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Research Article

Internal Quality Control of Neisseria Meningitidis Carriage in Kaya and Boussouma Region of Center-North Burkina Faso from 2016 to 2017

Sampo E[5]*, Sanou M[4], Sanon B[2], Somlare H[1], Ouattara K[1], Kienou M[1], Sawadogo M[1], Ouedraogo A[1], Kabre A[2], Bagaya S[2], Traore B[2], Ake F[3], Tarbangdo F[3], Nagalo BM[7], Kristiansen PA[6], Barro N[5], Ouedraogo R[4] and Sangare L[1]

[1]*Yalgado OUEDRAOGO University Hospital Center, Ouagadougou, Burkina Faso*

[2]*KAYA Regional Hospital Center, Center-Nord, Burkina Faso*

[3]*DAVYCAS International , Ouagadougou, Burkina Faso*

[4]*Charles DE GAULLE University Hospital Center, Ouagadougou, Burkina Faso*

[5]*Joseph KI-ZERBO University, (LaBESTA), Ouagadougou, Burkina Faso*

[6]*Coalition for Epidemic Preparedness Innovations (CEPI), Oslo, Norway*

[7]*Department of Hematology/Oncologie, Mayo Clinic Scottsdale, AZ, USA*

***Corresponding Author:** Sampo E, Joseph KI-ZERBO University, (LaBESTA), Ouagadougou, Burkina Faso.

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Abstract

Background: Internal quality control is essential for preventing inaccuracy of disease causing microbial diagnostic, poor reagent quality and equipment dysfunction in a meningeal bacteria carriage study. This study aimed to assess the quality of meningococcal carriage study results obtained from Boussouma and Kaya health districts in the Center-North of Burkina Faso from 2016 to 2017.

Methodology: During the *N. meningitidis* carriage study, internal quality control was performed on culture media, field working conditions, reagents, laboratory equipment and antibiotics discs.

Result: During the carriage study, an evaluation of culture media was made. Thus, no defective or contaminated modified Thayer-Martin (TMM) were not recorded. On the other hand, we noted 86 defective fresh sheep blood agar (GSF) media and 201 other contaminated. Evaluation of sample collection conditions showed that the field ambient temperature was between + 22-+ 37°C. The duration of field sampling varied respectively from 77-173 minutes, 74-232 minutes, 74-195 minutes and 75-210 minutes at first, second, third and fourth campaign. The transport duration between sampling sites and the laboratory varied between 26-118 minutes, 21-187 minutes, 27-121 minutes and 25-137minutes at first, second, third and fourth campaigns. Thus, the delay of samples transmission from the first sample collection until all TMM media laboratory incubation varied respectively 145-264 minutes, 120-264 minutes, 126-288 minutes and 120-301 minutes at first, second, third and fourth campaign. TMM and GSF sterility control did not show any contamination. We did not observe any colony on TMM media with *Proteus mirabilis* (NC 04175) and *Staphylococcus aureus* (ATCC 25923) sowed for selectivity control. On the other hand, growth colonies were observed on TMM and GSF media with *N. meningitidis* A (ATCC 13077) and *N. lactamica* (ATCC 23970) reference strains. Reagents quality control showed that *N. meningitidis* A is Gamma-glutamyl-beta-naphthylamide (GGT) and oxidase positive and O-Nitrophenyl β-D-galactopyranoside (ONPG) negative. On the other hand, *N. lactamica* hydrolyzes ONPG, possesses cytochrome oxidase and is devoid of GGT. Incubator temperature varied between + 36.3- +36.7°C. Bacteria identification control showed good growth and suitable antisera agglutination with *N. meningitidis*

A (ATCC 13077) reference strain. The strain storage temperature in the freezer ranged from -78--66°C. The refrigerator temperature used for reagents and media storage varied between + 2- +8°C. Finally, antibiotic discs tested gave good sensitivity with *N. meningitidis* A reference strain.

Conclusion: internal quality control system improved *Neisseria meningitidis* diagnosis in meningococcal A carriage study. It allowed assessing bacteriological diagnosis to obtain quality and reliable results in meningococcal A carriage study at CHR of Kaya laboratory in Burkina Faso from 2016 to 2017.

Keywords: Quality Control; Meningococcal A Carriage; Burkina Faso

Abbreviations

TMM: Modified Thayer-Martin; GT: Gamma-glutamyl-beta-naphthylamide; ONPG: O-Nitrophenyl β-D-galactopyranoside; MAVC: MenAfriVac; CHR: Regional Health Center; WHO: World Health Organization; LaBESTA: Laboratory of Molecular Biology of Epidemiology and Surveillance of Viruses and Bacteria Transmitted by Water and Food; CDC : Center for Disease Control and Prevention; GSF: Fresh Blood Agar; DGN: Gram-negative Diplococcus.

