TECHNICAL REPORT

External quality assurance scheme for *Neisseria meningitidis*

2009

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External quality assurance scheme for *Neisseria meningitidis* 2009

As part of the IBD-Labnet surveillance network

This report was commissioned by the European Centre of Disease Prevention and Control (ECDC), coordinated by Dr Lucia Pastore-Celentano and produced by Dr Steve Gray (Health Protection Agency, Meningococcal Reference Unit, Manchester, UK), on behalf of the IBD-Labnet consortium (referring to Specific Contract ECD.1027).

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Abbreviations

AMP	Agence de Médecine Préventive
BSAC	British Society for Antimicrobial Chemotherapy
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
CTX	Cefotaxime
DG SANCO	Directorate-General of Health and Consumers
DSN	Dedicated surveillance network
ECDC	European Centre for Disease Prevention and Control
EMGM	European Monitoring Group in Meningococci
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU-IBIS Network	European Invasive Bacterial Infections Surveillance
HPA	Health Protection Agency (UK)
I	Intermediate
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence type
MLST CC	Multilocus sequence type and clonal complex
MRU	HPA Meningococcal Reference Unit
PATH project	Program for Appropriate Technology in Health
PEN	Penicillin
PCR	Polymerase chain reaction
QMS	Quality management system
R	Resistant
RIF	Rifampicin
S	Susceptible
ST	Sequence type
SRGA	Swedish Reference Group for Antibiotics
SU	Sulphonamide
TESSy	The European Surveillance System
UA	Unassigned

Executive summary

Neisseria meningitidis is the major worldwide cause of meningitis and rapidly fatal sepsis in healthy individuals. The risk of meningococcal disease is higher among those with complement deficiencies, asplenia and other underlying conditions.

N. meningitidis is the only agent among the major bacterial agents causing meningitis that causes epidemic as well as endemic disease. The meningococcus is carried in the human nasopharynx asymptomatically by 5% to 10% of adults in non-epidemic periods. *N. meningitidis* accounts for morbidity and mortality within the cases and may result in sequelae. In addition, it is responsible for other infections, such as arthritis, osteomyelitis and cellulitis.

Meningococci are usually assorted according to serologic typing systems based on structural differences of capsule (serogroup), major outer membrane porin proteins (serotype), other outer membrane proteins (serosubtype) and lipooligosaccharide (immunotype).

Meningococcal disease surveillance is paramount and aims at different targets: early detection of cases to activate public health response (namely identification of close contacts and administration of chemoprophylaxis to prevent secondary cases of the disease, to evaluate trends and to act in outbreaks), surveillance with vaccination purposes and the estimation of the burden of meningococcal disease. Meningococcal surveillance systems are partially based on laboratory diagnoses, therefore, there is a need for accuracy and proficiency in surveillance laboratory performance.

ECDC promotes the performance of External Quality Assurance (EQA) schemes, in which laboratories are sent simulated clinical specimens or bacterial isolates for testing by routine and/or reference laboratory methods. EQA schemes or proficiency laboratory testing provides information about the accuracy of different characterisation and typing methods as well as antimicrobial susceptibility testing, and the sensitivity of the methods in place to detect a certain pathogen or novel resistance patterns. This means that quality assurance enables a laboratory performance to be assessed in comparison to reference methods and to other peer laboratories.

In March 2009, a collection of six viable isolates of *N. meningitidis* of the major disease-causing serogroups (A, B, C, Y and W-135) together with six simulated blood (non-culture) samples for molecular studies, was sent by UK-NEQAS to 27 participating Reference Laboratories (Annex 1) in the IBD-Labnet surveillance network for quality assurance testing. The laboratories were asked to perform phenotypic characterisation of viable isolates: serogroup, serotype, serosubtype and antimicrobial susceptibility testing (MIC results) on the viable isolates, and molecular characterisation both of the viable isolates and non-culture simulated septicaemia samples.

Overall, the EQA performance has shown that European Meningococcus Reference Laboratories differ in the level of characterisation of the strains. The phenotypic characterisation of viable isolates was quite successful with reports for serogroup received from all 27 participating laboratories for each sample.

However, the phenotypic serotyping and serosubtyping reports demonstrated limited discrimination due mainly to the limited resources or reactivity of the reagents. The EQA exercise pointed out that this is an area in which further work needs to be done.

The antimicrobial susceptibility testing exercise and determination of the minimum inhibitory concentration (MIC) pointed out that there are two major areas for consideration: the designated and reported MIC of the antimicrobial and the interpretation of susceptibility or resistance. The European Monitoring Group on Meningococci (EMGM) has recommended the utility of gradient diffusion methodology (such as by Etest) and a standardised agar plate medium (Mueller Hinton plus blood), but it seems that a number of laboratories may be using different methodologies, making comparisons more difficult. The range of MIC values and calculated modes suggest that laboratories are not all using Etest strips.

It is also apparent that the laboratories used a number of different guidelines to interpret the MICs as susceptible, intermediate or resistant. From the epidemiological point of view, it would be advisable to collect MIC values and then interpret them according to only one guideline for consistency. The MIC EQA reports suggest that CLSI currently predominates but a standardised methodology and the use of EUCAST breakpoints would be an appropriate future aim.

The MLST analysis of non-culture samples revealed more problems than the viable isolates MLST.

In conclusion, the results of the EQA exercise proved that the establishment of a regular EQA scheme for the reference laboratories is required in order to maintain the movement towards improved quality of epidemiological reports. It was also concluded that training might be requested to assist the laboratories setting up different techniques according to their particular needs.

Introduction

The European Centre for Disease Prevention and Control (ECDC) is a European Union (EU) agency with a mandate to operate dedicated surveillance networks (DSNs) and to identify, assess, and communicate current and emerging threats to human health from communicable diseases. Within its mission, ECDC shall 'foster the development of sufficient capacity within the Community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes.' (Article 5.3, EC 851/2004[1]).

External quality assurance (EQA) is part of quality management systems (QMS) and evaluates performance of laboratories by an outside agency on material that is supplied specially for the purpose. ECDC's disease specific networks organise a series of EQA for EU/EEA countries. In some specific networks, non-EU/EEA countries are also involved in the EQA activities organised by ECDC. The aim of the EQA is to identify needs of improvement in laboratory diagnostic capacities relevant to surveillance of disease listed in Decision No 2119/98/EC and to ensure comparability of results in laboratories from all EU/EEA countries. The main purposes of external quality assurance schemes include:

- assessment of the general standard of performance ('state of the art');
- assessment of the effects of analytical procedures (method principle, instruments, reagents, calibration);
- evaluation of individual laboratory performance;
- identification and justification of problem areas;
- provision of continuing education; and
- identification of needs for training activities.

