmX were calculated
332 according to a disease prevalence ranging between 0 and 100%.

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450 Table 1. Strains used in the study and their characteristics

| Strain reference | Serogroup:serotype/serosub-type |
|------------------|---------------------------------|
| 21525* | A:4:P1.9 |
| 21526 | A:4:P1.9 |
| 19256 | B:NT:P1.5,2 |
| 19257 | B:2a:P1.5,2 |
| 19324 | B:2b:P1.5,2 |
| 21721* | B:NT:P1.4 |
| 22733 | B:15:P1.4 |
| 22590 | B:14:P1.7,16 |
| 22644 | C:15:P1.7,16 |
| 22639* | C:2a:P1.5 |
| 20137 | C:2b: P1.5,2 |
| 19008 | C:2a: P1.5,2 |
| 20134 | C:NT:P1.10 |
| 19456 | Y:14:NST |
| 19336* | Y:NT:P1.5 |
| 19995* | W:2a:P1.5,2 |
| 19481 | W:NT:P1.5 |
| 19836 | W:NT:P1.6 |
| 19383 | E:NT: P1.5,2 |
| 19504* | X:NT: P1.5,2 |
| 24196 | X:4:P1.12 |
| 24287 | X:4:P1.16 |
| 23557 | X:NT:P1.5 |

451 NT: Nontypeable; NST: Nonsubtypeable.

452 * Strains that were used for capsular polysaccharide purification

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460 Table 2 Results of CSF samples obtained by PCR and by RDT

| PCR | Geographical origins | | | | Total | RDT | |
|----------------------|----------------------|--------|-----------|------------------|-------|------------------|------------------|
| | IP Paris | CERMES | CP Garoua | IP Côte d'Ivoire | | NmX ⁺ | NmX ⁻ |
| NmA | 6 | 15 | 6 | 0 | 27 | 0 | 27 |
| NmB | 6 | 0 | 0 | 2 | 8 | 0 | 8 |
| NmC | 7 | 0 | 0 | 0 | 7 | 0 | 7 |
| NmY | 2 | 0 | 0 | 0 | 2 | 0 | 2 |
| NmW | 6 | 10 | 4 | 18 | 38 | 0 | 38 |
| NmX | 7 | 80 | 5 | 0 | 92 | 86 | 6 |
| Nm NG | 0 | 0 | 16 | 1 | 17 | 0 | 17 |
| <i>S. pneumoniae</i> | 0 | 0 | 10 | 13 | 23 | 0 | 23 |
| <i>H. influenzae</i> | 0 | 0 | 1 | 3 | 4 | 0 | 4 |
| <i>S. agalactiae</i> | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| Negative* | 10 | 0 | 77 | 63 | 150 | 0 | 150 |
| Total | 45 | 105 | 119 | 100 | 369 | 86 | 283 |

461 *PCR Negative for *N. meningitidis*, *S. pneumoniae* and *H. influenzae*

462 CSF; Cerebrospinal fluid, RDT: Rapid Diagnostic Test, IP= Institut Pasteur, CP: Centre

463 Pasteur, Nm: *Neisseria meningitidis*, NG non groupeable

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472 Table 3 Performance of the RDT for NmX

| Test parameter | Value | 95% confidence interval |
|---|-------|-------------------------|
| Sensitivity (Se) | 0.94 | 0.86 to 0.98 |
| Specificity (Sp) | 1 | 0.99 to 1 |
| Positive Likelihood ratio (LH ⁺)* | 94 | 32 to 8252 |
| Negative Likelihood ratio (LH ⁻) | 0.07 | 0.03 to 0.15 |
| Positive predictive value (PPV) | 1 | 0.96 to 1 |
| Negative predictive value (NPV) | 0.98 | 0.95 to 0.99 |
| Diagnostic odd ratio (DOR)* | 1567 | 379 to 118420 |

473 Dividing by zero; the values of LH⁺ and DOR were calculated using a value for specificity that
474 corresponded to the lower 95% confidence interval (0.99).