Introduction

Meningococcal disease is, in general, a significant cause of morbidity and mortality in the world and particularly in the African meningitis belt [1]. *N. meningitidis***,** the etiological agent of meningococcal disease, possess 12 serogroups whose geographic distributions vary across the continents [2]. Six serogroups (A, B, C, W, X, and Y) are the main culprits of cerebrospinal meningitis [2]. In Burkina Faso, the serogroups most frequently found are NmA, NmC, NmW, NmX, and NmY. In the African meningitis belt, although large epidemics have generally been caused by NmA [6]. Other serogroups have often been at the cause of the epidemic: such as 13 735 NmW meningitis cases and 1640 deaths were reported in Burkina Faso in 2002 [3], NmC in Nigeria from 2013-2014 by localized outbreaks [4] and NmX in Niger from 1995 to 2000 [5].

N. meningitidis is a commensal bacterium of the human nasopharynx and was transmitted by direct contact or by respiratory droplets of oropharyngeal secretions suspended in the air [7]. Meningococcal bacteria colonizes the upper respiratory tract of a healthy person and constitute the primary source of bacteria's spread in the human population [8]. In Burkina Faso, the first national mass vaccination campaign was carried out in 2010 against *N. meningitidis* A with MenAfriVac® (MACV) with peoples aged from 1 to 29 years [9]. MACV is a safe, immunogenic, and accessible conjugate vaccine [10] to reduce the NmA prevalence [11]. After MACV mass vaccination, other epidemiologic serogroups were reported in cerebrospinal meningitis outbreaks in health districts of Burkina Faso [12] and elsewhere. This was the NmC case that has been reported in Burkina Faso, indicating a risk of a large-scale epidemic such as those in Niger and Nigeria in 2015 [13]. Through a total of 6588 cerebrospinal fluid (CSF) samples collected from 15 to 19 countries participating in the vaccination campaign in 2015, laboratory results reported 2364 (36%) bacterial agents' cases [18]. Among the positive cases, 74 Nm A cases were confirmed in Guinea (in areas that were not yet covered by the MACV immunization campaign) and 3 Nm A cases in Burkina Faso in 2015 [18]. This suggested a decrease in group immunity. Thus, a second catch-up vaccination with MACV with children aged from 1 to 5 years old was carried out in 2016 and integrated into the national routine vaccination program in Burkina Faso [14].

A second *N. meningitidis* A carriage study was carried out in the health districts of Kaya and Boussouma of Burkina Faso from 2016 to 2017. It was organized by the Health Ministry of Burkina Faso, in collaboration with the Center for Disease Control and Prevention/Atlanta (CDC), Coalition for Epidemic Preparedness Innovations (CEPI), World Health Organization (WHO) and Davycas International to assess the impact of NmA conjugate vaccine. Operational procedures harmonization and reliable results insurance were a significant challenge [15]. The quality control system was an essential tool to identify in real-time: logistical problems, optimal control of inputs, and the influence of ambient temperature to ensure the accuracy of final data. Given the renewed interest in the herd immunity assessment by meningococcal carriage studies means, we focused our study on the internal quality control of culture media, reagents, antibiotics discs, laboratory equipment, and operational conditions in the field during the 2016 and 2017 NmA carriage study in Burkina Faso.

Material and Methods

Culture media preparation and evaluation

We used modified Thayer-Martin Selective Medium (TMM) for *Neisseria meningitidis* isolation from salivary swab subculture and

fresh blood agar (GSF) for confirming the diagnostic of meningococcal suspect's strains. We prepared these media at the Health Sciences Training and Research Unit (UFR/SDS) of Joseph KI-ZERBO University in Ouagadougou, Burkina Faso, from 2016 to 2017. During the Preparation, measures of safety and precautions were observed.

For TMM agar preparation, we weighed 42.5g of Columbia agar base (Oxoid, cat. No. CM0331) and 5g of bovine hemoglobin (BBL, ref. 212392) with a precision balance for a total volume of 1 liter. Each component was put in a bottle containing 500ml of distilled water (pH 7) and brought to a boil on a hot plate. After autoclaving at 121 ° C for 15 minutes, the medium was brought to 45-50°C at room temperature. The two components were mixed and added to 10ml of IsoVitaleX ™ enrichment solution (Oxoid, cat.no.SR0090A) and 2ml of V-C-N-T inhibitory antibiotic solution (BBL, ref 212408). The culture medium thus prepared was conditioned in 90mm of sterile Petri dishes about 25ml per dish.

For fresh blood agar preparation, we weighed 20g of trypticase soy agar powder (BD BBLTM Dehydrated Culture Media cat n. B12305) on a precision balance mixed in 500ml of distilled water (pH 7) contained in a bottle of 1 liter. After boiling on a hot plate and autoclaving at 121 °C for 20 minutes, the agar was cooled to 45-50 °C at room temperature before adding 5% of sterile and defibrinated sheep blood. We distributed this mixture in 90mm of sterile Petri dishes, about 25ml per Petri dish.

Both media were subjected to growth quality control using *N. meningitidis* (ATCC 13077) and *N. lactamica* (ATCC 23970) reference strains to ensure appropriate quality.

For the sterility control, one medium agar of each media lot was incubated at 37 °C for 48-72 hours with observation every 24 hours. The culture medium was considered sterile when no growth was observed within the incubation period.