N. meningitidis is a selective commensal and pathogen of humans. The meningococcus is carried in the human nasopharynx asymptomatically by 5% to 10% of adults. Nasopharyngeal colonisation is an important immunising process that may protect against future illness. Meningococci are transmitted directly by contact with nasal or oral secretions or through inhalation of large droplets. The meningococcal disease has a major impact among children: in this group the attack rate and case-fatality ratio can be 20 times that of the adult population.

In outbreaks it affects mostly older children, adolescents and adults. The epidemiology of the disease varies in different countries. In general, there is a pattern of certain endemicity interspersed with unpredictable outbreaks.

The meningococcal outer membrane containing *pili* and other proteins, a lipopoly(oligo)saccharide, phospholipids, and a capsular polysaccharide, is a major contributor to the virulence of *N. meningitidis*.

The development of the serological typing of meningococci and the immunological characterisation of the highly diverse subcapsular antigens is the basis of the serogrouping and classification of meningococci. Of the 13 recognised serogroups, only five (serogroups A, B, C, Y, and W-135) are associated with disease. The geographical distribution of the serogroups shows that serogroup A strains cause most epidemics in the so called 'meningitis belt' (the Sahel region of the sub-Saharan Africa) and Asia, but more localised epidemics of serogroup C also occur. In the Americas, Europe and Australasia, the disease follows a seasonal pattern at lower rates, being C and especially B the most common serogroups. Serogroup Y infections have emerged as a significant cause of morbidity in the USA in recent years.

Molecular techniques enable the comparison of genetically and pathogenically distinct meningococci. The use of these sophisticated techniques will provide an increase in the understanding of the epidemiology of meningococcal disease.

The European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) has undertaken successfully the surveillance of invasive diseases caused by *Neisseria meningitidis* and *Haemophilus influenzae*. EU-IBIS has been coordinated by the Health Protection Agency (HPA) in London since 1999 and funded by the European Commission (DG SANCO) until September 2006. Since October 2006, the network was funded by ECDC until October 2007 when the epidemiological and laboratory surveillance was integrated into ECDC.

The network has worked in close collaboration with the European Monitoring Group on Meningococci (EMGM) to integrate epidemiological and molecular components of meningococcal disease in Europe.

The implementation of laboratory surveillance methods has been outsourced to a consortium of experts that constitute the IBD-Labnet. The IBD-Labnet consortium has achieved consensus for the laboratory methods and variables to be used for the characterisation and discrimination of circulating meningococcal strains.

[1] Regulation (EC) no 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for Disease Prevention and Control

The consortium concluded that the laboratory surveillance should rely only on molecular and sequence-based typing data. Molecular typing schemes have proved superiority when compared to serological typing.

Based on previous published recommendations of the European Monitoring Group on Meningococci, the IBD-Labnet consortium agreed on a molecular typing scheme for *N. meningitidis*:

Serogroup:PorA(vr1):PorA(vr2):FetA(vr1):clonal complex (MLST)

This scheme provides highest resolution with lowest sequencing efforts and costs and hence, it was recommended as the laboratory variables to be included in the TESSy database. Consensus was also achieved on antimicrobial susceptibility testing. The MICs for RIF, PEN, CIP and CTX will be the laboratories variables for meningococci.

Some countries might no be able to provide the molecular typing data due to economical reasons. To support the Member States, ECDC promoted the performance of this EQA exercise to ensure high quality results to be reported as part of the laboratory surveillance and to assess the training needs for capacity building. In addition, some countries processing a large number of samples, offered their help to those countries that are not able to implement the molecular typing methods yet.

1 Material and methods

The objectives of this exercise were:

- to design an EQA scheme utilising a small panel of material comprising viable *Neisseria meningitidis* isolates and non-viable simulated clinical samples for genotypic and phenotypic characterisation (where possible) to all EU Member States and candidate countries with suitable reference facilities; and
- to improve quality of data, assisting in the standardisation of techniques and thereby facilitating consistent epidemiological data for submission to the ECDC TESSy database.

1.1 Study design

The design of the project allowed individual reference laboratories to test the material using their routinely available techniques in order to complete some or all of the following criteria in the allocated time period. The reference laboratories were able then to compare their own submitted results to the consensus results (of all the testing laboratories) to determine differences, if any.

An anonymised summary was produced showing the submitted results, the consensus by interpretation and the number of laboratories with each submitted result.

The EQA distribution utilised the availability of the large collection of *N. meningitidis* isolates, molecular facilities and expert knowledge at the Health Protection Agency's Meningococcal Reference Unit (MRU, Manchester, UK), with the expert knowledge of Dr Vivienne James (UK NEQAS for Microbiology), and facilities in the external Quality Assurance Department (eQAD) Centre for Infections, Colindale, London. UK NEQAS for Microbiology undertake several international EQA schemes for other organisms that also require freeze-drying, distribution, results analysis and web-based reporting.

The characterisations (test results) requested of the participating reference laboratories are described in Table 1.

Table 1 Tests requested to the participating laboratories

Procedure	Isolates	Non-culture (simulated septicaemia)	Technique name
Phenotype	serogroup, serotype, serosubtype	-	
MIC	PEN, CTX, CRO, RIF, CIP, SU	-	
Genotype		Species DNA detection	
		Serogroup	
	MLST, MLST CC	MLST, MLST CC	Multilocus sequence type and clonal complex
	porA VR1,VR2,VR3	porA VR1,VR2,VR3	porA sequence typing variable regions
	FetA	FetA	FetA iron-binding protein variable region
	porB*	porB	porB sequence typing
	penA*	penA	Penicillin-binding protein sequence variants

** porB and penA sequence-based typing were included to the primary dataset for added value and to extend the characterisation of the distributed material. It was anticipated that only a few laboratories would consider testing and reporting the sequence-based porB and penA designations.*

Laboratories were able to report via the web (using their unique identifiers) into a specifically designed report on the UK NEQAS website.

The report also allowed for the collection of additional supportive information relating to the gene (molecular) targets used for detection and serogroup designation. Including the option for reporting of the techniques used for nucleic acid extraction, amplification and detection allowed for a simple (but anonymous) survey of the facilities available within the European laboratories. In addition, methodological information may help to assess how a technique(s) is performing alongside others in different laboratories.