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Fig. 1

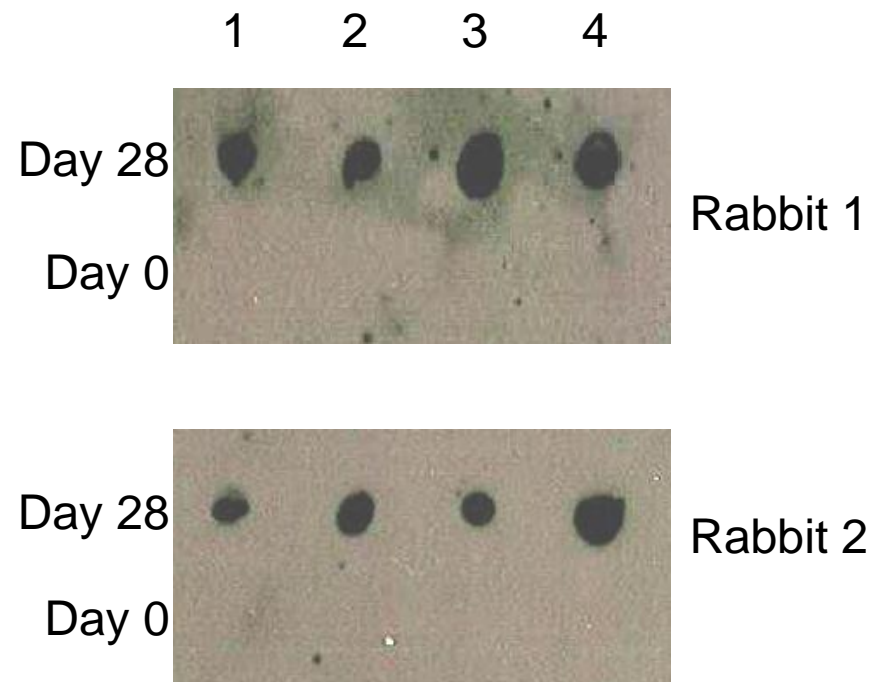


Fig. 2