The selectivity control was carried out by sowing *Staphylococcus aureus* (ATCC 25923), and *Proteus mirabilis* (NC 04175) reference strains on TMM agars. These media were considered selective if none of these species formed colonies due to the inhibitory action of antibiotics.

Activities on the sampling sites

The sampling sites (villages) concerned by this carriage study were Tamdaogo, Terrin Mossi, Mastenga, Noungou, Foulla, and Iryastenga for Kaya health district; Forgue and Bouldin for Boussouma health district (Figure 1). Every morning, a recruiters' team went at 5 AM to one of the selected villages for volunteers' recruitment. Another group of samplers composed of nurse and laboratory staff went to this same sampling site at 6 AM to collect oropharyngeal samples. After installing sampling equipment on the site, TMM agars were removed to the media rack, brought to field temperature. After that its were labeled with a barcode corresponding to the identification number of each sample taken. Each sample collected by swab was sown immediately in TMM agar close to Bunsen burner flame and placed in a jar (Thermo Fisher Scientific, Waltham, MA, USA). When the jar was full of sowed media, CO2 generator bags about two (Pack-CO2 No.10-07 20/Box, MITSUBISHI GAS CHEMICAL) and wet pad were introduced before hermetically closing with lid. We then placed the filled jar in a large transport box.

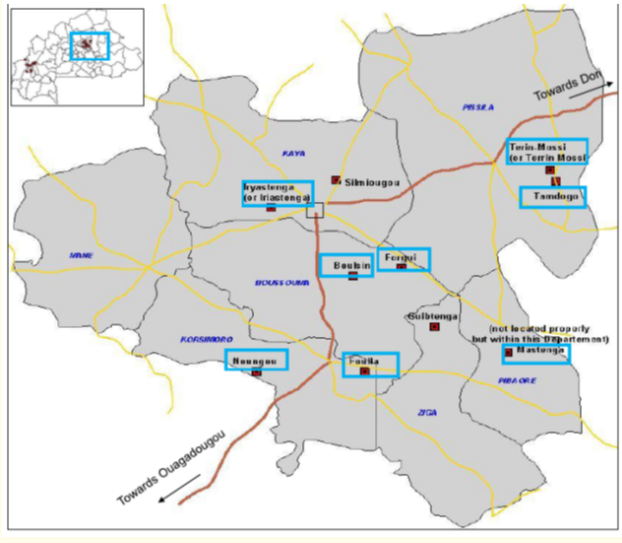

Figure 1: Survey sites (blue boxes) in the health districts of Kaya and Boussouma.

The sampling duration was determined between the time of first and that of the latest samples daily taken on the field. The minimum and maximum temperatures in the TMM media transport box were collected each day, using a digital display thermometer (digital thermometer T°int/ext 2041/2M, Moineau instruments) whose probe was housed in the transport box. The delay of sample transmission was defined between the first sample taking time on the sampling site until the all samples incubation at CHR of Kaya laboratory. The acceptance limits of delay set for the carriage study was 6 hours.

Activities at CHR of Kaya laboratory

At the laboratory, we incubated jars containing sowed or not TMM media at 37 °C for 18-48 hours. During each of these incubations, sterility, growth, and selectivity TMM and GSF media control were carried out, according to *N. menigitidis* A carriage study procedures. The sowed media were examined in a flulaminar hood at the end of each incubation for suspicious *Neisseria* colony selection.

Selection of suspicious *Neisseria* colonies

Suspicious colonies were observed to be oxidase tested on an oxidase strip (BD, Franklin Lakes, NJ, USA). A Gram-staining was carried out from suspect colonies to suggest Gram-negative diplococci (DGN) evoking the *Neisseria* genus. *Neisseria* suspected colonies and those of *N. meningitidis* A and *N. lactamica* reference strains were resowed on GSF media and incubated at 37 °C under CO2 for 18-48 hours to continue biochemical and antigenic identifications such as described in (Figure 2).

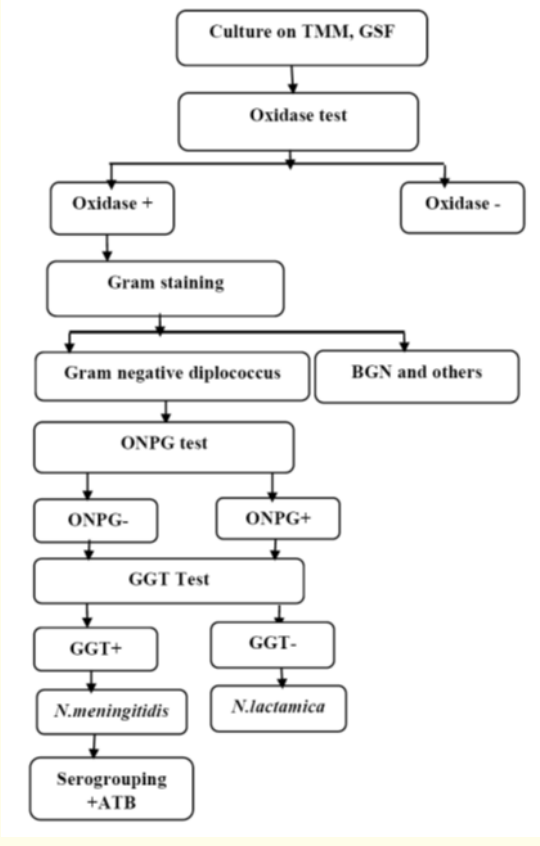

Figure 2: Isolates identification procedure.