1.2 Participants

The list of the participating reference laboratories can be found in Annex 1.

All participants were contacted prior to the EQA distribution to confirm the address and contact details for despatch of the potentially hazardous material. At the same time, the HPA business and legal department required the agreement of participants to the terms and conditions of the ECDC EQA distribution (Annexes 2 and 3). In essence, it confirmed the recipient's details and their responsibility for safe handling of the material. Also included were clauses relating to the retention and further use of the material with specific restrictions upon third-party distribution and the necessity for review of any publications relating to the EQA material.

It was envisaged that the reference laboratories would wish to store the viable cultures and retain any unused material for their own quality processes. It was hoped that the distribution of the well-characterised material would become a resource within and between the reference laboratories.

1.3 Timelines

Table 2 Timelines for the EQA exercise

Event	Dates
Selection of EQA strains (inc. MIC)	September–October 2008
Assessment of material (inc. non-cult)	November 2008–January 2009
Building participants list	January 2009
Frozen transport of material to eQAD UK NEQAS	February 2009
Freeze-dry panel (eQAD UK NEQAS)	February–March 2009
Pre-despatch checks (MRU)	March 2009
Confirmation of terms and conditions document	January–March 2009
Distribution and collation of replies to terms and conditions	March 2009
Interim progress presentation ECDC Stockholm	March 2009
EQA panel despatch UK NEQAS EQA distribution No. 2452	30 March 2009
Reference lab testing	April—May 2009
Final return of results	11 May 2009
Analysis and collation of consensus results	May–June 2009
Producing reports	May—June 2009
Consensus summary released	14 May 2009
Individual results (compared to all reports) released on UKNEQAS website https://results.ukneqas.org.uk	16 June 2009
IBD-Labnet EQA workshop	17 June 2009, Manchester, UK
Email reminder to check website for individual lab results	22 June 2009

1.4 The EQA panel material

Isolates

The EQA panel consisted of six viable isolates of *N. meningitidis* that were selected to be representative of the major disease-causing serogroups (A, B, C, Y and W-135) and to demonstrate significant MICs to commonly used antimicrobials (UK NEQAS EQA distribution No. 2452, reference numbers 9199–9204). Five of the six isolates for MIC testing were kindly supplied by Dr Muhammed-Kehir Taha, from the Institute Pasteur, Paris (France), where expert MIC determination and sequence-based characterisations are performed. The isolates also exhibited a diversity of genotypic and phenotypic characterisations.

The sixth isolate, serogroup Y, was included as representative of similar organisms (MLST ST-23, clonal complex ST-23) that have caused significant disease in the USA. The isolate's reaction with serotype 2c monoclonal antibody would assess the availability of this potentially useful reagent within Europe and in comparison to the molecular characterisations.

Non-culture simulated septicaemia samples

The six simulated blood (non-culture) samples for PCR (or other molecular studies) – UK NEQAS EQA distribution No. 2452 – were prepared from heat-treated meningococcal case isolates. One sample was designated as a

negative control (9207), it contained no meningococcal or any other target DNA. All five positive *N. meningitidis* samples used isolates initially obtained from cases of invasive meningococcal disease. Four of the samples utilised isolates from UK cases of serogroup A (9206), B (9205 and 9210) and C (9209).

The fifth sample (9208), a serogroup X isolate (from an African meningitis case), was kindly provided by Agence de Médecine Préventive (AMP) for inclusion in the EQA panel. Serogroup X meningococci have the potential to be detected in European cases as there have been reports of outbreaks or clusters of serogroup X in African countries. Serogroup X cases are very unusual in the UK. Although a few other serogroup X isolates are available in the HPA MRU archive they are not of the specific multilocus sequence type and clonal complex (MLST CC).

The serogroup X sample was included in the panel for two other reasons. Firstly, the requirement for laboratories to test the sample with their complete panel of molecular assays once it was confirmed positive for meningococcal DNA. Probably then attempting PCR assays for serogroups B, C, Y, W-135 and A before considering serogroup X or 29E (if the assays were available). Secondly, including an African serogroup X in the EQA panel makes the ECDC EQA panel useful to colleagues involved in the molecular surveillance of meningococcal disease cases in Africa pre- and post- the introduction of serogroup A conjugate vaccine (PATH project).

One serogroup B sample (9205) was prepared from an isolate that was not detectable using the initially published *ctrA* primer set. A small subset of CC ST-269 meningococci were found to require modified primers that have since been used at the HPA MRU. To address sensitivity issues found during the previous EU-IBIS-sponsored EQA distributions, sample 9210 (serogroup B) was diluted considerably more than the other samples, although at a dilution that was consistently detectable using ABI Taqman™ assays at the HPA MRU.

Preparation of the simulated septicaemia (non-culture) samples for molecular investigation

In order to provide sufficient standardised material and not to incur ethical or blood safety issues, it was decided not to use actual human clinical (blood) samples. Safety considerations necessitated the use of heat-treated suspensions of meningococci in a protein matrix or diluent – ideally, one that was suitable for freeze-drying and acceptable for import into all States. For that reason horse (equine) blood rather than bovine was used.

During the extensive assessment of suitable positive dilutions, it was noticed that the horse blood would, on occasion, be lysed and that one of the locally used semi-automated nucleic acid extraction instruments (based on capture column technology) yielded poor or inconsistent results. To overcome this problem, 'fresh' defibrinated horse blood was used and the more reproducible diluent (horse serum) was used as the diluent for the serogroup X (9208), serogroup C (9209) and serogroup B (9210) samples.

On receipt of the freeze-dried samples, it was necessary for the laboratories to re-constitute the material with 1mL of sterile water inside a microbiological safety cabinet before commencing the local nucleic acid extraction procedure(s). It should be noted, that the heat-treated suspensions of meningococci were not checked to ensure non-viability, although the heating process and equipment used had previously been validated to kill meningococci.

Summary of the processes involved in sample preparation:

- standardised saline suspensions of live meningococci (using a spectrophotometer) were diluted in a microbiological safety cabinet;
- estimation of viable cell count by Miles & Misera;
- ~107–108 viable orgs/mL = Stock;
- suspension was heat-killed (100°C for 5 mins) = Stock suspension;
- dilutions of stock suspension in sterile defibrinated horse blood or serum;
- use ABI Taqman™ assays to assess suitable dilutions for EQA panel simulating typical clinical samples;
- ~103–105 viable orgs/mL;
- frozen stock suspension was transported to UK NEQAS for MRU specified dilution in defibrinated horse blood or serum, freeze-drying and international distribution.