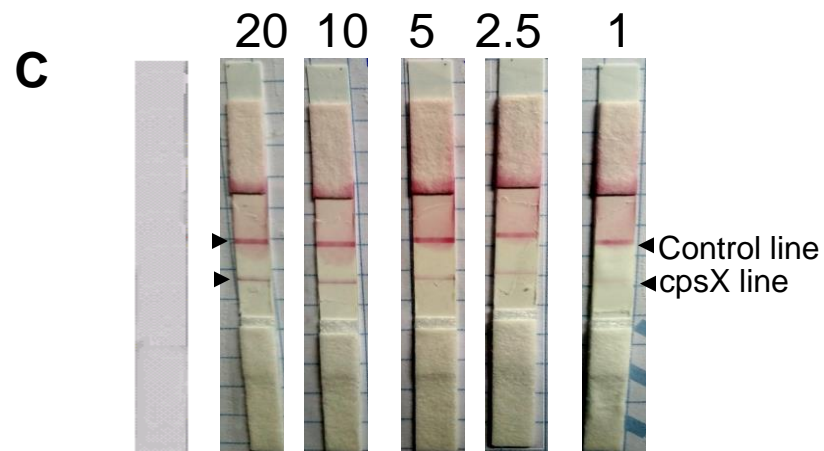
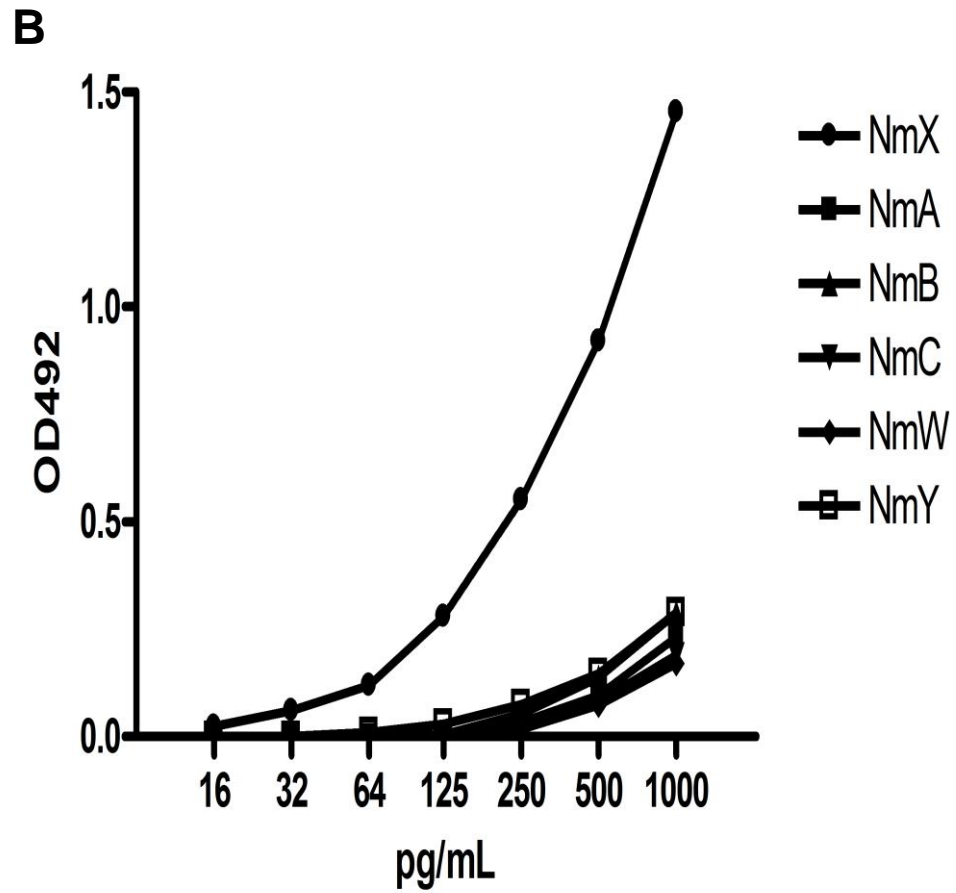
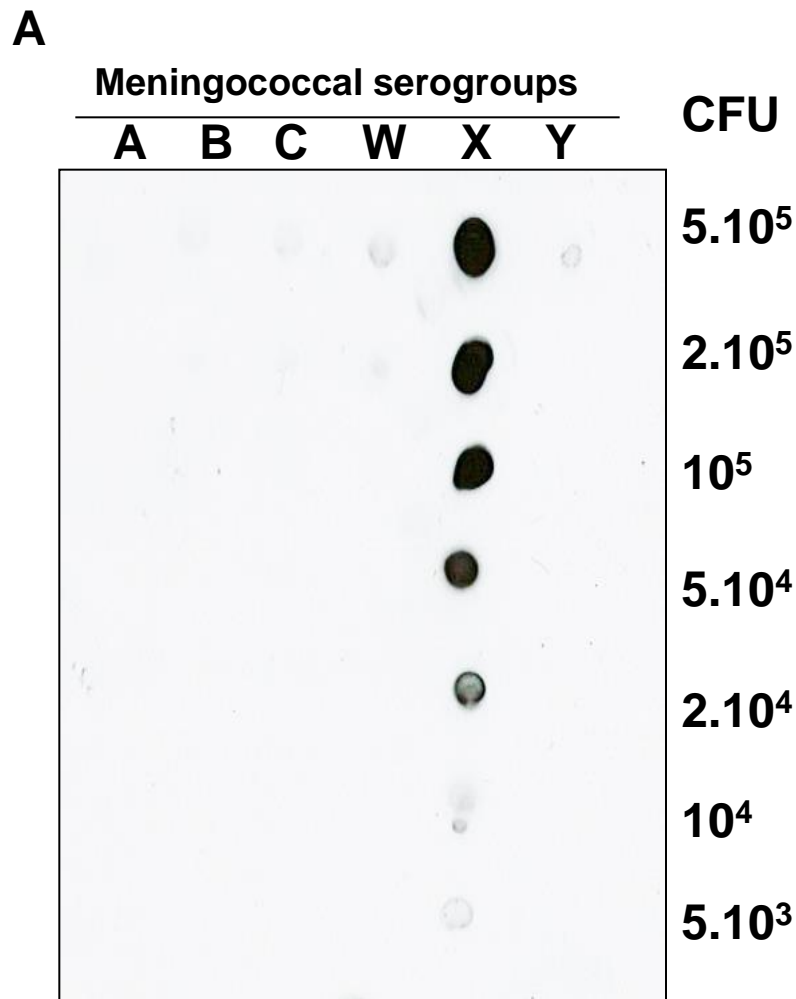


Fig. 3