ONPG test

we used suspicious *Neisseria* colonies, and those of *N. meningitidis* A and *N. lactamica* reference strains to prepare bacteria suspensions equivalent to MacFarland 4.0 turbidity in hemolysis tubes, each containing 250 µl of 0,7% saline solution for O-Nitrophenyl β-D-galactopyranoside (ONPG) test. One disc of ONPG (ONPG; Rosco Diagnostica, Taastrup, Denmark) was put in each tube, and the whole was incubated at 37°C for 4 hours. After 4 hours of incubation, we did an early first visual reading, and the tubes were reincubated for a final reading after 24 hours of incubation. This test was positive when the bacterial suspension turned yellow evoking *N. lactamica*, and negative when the suspension remained white, evoking *N. meningitidis*.

Test GGT

Other bacterial suspensions equivalent to MacFarland 4.0 turbidity were carried out in hemolysis tubes containing 250µl of 0,7% saline solution with these same suspect colonies and reference strains for GGT (Gamma-glutamyl-beta-naphthylamide) quality control. GGT (Key Scientific - Rosco Diagnostica RO46711, 50 discs/tube) was added to each tube and incubated at 37°C for 4 hours. At the end of this first incubation, three aminopeptidase drops (Key Scientific - Rosco Diagnostica RO92231 20ml/bottle) were added to each tube for 5 minutes GGT revealing. A second incubation was done for a second visual comparative reading after 24 hours. GGT test was positive when bacterial suspension turned bright red, evoking *N. meningitidis* and negative when it remained colorless, it evoked *N. lactamica*.

Antigen agglutination test

Serogrouping was done with specific antisera A, C, W, X, and Y (Thermo Fisher Scientific, Remel Products, Lenexa, KS, USA) on suspected colonies of *N. meningitidis* from carriage study. Likewise, serogrouping was carried out on reference strain *N. meningitidis* A for quality control with the same antisera. Serogrouping was carried out on glass slides, each divided into four chambers. 1% formaldehyde solution and 0.7% saline solution were mixed to use for bacterial suspension on each slide chamber. For each suspect or reference strain, we used two slides for antigen agglutination.

Thus, under a flulaminar hood, a drop of formalized saline solution was put in each chamber of the glass slide. With a 10µl sterile calibrated sower, suspected *Neisseria* colonies or those of *N. men-*

ingitidis A reference strain resulting from a 24hours incubation on GSF was taken and then suspended in the lower part of each slide chamber. Colonies and formalized saline solution drop were homogenized in all the slide chambers by a slow rotating movement with the sterile calibrated sower. Self-agglutination was observed. In case of self-agglutination absence, one drop (10µl) of each specific antiserum was added in each slide chamber except the one reserved for negative control (formalized saline solution). The content of each slide chamber was homogenized for 1-2minutes by using a sterile sower. The agglutination results were obtained by visual observation. Agglutination between the capsular antigen of *N. méningitidis* A reference strain and antiserum A was observed in the chamber which contained this mixture.

For suspected colonies serogrouping:

- The test was said auto agglutinated in case of agglutination between suspected colonies and formalized saline solution;
- The test was positive for a serogroup when agglutinated with one of the specific antisera was observed;
- The test was non-agglutinated in the case of agglutination absence to all of the meningeal antisera;
- The test was said polyagglutinated in the case of agglutination with several specific antisera.

Temperature control

For isolation conditions, the field temperature was recorded. The acceptance limits of field temperature set for the study in the sampling site was between +25-+37°C. If field temperature in the transport box (T>+37°C) it could be cooled with ice-pack but not beyond +25°C. The incubator temperature was recorded each morning on a temperature log sheet. In general, the acceptable tolerance of incubator temperature variation limit is + 35 ± 2°C. Likewise, for reagents and culture media of carriage study storage conditions in refrigerators (IGMIS, BEKO, and Electrolux) were recorded. The tolerance limit generally suggested for controlled refrigerators temperature is + 5°C ± 3°C. Then, the acceptance limits of reagents and media storage set for the study was +4°C. Freezer temperatures were recorded in the same way. The acceptance limits of Strains storage set for the study was -80°C.

Neisseria strains storage

All agglutinated colonies to one or more antisera, all non-agglutinated colonies, all ONPG +/GGT + and ONPG-/GGT- colonies and 10% of ONPG +/GGT- colonies were collected in cryotubes. Each containing 0.5ml of Greaves medium was labeled with corresponding sample numbers and stored in a freezer at -80 ° C. Greaves medium is composed of 5% bovine serum albumin and 5% monosodium glutamate 10% glycerol (produced at NIPH, Oslo, Norway). For storage quality control, aliquots of *N. meningitidis* and *N. lactamica* reference strains were kept in the freezer under the same storage conditions as the previous ones. 10% of swabs were also placed in cryotubes containing 0.5ml of Greaves' medium. A freezer temperature reading was taken every morning to check the storage quality of the isolated strains.