2 Results

UK NEQAS released two reports to each laboratory. The final summary report (Annex 5) is comprehensive, indicating the individual laboratory's results compared to all other submitted results and took more time to produce than the interim report. Thus, it was only available to participants via the UKNQAS website (https://results.ukneqas.org.uk) from 16 June 2009.

An email reminder was distributed to all the reference laboratories on 22 June 2009 drawing attention to the UK NEQAS website and accessibility to the final report using their unique code.

2.1 Part 1: Phenotypic characterisation of viable isolates

The phenotypic characterisation of the six viable isolates (Nos. 9199–9204) was generally quite successful with reports for serogroup received from 27 laboratories for each sample.

The consensus results are shown as Table 3, compiled from the interim report and final summary report (Annexes 4 and 5).

Table 3 Isolate phenotypic characterisations

EQA number	Serogroup	Serotype	Serosubtype		
			VR1	VR2	VR3
9199	W135	NT	P1.5	NT	NT
9200	Y	NT (2c)*	P1.5	P1.2	NT
9201	B	14	P1.7	P1.16	NT
9202	C	2a	P1.5	NT	NT
9203	B	1	NT	NT	P1.6
9204	A	21	NT	P1.9	NT

**9200 serotype = 2c+ if reagent available;*
NT = Not typable/serosubtypable.

Serogroup

Sample 9199, serogroup W135, was successfully determined, but it appears that one laboratory does not have access to the specific reagent but the pooled Y/W135. The reagent availability problem was also noted for sample 9200, the serogroup Y isolate.

Sample 9200 was reported as non-groupable by two laboratories. Sample 9201, serogroup B was confirmed by 23 (of 27) laboratories, but there were three non-groupable and one serogroup Y reports. Samples 9202 serogroup C, 9203 Serogroup B and 9204 Serogroup A were designated correctly by all 27 laboratories.

Serotype

Out of 27 laboratories reporting serogroup, 15 laboratories reported serotyping, often as not typable. Where reagents and testing were available, the consensus was achieved. However, there were different notations used to describe the same result, e.g. sample 9202 was reported as serotype 2a or P2.2a, both of which are correct.

Similarly, for sample 9203, the serotype was reported as 1 or P3.1. Sample 9204, serogroup A probably reacted with both serotype 4 and serotype 21 antibodies, but caused problems with standardised notation and reporting dual reactions. Thus, the serotype 4 result may be a genuine cross-reaction. Sample 9200, serogroup Y, demonstrated the restricted availability of the serotype 2c monoclonal antibody. Serotype 2c could be a useful marker of serogroup Y meningococci associated with significant disease in the USA.

Serosubtype

The serosubtype reporting shows that 12 or 13 laboratories can confirm phenotypic serosubtype designations, but that the EQA panel demonstrates that over half of the laboratories do not have access to or are unable to serosubtype phenotypically. The notation problem as seen for serotype was not observed for serosubtype, possibly due to the separate reporting of VR1, VR2 and VR3. P1.xx was used consistently. However it was apparent that one laboratory had access to VR3 specific reagents for P1.35, P1.36 and P1.37.

Table 4 Results of phenotypic characterisations

EQA number	Identification			Ratio reporting consensus (%)			Non-consensus results reported		
Serogroup									
9199	W135			26/27(96%)			Y/W135		
9200	Y			24/27(89%)			Not groupable, Y/W135		
9201	B			23/27(85%)			Not groupable/Y		
9202	C			27/27(100%)					
9203	B			25/27(93%)			Not groupable		
9204	A			27/27(100%)					
Serotype									
9199	NT			8/15(53%)			15,P3.15,1.15		
9200	NT (2c)*			10/15(67%)			2c,21,4		
9201	14			9/15(60%)			P3.14, Not typable/serosubtypable		
9202	2a			13/15(87%)			P2.2a		
9203	1			13/15(87%)			P3.1		
9204	21			6/15(40%)			4,P3.4,21; 4,21; Not typable/serosubtypable		
Serosubtype									
	VR1	**VR2**	**VR3**	**VR1**	**VR2**	**VR3**	**VR1**	**VR2**	**VR3**
9199	P1.5	NT	NT	13/13(100%)	9/10(90%)	9/10(90%)	-	P1.10	P1.37
9200	P1.5	P1.2	NT	12/13(92%)	12/12(100%)	9/10(90%)	P1.2,10	-	P1.36
9201	P1.7	P1.16	NT	12/13(92%)	11/11(100%)	9/10(90%)	P1.7,16	-	P1.35
9202	P1.5	NT	NT	13/13(100%)	8/10(80%)	9/10(90%)	-	P1.2	P1.36
9203	NT	NT	P1.6	9/11(82%)	9/11(82%)	10/12(83%)	P1.6,P1.18	P1.16,P1.25	NT,P1.38
9204	NT	P1.9	NT	9/11(82%)	11/12(92%)	9/10(90%)	P1.9,P1.20	NT	P1.35

**9200 serotype: 2c+ if reagent available;*
NT= Not typeable/serosubtypeable

Table 5 Overview of the number of participating laboratories per phenotypic method

Method	Participants
Serogroup	27
Serotype	15
Serosubtype	
– VR1	13[a]
– VR2	12[b]
– VR3	10[c]

[a] *Only 11 participants for EQA numbers 9203, 9204.*
[b] *Only 10 participants for EQA numbers 9199, 9202; 11 participants for EQA numbers 9201, 9203.*
[c] *12 participants for EQA number 9203.*

2.2 Part 2: MIC results

The MIC (minimum inhibitory concentration) results were summarised within the interim report only whether the consensus categorised the results as susceptible, intermediate or resistant to the five antimicrobials.

The final summary report presents the interpreted categorised results comparing the consensus to the individual laboratory's report, but also showing the number and percentage of correct reports. From the final summary report it may be observed that 26 of the 27 laboratories are able to carry out and report a penicillin MIC but only a maximum of 24 test for ciprofloxacin, 22 for rifampicin, 16 for cefotaxime, 16 for sulphonamide and only 9 for ceftriaxone.

There are two main areas for consideration: the designated and reported MIC of the antimicrobial and the interpretation of susceptibility or resistance. The EMGM has recommended the utility of gradient diffusion methodology (such as by Etest) and a standardised agar plate medium (Mueller Hinton plus blood), but it appears that a number of laboratories may be using different methodologies making comparisons more difficult. The range

of MIC values and calculated modes suggest that not all laboratories are using Etest strips. Also noted in the comments section of the final summary report was the removal of operators (< or >) implying breakpoint plates or results at the limits of the gradient diffusion (Etest) strips. This may also be evidence of the variety of techniques used.