Antibiotic discs quality control

Antibiotic discs quality control was carried out when clinical and carriage strains antibiogram was performed. Bacterial suspensions with young colonies from 24 hours incubation of groupable, non-groupable, reference, and clinical *Neisseria meningitidis* were carried out in tubes, each containing 3 ml of 0,7% saline solution. Fresh blood agar sowing by using a sterile swab was performed with each bacterial suspension. The modified Kirby Bauer method [16] was applied for antibiotic discs diffusion on blood agar.

Results

Evaluation of culture media

Table 1 shows that during the four campaigns, no TMM agar was defective or contaminated. On the other hand, 35 defective and 199 contaminated blood agar were identified and removed in the first campaign against 45 defective GSF and one contaminated in the second campaign. Likewise, one defective was noticed and removed in the third campaign against 5 defective and 1 contaminated GSF in the fourth campaign. The average media loss rate was 4,05 % for blood agar against 0% for TMM.

Media		C 1	C 2	C 3	C 4	Totals
	Quantity	1815	2074	1896	1820	7605
Modified Thayer-Martin agar (TMM)	Contamination	0	0	0	0	0
	Défective	0	0	0	0	0
	Loss rate (%)	0	0	0	0	0%
	Quantity	1719	2104	1579	1715	7117
	Contamination	199	1	0	1	201
Fresh Blood Agar (GSF)	Défective	35	45	1	5	86
	Loss rate (%)	13,61	2 ,19	0,06	0,35	4,05 %

Table 1: Evaluation of TMM and GSF media.

C : Campaign.

Field conditions quality control

Ground temperature

Table 2 shows temperature variation during the sample collection in the field according to campaigns. First campaign maximum temperature varied between + 32- + 37°C and minimum between + 26- + 32°C while second campaign maximum temperature varied between + 29- + 35°C and minimum + 22- + 28°C. As far as third campaign maximum temperature varied between 31-36°C and minimum + 26- + 33°C while fourth campaign maximum temperature varied between + 27- + 35°C and minimum + 22- + 29°C.

Jours	Campaign 1		Campaign 2		Campaign 3		Campaign 4	
	TM	Tm	TM	Tm	TM	Tm	TM	Tm
J1	37	29	29	22	32	30	30	28
J2	35	29	30	22	32	31	31	28
J3	37	30	30	22	36	33	22	32
J4	36	32	29	22	37	33	27	26
J5	35	30	33	26	36	32	28	27
J6	36	30	34	27	35	33	35	26
J7	35	30	31	26	35	30	34	26
J8	34	30	31	26	33	31	33	26
J9	33	30	31	25	34	29	32	25
J10	34	26	31	28	33	29	34	27
J11	32	27	34	24	34	26	35	25
J12	32	28	35	23	33	31	34	24
J13	34	30	32	28	34	31	34	26
J14	32	27	30	27	33	31	34	26
J15	32	27	34	28	32	31	32	26
J16	32	28	33	28	31	30	32	28

Table 2: Temperature control on the sampling site

TM: Maximum Temperature; Tm: Minimum Temperature, C: Campaign.

Sample collection duration

According to figure 3, sampling time control showed that sampling time varied by campaign respectively from 77-1731minutes,74-232 min,74-195minutes, and 75-210minutes at first, second, third and fourth campaigns. The transport time varied respectively between 26-118minutes, 21-187minutes, 27-121minutes, and 25-137minutes at first, second, third, and fourth campaigns. Transmission delay was between the first sample taking time in the field until the incubation of all TMM agars sown to the laboratory. This delay varied respectively from 145-264 minutes,120-264 minutes, 126-288 minutes and 120-301 minutes at first, second, third and fourth campaigns.

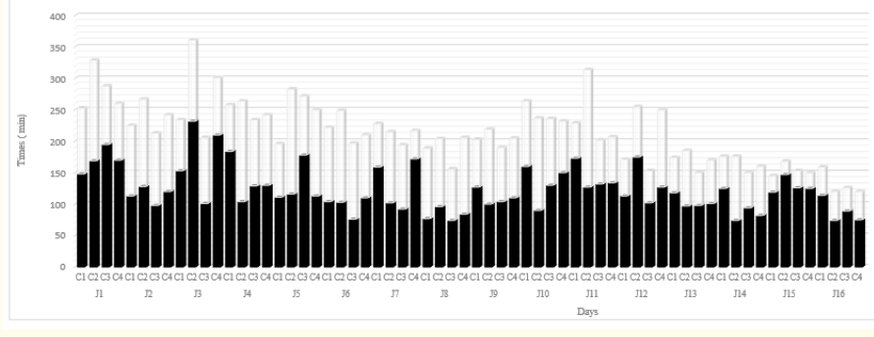

Figure 3: Time control on the sampling site.

TP: Sampling time; Tr: Transfer time; D: Time for transmission of samples to the laboratory = TP + T.

Incubation quality control

According to table 3, isolation conditions control showed that the Incubator temperature was between + 36.3- + 36.7°C (table 3).

Media quality control

According to table 4, the media sterility control was positive by the absence of any colony on TMM and GSF media during the four carriage studies. *Proteus mirabilis* and *Staphylococcus aureus* sowed on TMM agar did not show any growth colonies for selectivity control. On the other hand, *N. meningitidis* A and *N. lactamica* grew well on TMM and GSF media for growth control (table 4).

Reagent quality control

According to table 5, Reagents quality control showed that the oxidase test was positive (+), and the Gram staining showed Gram-negative diplococci (DGN) evoking the *Neisseria* genus. Differential tests: ONPG negative (-), GGT positive (+) evoked *N. meningitidis* while *N. lactamica* was ONPG positive (+), GGT negative. According to serogrouping, antisera used quality control showed suitable identification with *N. meningitidis* A by a slide agglutination between antiserum A and capsular antigen A. The other antisera C, X, W, and Y agglutination were negative because of capsular antigen A.

Jours	Temperature C1	Temperature C2	Temperature C3	Temperature C4
J1	36,5	36,5	36,5	36,5
J2	36,5	36,5	36,5	36,5
J3	36,5	36,5	36,5	36,5
J4	36,5	36,5	36,5	36,5
J5	36,4	36,6	36,6	36,4
J6	36,5	36,5	36,5	36,5
J7	36,5	36,5	36,4	36,5
J8	36,5	36,5	36,3	36,5
J9	36,5	36,5	36,5	36,5
J10	36,7	36,5	36,5	36,5
J11	36,5	36,5	36,4	36,5
J12	36,5	36,5	36,5	36,5
J13	36,5	36,5	36,5	36,6
J14	36,5	36,5	36,5	36,5
J15	36,5	36,5	36,5	36,5
J16	36,5	36,5	36,5	36,6
J17	36,5	36,6	36,5	36,5
J18	36,5	36,5	36,5	36,5
J19	36,5	36,6	36,5	36,5
J20	36,5	36,7	36,6	36,5
J21	36,5	36,6	36,5	36,5
J22	36,5	36,6	36,5	36,5
J23	36,5	36,6	36,5	36,5
J24	36,5	36,5	36,5	36,5
J25	36,5	36,5	36,5	36,6
J26	36,5	36,6	36,5	36,5
J27	36,5	36,6	36,4	36,5
J28	36,5	36,5	36,5	36,5
J29	36,5	36,5	36,5	36,5
J30	36,5	36,5	36,5	36,5
J31	36,5	36,5	36,5	36,5

Table 3: Incubation quality control.

C1: Campaign 1 from May-June 2016, C2: Campaign 2 from October-November 2016, C3: Campaign 3 from April-May 2017, C4: Campaign 4 from October-November 2017.

Media and culture	C 1	C 2	C 3	C 4	Totals
Batches Number of culture media					
Modified Thayer-Martin Agar (TMM)	6	9	7	6	28
Fresh Blood Agar (GSF)	15	13	14	14	56
24h-48h sterility control					
Modified Thayer-Martin Agar (TMM)	0	0	0	0	0
Fresh Blood Agar (GSF)	0	0	0	0	0
Contrôle de pousse 24h-48h					
On Modified Thayer-Martin Agar (TMM)					
Neisseria meningitidis A	6	9	7	6	28
Neisseria lactamica	6	9	7	6	28
On Fresh Blood Agar (GSF)					
Neisseria meningitidis A	15	13	14	14	56
Neisseria lactamica	15	13	14	14	56
24-48h selectivity control on TMM					
Proteus mirabilis	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0

Table 4: Quality control of culture media.

C: Campaign.

Reference strains	*N. meningitidis* A	*N. lactamica*
Morphological identification	DGN	DGN
Biochemical identification		
Oxidase	Positive	Positive
ONPG test incubation 5min -24 hours	Negative	Positive
GGT test incubation 4-24 hours	Positive	Negative
Serogrouping		
Antiserum A	Positive	
Antiserum C	Negative	
Antiserum W	Negative	
Antiserum X	Negative	
Antiserum Y	Negative	

Table 5: Reagents quality control.

DGN : Gram negative diplococcus.

Reagents and media storage control

Table 6 showed that reagents and media average storage temperature in the three refrigerators used was between + 3.22- + 7.38°C.