The final summary report does not state the specific laboratory's reported MICs compared to the mode, but that may readily be done by the laboratory themselves. What is apparent is that a number of guidelines were used to interpret the MICs as susceptible, intermediate or resistant. Nineteen of the 27 laboratories used the CLSI *N. meningitidis* MIC interpretation guidelines, with BSAC, EUCAST and SRGA used each by one laboratory; another used a combination. Interestingly, four laboratories used other interpretations (breakpoints). It was noted in some instances that although a standard guideline has been stated, the laboratory's interpretation of their stated MIC was at odds with this guideline. Whether this was due to a misunderstanding or clerical error is not clear.

With regard to the specific antibiotics, there should be some concern that some (2) laboratories reported cefotaxime intermediate susceptibility for sample 9202 (serogroup C) and one laboratory for 9203 (serogroup B) and whether that was a technical or interpretation issue is not known. It was reassuring that all laboratories were able to test and report all the isolates to be susceptible to ceftriaxone.

Penicillin MIC interpretation is probably the major problem at present and is related to the guideline chosen. Specifically, at which MIC level should intermediate (or reduced) susceptibility be interpreted and reported. There was one report of penicillin resistance for sample 9203 (serogroup B), which will hopefully be reviewed by the reporting laboratory as to whether it was due to a technical issue.

Sulphonamide MICs were included in the EQA as they are used as an epidemiological marker on occasion. Determination of the sulphonamide MIC can be problematic due to the bacteriostatic action of the antimicrobial. Particular difficulties were encountered testing and reporting the sulphonamide MIC for sample 9200 (serogroup Y).

Only one interpretation of rifampicin MIC was inconsistent (sample 9201), classifying the result as susceptible. Interestingly, the range of MICs reported by all laboratories for the rifampicin was 10-fold (0.32–32.0 mg/L).

Table 6 Results of antimicrobial susceptibility testing

Antimicrobial agent	Correct result	Number of laboratories reporting as:			% laboratories with correct result
		S	M / I	R	
EQA No. 9199					
Ciprofloxacin	resistant	2	7	15	62.5
Ceftriaxone	susceptible	14	0	0	100
Cefotaxime	susceptible	20	0	0	100
Penicillin	susceptible	21	4	0	80.8
Rifampicin	susceptible	23	0	0	100
Sulphonamide	resistant	0	1	16	94.1
EQA No. 9200					
Ciprofloxacin	susceptible	24	0	0	100
Ceftriaxone	susceptible	14	0	0	100
Cefotaxime	susceptible	20	0	0	100
Penicillin	susceptible	13	12	0	50.0
Rifampicin	susceptible	23	0	0	100
Sulphonamide	susceptible	12	3	2	70.6
EQA No. 9201					
Ciprofloxacin	susceptible	24	0	0	100
Ceftriaxone	susceptible	14	0	0	100
Cefotaxime	susceptible	20	0	0	100
Penicillin	susceptible	22	3	0	84.6
Rifampicin	resistant	1	0	22	95.7
Sulphonamide	resistant	0	0	17	100
EQA No. 9202					
Ciprofloxacin	susceptible	24	0	0	100
Ceftriaxone	susceptible	14	0	0	100
Cefotaxime	susceptible	18	2	0	90.0
Penicillin	intermediate	0	20	5	76.9
Rifampicin	susceptible	23	0	0	100
Sulphonamide	resistant	0	0	17	100
EQA No. 9203					
Ciprofloxacin	susceptible	24	0	0	100
Ceftriaxone	susceptible	14	0	0	100
Cefotaxime	susceptible	19	1	0	95.0
Penicillin	intermediate	5	19	1	73.1
Rifampicin	susceptible	23	0	0	100
Sulphonamide	resistant	1	0	16	94.1
EQA No. 9204					
Ciprofloxacin	resistant	2	6	16	66.7
Ceftriaxone	susceptible	14	0	0	100
Cefotaxime	susceptible	20	0	0	100
Penicillin	susceptible	22	3	0	84.6
Rifampicin	susceptible	23	0	0	100
Sulphonamide	resistant	0	0	17	100

2.3 Part 3: Molecular characterisations

The consensus molecular characterisations for the EQA panel taken from the interim report are summarised in Tables 7 and 8.

Table 7 Molecular geno-serogroup, genotype (porB) and geno-subtype (porA) designations

EQA number	Genogroup	Genotype*	*porA*		
			VR1	VR2	VR3
9199	W135	3-291	5-3	10-65	37-1
9200	Y	2-55	5-1	2-2	36-2
9201	B	3-36	7	16	35
9202	C	2-2	5	2-1	36-2
9203	B	3-82	18	25	38-1
9204	A	3-47	20	9	35-1
9205	B	ND	22	9	35-1
9206	A	ND	5-2	10	37-1
9207	Negative	-	-	-	-
9208	X	ND	5-1	10-1	36-2
9209	C	ND	7-4	14-6	35-1
9210	B	ND	19-2	13	36

**Only two laboratories reported porB sequence typing designations for the viable isolates only. There were no porB sequence typing reports for the non-culture material.*
ND: not determined

Table 8 Molecular MLST, clonal complex (CC), fetA and penA designations

EQA number	Group	MLST		*fetA*	*penA*	Est. viable count
		ST	CC			
9199	W135	6361	174	1-7	1	
9200	Y	23	23	1-7	22	
9201	B	32	32	3-3	3	
9202	C	11	11	5-5	9	
9203	B	414	41/44	1-5	15	
9204	A	4789	5	3-1	4	
9205	B	1195	269	5-1	27	$6.0x10^4$ ++
9206	A	75	1	3-5	83	$5.0x10^5$ +++
9207	Negative	-	-	-	-	-
9208	X	751	UA	4-23	57	$2.7x10^5$ +++
9209	C	1031	334	3-9	22	$2.6x10^4$ ++
9210	B	461	461	1-45	9	$1.4x10^3$ +

The consensus ST for 9208 was designated ST-751 (Table 8) even though it was only determined by three laboratories.

The estimated viable count (Table 8 – not stated in the interim or final reports) indicates the minimum concentration of *N. meningitidis* genomes in the sample and corresponds to the degree of positivity determined. That would equate to the intensity of fluorescent band if using gel electrophoresis or the cycle threshold (CT value) for real-time PCR detection.

N. meningitidis confirmation and geno-serogroup determination

Extraction, amplification and detection methods

Participants were requested to report the extraction, amplification and detection methods used on both viable isolates and non-culture (horse blood and horse serum) samples. A number of combinations of techniques were used but the predominant method of isolate DNA extraction was by heating (boiling). Magnetic beads and spin columns (capture columns) were also used.

The DNA from the non-culture blood sample was extracted most often by means of spin columns (capture columns) and then magnetic beads. Similar methods were used for the serum samples. Amplification was

predominantly by real-time PCR but a number of laboratories are using conventional thermal cyclers and gel electrophoresis. The real-time PCRs are detected either by Taqman probes or by fluorescence as stated by the participants.

It should be noted that it was not possible to elucidate whether the laboratories using conventional amplification and gel-based detection were those with negative results from the anonymised summary. It was left for the individual laboratories to assess their own results.

This may be an aspect that could be investigated by UK NEQAS from the submitted reports.

Viable isolates

Fifteen laboratories undertook the molecular confirmation of the viable isolates. Whether that is their routine practice was not stated. A limited number of gene targets were used for PCR assays, namely *ctrA*, *crgA*, *porA* and *siaD*/*porA*/orf2. *CtrA* was the predominant assay to confirm capsulated meningococci. All reports were positive for samples 9199–9204, confirming *N. meningitidis* DNA.

The consensus report for 9199 was W135 based on *siaD* PCR, but one laboratory using *siaD* reported genogroup Y. One report was W135 using *synG*. Three laboratories reported not serogroup B or C. 9200 was reported as serogroup Y (*siaD*) by 10 laboratories, but by one as serogroup W135. Three laboratories reported not serogroup B or C. One report was Y using *synF* (a syonym for *siaD*).

Samples 9201, 9202 and 9203 were all confirmed correctly as serogroups B, C and B, respectively, using *siaD* based assays.

Sample 9204, serogroup A, highlighted the variety of assays and synonymous terminology. Thirteen correct serogroup A reports were submitted attributed to *mynA* (2), *sacC* (2), *siaD*/orf2 (1), orf2 (4), *sacB* (1) and siaD (3), with three reports stating not serogroups B or C.

Non-culture simulated septicaemia samples

A number of PCR assays and combination of assays were reported. For detection: 16S rRNA, *crgA*/16S rRNA, 16SrDNA, *crgA*, *ctrA*, *porA*, *ctrA/porA* and *siaD/porA*/orf2 assays were reported.

For genogroup, a number of different gene targets were recorded, of which some were synonymous and dependent on the sample under investigation.

Sample 9205 genogroup B was reported as positive for *N. meningitidis* DNA by 12 laboratories, but eight reported negative, seven of which were using the *ctrA* assay. The sample was prepared for the EQA as the isolate was one of a small subset of serogroup B CC ST 269 organisms that were not detected by the initial set of published *ctrA* primers. Serogroup B was confirmed for 9205 by 12 laboratories, with one reporting negative and one reporting Non-A, B, C, Y or W135. The positive assays were all *siaD*-based.

Sample 9206 a strongly positive serogroup A preparation (Table 8) was reported as positive for *N. meningitidis* DNA by 18 laboratories and only reported negative by two. Serogroup A was confirmed by 14 laboratories using a variety of targets some of which are synonymous: *mynC/sacC* (1), *sacC* (1), *siaD*/orf2 (1), *mynA* (2), *mynB* (1), *orf2* (4), *sacB* (2), *siaD* (2).

Unfortunately, one laboratory reported serogroup C from a *siaD* assay. Two others quite correctly reported *siaD* non-B and non-C.

Sample 9207 was correctly reported as negative for *N. meningitidis* DNA by 17 laboratories, eight using *ctrA* assays, although three laboratories reported positive *ctrA* results for the negative control sample. Seven serogroup reports were submitted, six of which were negative or stated all the serogroups checked. One report was submitted confirming serogroup B by *siaD* assay.

Sample 9208 a strongly positive preparation of serogroup X (Table 8) was reported as positive for *N. meningitidis* DNA by 19 laboratories, 13 using *ctrA* assays. One laboratory reported a negative *ctrA* result. Serogroup X was confirmed by eight laboratories using the following assays: *ctrA* (2), *xcbA* (2), *xcbB* (1), *siaD*/orf2 (1) and *siaD* (2). Two laboratories reported serogroup B and five laboratories were unable to confirm the serogroup (other than state what had been tested). Confirmation of serogroup X genotypically was only possible (or available) in 53% (8) of the 15 laboratories reporting a result of testing. For such a rare cause of systemic meningococcal disease, this is an interesting finding.

Sample 9209 a serogroup C preparation was recorded as positive for *N. meningitidis* DNA by all 20 reporting laboratories. Serogroup C was confirmed using *siaD* assays by 19 reporting laboratories. There were no conflicting serogroup results for sample 9209.

Sample 9210 a weakly positive serogroup B preparation (Table 8) was reported as positive for *N. meningitidis* DNA by all 20 reporting laboratories. This was a surprisingly good result given the estimate of 103 cfu/mL (Table 8) equating to a low-level positive clinical sample.

Although the majority of laboratories used *ctrA* (14), all the other assays – 16S rRNA (1), *crgA*/16S rRNA (1), *crgA* (2), *porA* (1) and *siaD/porA*/orf2 (1) – proved sensitive. Serogroup B was confirmed by 17 of the 18 reporting laboratories using *siaD* assays. One laboratory reported serogroup A.

Genotype – porB sequencing

Only two laboratories reported *porB* sequence typing results and even then only for the viable isolates (Annex 5). There was agreement between the two for samples 9199, 9201, 9202, 9203 and 9204. 9200 was only determined or reported by one laboratory.

Geno-serosubtype – porA sequencing

There was excellent agreement for those laboratories reporting *porA* sequencing typing variable regions (VR1, VR2 and VR3). Differing numbers of reports were made for each viable isolate sample, but there were a maximum 18 reports (Annex 4).

There were some notational errors or confusions, such as sample 9200, where *porA* VR1 was recorded as 5-1 (16) and 1.5-1 (1). A further submission of 5-11 may possibly be a transcription error.

Sample 9203 VR1 was reported as consensus 18 (by 18 laboratories), but also reported as 7-4 by two laboratories. The two laboratories should be able to review their sequencing traces and base calling in the light of the consensus sequence to re-assess their results.

The differences between VR1 18 and 7-4 at the amino acid and nucleotide levels can be determined by using the *Neisseria* typing website.