Jours	T.av F 1	T.av F 2	T.av F 3
J1	5,875	6	4,75
J2	5,5	5,375	5,875
J3	5,175	5,5	5,125
J4	5,75	5,25	5,375
J5	4,6	5,75	5,775
J6	5,325	6	5,125
J7	5,225	5,375	5,125
J8	5	5,25	3,875
J9	5,25	5,75	4,375
J10	5,875	5,875	5
J11	5	6,125	5,675
J12	5,125	5,875	4,675
J13	4,55	6,25	6
J14	5,925	5,75	5,75
J15	5,925	6,5	5,625
J16	5,45	6,875	5,325
J17	6,4	7	5,425
J18	4,675	5,75	5,575
J19	6,05	5,5	5,375
J20	4,8	4,625	5,7
J21	5,7	6,3	6,05
J22	7,375	6,25	5,1
J23	6,25	5,175	3,225
J24	5,075	6	4,95
J25	6,3	6,05	4,05
J26	4,025	6	4,65
J27	5,05	5,25	5,775
J28	5,975	6,475	5,95
J29	4,425	6,25	5,25
J30	5,575	6,05	5,925
J31	5,775	6,375	5,625

Table 6: Conservation control of reagents and culture media.

T.av F: Average refrigerator temperature.

Strains storage quality control

According to table 7, strains storage temperature control in the freezer was between -78--66°C during the four campaigns.

Days	Temperature °C			
	Campaign 1	Campaign 2	Campaign 3	Campaign 4
J1	-66	-70	-70	-74
J2	-71	-74	-74	-75
J3	-72	-73	-74	-75
J4	-75	-75	-73	-75
J5	-75	-70	-70	-73
J6	-75	-75	-73	-75
J7	-73	-75	-75	-75
J8	-73	-75	-73	-75
J9	-72	-74	-73	-75
J10	-73	-75	-75	-75
J11	-75	-75	-70	-75
J12	-74	-70	-70	-75
J13	-73	-75	-73	-72
J14	-72	-74	-75	-74
J15	-73	-73	-75	-75
J16	-73	-73	-71	-74
J17	-75	-73	-73	-74
J18	-74	-75	-73	-74
J19	-75	-70	-73	-75
J20	-75	-73	-74	-74
J21	-75	-73	-70	-75
J22	-73	-75	-72	-74
J23	-75	-70	-75	-74
J24	-73	-73	-75	-74
J25	-72	-76	-78	-74
J26	-75	-74	-78	-74
J27	-74	-76	-78	-74
J28	-75	-75	-78	-75
J29	-75	-75	-78	-75
J30	-76	-71	-76	-76
J31	-74	-76	-75	-76

Table 7: Bacteria conservation control.

Antibiotic discs quality control

Table 8 showed that chloramphenicol and oxacillin diffusion diameters were between 32-36 and 22-25 millimeters.

Discussion

An evaluation of culture media was carried out during the four carriage campaigns. At the end of the *N. menigitidis* A carriage study, no defective and no contaminated TMM agar were not recorded. The lack of contamination was due to antibiotics and antifungals in TMM agar that inhibited other germs growth except *Neisseria* [17]. On the other hand, 86 defective, and 201 contaminated Fresh blood agars (GSF) were recorded and eliminated during the four campaigns. Therefore, the average loss rate was 4,05% for fresh blood agar against 0% for TMM. GSF contamination was due to the media's long storage and transport time before transferring to the Kaya laboratory.

Field temperature monitoring during TMM agar sowing showed a maximum average temperature between + 30- + 36°C, a minimum average between + 24- + 31°C. The acceptance limits of field temperature set for the study in the sampling site was between +25- +37°C. Kristiansen., *et al*. in 2012 in Burkina Faso, found that the maximum temperature during sample sampling and TMM agars sowing on the field was between + 25- + 38.5°C [15]. This difference of temperature was due to sampling period variations. So, the first campaign's maximum temperature from April to June 2016 was between + 32- + 37°C and the minimum temperature was + 26- + 32°C. Those recorded at the second campaign from November to December 2016 were between + 29- + 35°C for maximum temperature and + 22- + 28°C for minimum temperature. Likewise, at the third campaign, the maximum temperature from April to May 2017 was between + 31- + 36°C and the minimum temperature between + 26- + 33°C. Those recorded at the fourth campaign from October to November 2017 were between + 27- + 35°C for maximum temperature and between + 22- + 29°C for the minimum temperature. Ambient temperature in the field was favorable for starting already oropharyngeal germs growth. Thus, oropharyngeal samples sowed on TMM agars in hermetically sealed jars with a humid atmosphere enriched to CO2 at field temperature constituted necessary conditions for carriage *Neisseria* development.

Antibiotics	Critical diameter (mm)	Inhibition diameter (mm)								
		T1	T2	T3	T4	T5	T6	T7	T8	T9
Oxacilline 5µg	18	22	25	22	24	25	24	22	24	22
Chloramphénicol 30g	30	35	36	34	36	36	34	32	35	32

Table 8: Antibiotics quality control.