The non-culture samples (9205–9210) proved slightly more exacting (Annex 5), with 11 laboratories reporting the consensus results for samples 9206, 9208 and 9209.

However, for samples 9205 and 9210 only eight and nine reports were submitted, respectively. This suggests that the weaker serogroup B samples could be more problematic.

fetA sequence typing

All nine submitting laboratories confirmed the consensus *fetA* results for the viable isolates 9199–9204 (Table 9, Annex 5). The *fetA* typing submissions for the non-culture samples (9205–9210) varied from six and four for the strong positive serogroup X and A samples 9208 and 9206 to three laboratories for the weaker samples 9205, 9209 and 9210.

penA sequence typing

The *penA* typing consensus was reported for all the viable isolates 9199–9204 samples by eight laboratories. Three laboratories reported the *penA* for the non-culture samples 9205–9210 with only one discrepant result for sample 9210.

Table 9 Results of genotypic characterisations

EQA number	Identification	Ratio reporting consensus (%)	Non-consensus results reported
DNA identification			
9199	Positive	15/15 (100%)	-
9200	Positive	15/15 (100%)	-
9201	Positive	15/15 (100%)	-
9202	Positive	15/15 (100%)	-
9203	Positive	15/15 (100%)	-
9204	Positive	15/15 (100%)	-
9205	Positive	12/20 (60%)	Negative
9206	Positive	18/20 (90%)	Negative
9207	Negative	17/20 (85%)	Positive
9208	Positive	19/20 (95%)	Negative
9209	Positive	20/20 (100%)	-
9210	Positive	20/20 (100%)	-
Genogroup			
9199	W135	11/15 (73%)	Y, non-B, non-C
9200	Y	11/15 (73%)	W135, non-B, non-C
9201	B	15/15 (100%)	-
9202	C	15/15 (100%)	-
9203	B	15/15 (100%)	-
9204	A	13/16 (81%)	Non-B, non-C
9205	B	12/14 (86%)	Negative; non A,B,C,Y or W135
9206	A	14/17 (82%)	C, non-B, non-C
9207	Negative	6/7 (86%)	B; non A,B,C,Y or W135
9208	X	8/15 (53%)	B, not groupable, non-B, non-C
9209	C	19/19 (100%)	-
9210	B	17/18 (94%)	A
Genotype *por B* sequencing			
9199	3-291	2/2 (100%)	
9200	2-55	1/1 (100%)	
9201	3-36	2/2 (100%)	
9202	2-2	2/2 (100%)	
9203	3-82	2/2 (100%)	
9204	3-47	2/2 (100%)	
9205	ND	-	
9206	ND	-	
9207	-	-	
9208	ND	-	
9209	ND	-	
9210	ND	-	

Table 10 Number of laboratories submitting specific molecular reports compared to the percentage agreeing with the consensus

EQA number and genogroup	MLST		CC		*fetA*		*penA*	
9199 W135	12	100%	12	100%	9	100%	8	100%
9200 Y	12	83%	12	100%	9	100%	8	100%
9201 B	12	92%	12	100%	9	100%	8	100%
9202 C	12	92%	12	100%	9	100%	8	100%
9203 B	12	92%	12	100%	9	100%	8	100%
9204 A	12	67%	12	100%	9	100%	8	100%
9205 B	3	100%	4	100%	3	100%	3	100%
9206 A	5	60%	6	100%	4	100%	3	100%
9208 X	14	36%	5	80%	6	100%	3	100%
9209 C	5	40%	6	83%	3	100%	3	100%
9210 B	4	50%	6	83%	3	100%	3	67%

Figure 1 Multilocus sequence type (MLST)

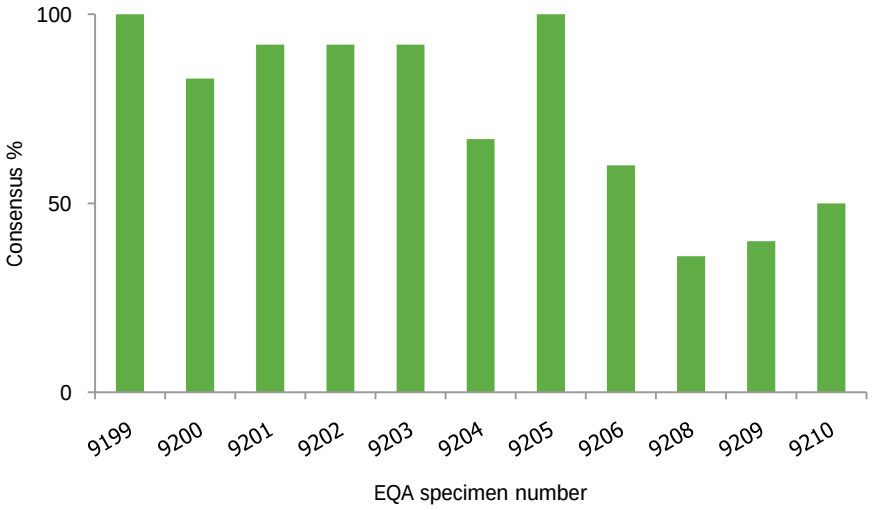

Figure 2 Clonal complex

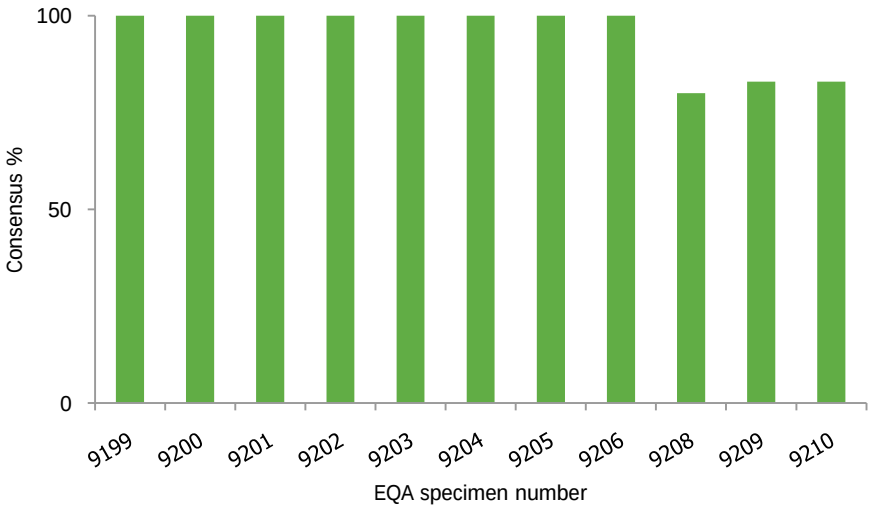

Table 11 Number of laboratories reporting specific MLST and CC designations for each sample