According to sampling time control, the sampling time varied respectively from 77-173minutes, 74-232minutes, 74-195minutes, and 75-210minutes at first, second, third and fourth campaign. The transport time changed between 26-118minutes, 21-187minutes, 27-121minutes, and 25-137minutes at the first, second, third, and fourth campaigns. Therefore, sample transmission delay to the kaya laboratory varied from 145-264 minutes, 120-264 minutes, 126-288 minutes, and 120-301 minutes at the first, second, third, and fourth campaigns. Our results were different from those found by Kristiansen., *et al*. in 2012 in Burkina Faso, who showed a delay between 60 to 370 minutes [15]. The sampling time variation was due to the distance of certain villages which were far from sampling sites and the slowness of volunteers recruited. Likewise, the variation of transport time was due to distance difference from certain elected village to Kaya laboratory. Transmission delay had no negative effects, such as media drying. It was lower than the maximum time limit authorized between the sampling and the incubation of all samples in the laboratory, which is set at 360 minutes (6 hours) [19]. According to media quality control, a TMM and GSF agar from different preparation batches subjected to sterility control showed no growth colony during the four carriage campaigns. *Proteus mirabilis* and *Staphylococcus aureus* sowed on TMM agar did not show any growth colony on this medium after 24 hours of incubation for selectivity control. Thought, NmA and *N. lactamica* reference strains sowed on TMM and GSF agar showed good growth control. According to Massicotte in 2003, each batch of media must be controlled, and its nutritional capacity must be verified with controled strains known and accepted in public health laboratories in Quebec [20]. TMM media selectivity was due to inhibitory action of vancomycin on (Gram-positive Cocci), colistin on (Gram-negative bacilli), and nystatin on (yeasts) and trimethoprim on (*Proteus)* [21]. Quality control of reagents showed that *N. meningitidis* A and *N. lactamica* possessed cytochrome C oxidase in their respiratory chain allowing tetramethyl-p-phenylenediamine hydrochloride to oxidize a violet compound on oxidase paper. Gram staining showed DGN evoking the *Neisseria* genus. These two tests constituted a differential diagnosis for *Neisseria* genus detection. *N. meningitidis* A was ONPG negative, GGT positive. At the same time, *N. lactamica* was ONPG positive and GGT negative. GGT is an enzyme, which hydrolyzes gamma-glutamyl-beta-naphthylamine detectable by reactions with an aminopeptidase reagent giving a red color, allowing the proliferation of *N. meningitidis* in cerebrospinal fluid (LCS) [22]. Our results corroborated with Kristiansen., *et al*. in 2014 in Burkina Faso, who found Oxidase positive, ONPG negative, and GGT positive for *N. meningitidis* isolates whose Gram staining evoked Gram-negative diplococci [23]. All these tests allowed to reveal reference strains' biochemical and bacteriological characteristics showed that reagents were in good quality. According to incubator operating quality control, we noted that the incubator temperature varied between + 36.3- + 36.7°C. These results were very close to those of Ryan., *et al*. in 2004 in the United States, who demonstrated that the optimal overnight incubation temperature for meningococcal growth was + 35- + 37°C [24]. In general, the acceptable tolerance of incubator temperature variation limit is + 35 ± 2°C [25,26]. This temperature made it possible to obtain good colonies growth characteristic of *N. meningitidis* A or *N. lactamica* reference strains sowed on TMM agar and GSF. For the three refrigerators operating quality control, the four campaigns' reagents and media average storage temperature were between + 3.22 -+ 7.38°C. Our results were close to the tolerance limit generally suggested for controlled refrigerators temperature + 5°C ± 3°C [25,26]. All of this evoked a good operating quality of incubator equipment.

For freezer operating control, we found that equipment temperature was between -78--66°C. Our results were close to the tolerance limit generally suggested for a controlled freezer temperature, which is -65°C ± 10°C [25,26]. Controlled strains are freezing at -70˚C remains the most widely used method for long-term storage. At -70°C, the chemical changes in bacteria are prolonged. Study strains can be stored indefinitely at this temperature [26]. *N. meningitidis* A and *N. lactamica* reference strains stored at – 70˚C and subcultured on TMM and GSF media had grown well.

That testified a good operating quality of freezer for carriage and references strains storage.

For quality control of antisera used during four campaigns, serogrouping showed good agglutination on a slide chamber where it was monovalent antiserum A and N. meningitidis A capsular antigen. Agglutination with the other specific antisera C, X, W, and Y with the same capsular antigen remained homogeneous and negative. That explains why the particular antisera used for carriage strains identification were in good quality. Antibiotic disc quality control was carried out with *N. meningitidis* A reference strains when performed clinical, and carriage strains antibiogram. Chloramphenicol and oxacillin used for *N. meningitidis* A antibiogram showed respectively an inhibition zone or inhibition diameter between 32-36mm and 22-25mm. These results explained that antibiotic discs used were in good quality. Our results were closed to the sensitivity limit for chloramphenicol 30µg and oxacillin 5µg, which are respectively 30mm and 18mm for *N. meningitidis* [27].

Conclusion

Internal quality control was necessary for *N. meningitidis* carriage study diagnostic. It constituted a means for early warning of inaccuracies diagnostic, poor reagent quality, and equipment failure. This control improved bacterial diagnostic quality and *N. meningitidis* sensitivity profile detection. It allowed obtaining reliable carriage study results at Kaya laboratory in Center-North of Burkina Faso from 2016 to 2017.

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Conflict of Interest

I declare that any financial interest or any conflict of interest do not exists.

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