EQA number and genogroup	MLST (number of laboratories)	CC (number of laboratories)
9199 W135	6361 (12)	174(12)
9200 Y	23(10), 3402(1), 3171(1)	23(12)
9201 B	32(11), UA*(1)	32(12)
9202 C	11(11), 3410(1)	11(12)
9203 B	414(11), 247(1)	41/44(12)
9204 A	4789(8), UA(3)	5(12)
9205 B++	1195(3)	269(4)
9206 A+++	75(3), UA(2)	1(6)
9208 X+++	UA(9), 751(3)	UA(4), 751 (1)
9209 C++	1031(3), UA(2)	334(5), UA (1)
9210 B+	461(2), 3413(1), UA(1)	461(5), UA(1)

**UA: unassigned*

Viable isolate MLST

Looking more closely at the MLST and CC results (Table 11), it may be seen that for the viable isolates (9199–9203) the clonal complex designations were achieved by all 12 submitting laboratories with often only one laboratory not agreeing with the consensus ST. However, the serogroup A isolate 9204 appeared more problematic. Only eight of the submitting 11 laboratories confirmed the ST but 12 submitting laboratories confirmed the CC.

Non-culture MLST

The serogroup B sample 9205 (designed for ++ positivity) was only designated a ST by three laboratories and a CC by four laboratories.

Only five laboratories submitted an ST designation for the serogroup A sample 9206 (designed for +++ positivity) of which only three agreed. However, all six submitting laboratories agreed with the CC designation.

The serogroup X sample 9208 (designed for +++ positivity) was poorly designated, although an encouraging number of submission were received: 12 for the ST (of which only three agreed).

Most ST reports designating unassigned (UA) lead to a confused designation for the CC. It is likely that the sample was ST 751 and should therefore be CC unassigned (currently).

Detailed review and analysis of MLST results

Examples of detailed discrepant MLST reports analysis are provided in Annex 5. The examples described were presented by Dr Gray in the IBD-Labnet annual meeting in Manchester, in June 2009.

If a laboratory submitted a value at odds with the consensus MLST, they can specifically compare the nucleotide sequences to locate any base differences, thereby revealing clerical or technical errors. It is suggested that any inconsistencies are best remedied by a complete repeat of the process, re-amplifying products from the original material.

3 Conclusions

The establishment of a regular EQA scheme for the reference laboratories is required in order to maintain the improvement of quality of epidemiological reports. Where possible, an EQA scheme should be frequent (twice a year) but with fewer samples than in this panel, possibly two isolates and three non-culture samples.

The performance of this EQA exercise has called attention to a potentially broad range of topics that could be considered for a training workshop, not all of which are appropriate to each of the reference laboratories. One laboratory may require assistance setting up gel-based PCR assays, but another may wish for refined sequence analysis training.

This EQA scheme has pointed out that the phenotypic serotyping and serosubtyping demonstrated limited discrimination due to the limited panels of reagents, both their reactivity and availability.

Penicillin MIC interpretation is probably the major problem at present concerning antimicrobial susceptibility testing, and is related to the guideline chosen, specifically at which MIC level should intermediate (or reduced) susceptibility be interpreted and reported.

For European MIC epidemiology, it would be preferable to collect the MIC values and then interpret according to one guideline for consistency. The MIC EQA reports suggest that CLSI currently predominates, but a standardised methodology and working with EUCAST to assign breakpoints should be the aim.

According to the results, *porB* sequencing is probably likely to remain an activity achievable by a minority of laboratories due to the complexity of the procedure and the practicalities of non-culture application.

The non-culture material revealed more problematic for designating MLST and CC (Table 10). This activity deserves dedicated training.

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Annex 1: Participating reference laboratories

Country	Contact person	Institution
Austria	Dr Sigrid Heuberger	National Reference Centre for Meningococci, Pneumococci and *Haemophilus influenzae* Austrian Agency for Food and Health Safety Beethovenstraße 6 8010 Graz, Austria
Belgium	Dr Françoise Carion	National Meningococcal Reference Laboratory Scientific Institute of Public Health, Bacteriology Division Rue Juliette Wytsman 14-16 1050 Bruxelles, Belgium
Czech Republic	Dr Pavla Krizova	National Reference Laboratory for Meningococcal Infections Centre for Public Health Laboratories National Institute of Public Health Srobarova 48 100 42 Prague 10, Czech Republic
Denmark	Dr Lotte Lambertsen	Neisseria and Streptococcus Reference Laboratory Department of Bacteriology, Mycology and Parasitology Statens Serum Institut 5 Artillerivej, building 211/117B 2300 Copenhagen, Denmark
England and Wales	Dr Eduard Kaczmarski	HPA Meningococcal Reference Unit Manchester Royal Infirmary Oxford Road, Manchester M13 9WZ, UK
Estonia	Dr Inna Sarv	Head, Central Laboratory for Microbiology Health Protectorate Inspectorate Kotka 2 11315 Tallinn, Estonia
Finland	Prof Dr Helena Käyhty	Immune Response Unit National Institute for Health and Welfare (THL) PO Box 30 Fi-00271 Helsinki, Finland
France	Dr Muhamed-Kheir Taha	Unit Invasive Bacterial Infections National Reference Centre for Meningococci Institut Pasteur 26 rue de Dr. Roux 75724 Paris Cedex 15, France
Germany	Prof Dr Matthias Frosch and Prof Dr Ulrich Vogel	Institute for Hygiene and Microbiology University of Würzburg Josef-Schneider-Straße 2 97080 Würzburg, Germany
Greece	Dr Georgina Tzanakaki	National Meningitis Reference Laboratory National School of Public Health 196 Alexandras Avenue 115 21 Athens, Greece
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Iceland	Dr Hjordis Hardardóttoir	Department of Clinical Microbiology Institute of Laboratory Medicine Landspitali University Hospital Baronsstigur, 101 Reykjavik, Iceland
Ireland	Prof Mary Cafferkey	Irish Meningococcal and Meningitis Reference Laboratory Children's University Hospital Temple Street Dublin 1, Ireland
Italy	Dr. Paola Mastrantonio	Department of Infectious, Parasitic and Immunomediated Diseases. Instituto Superiore di Sanitȧ Viale Regina Elena 299 00161 Rome, Italy
Latvia	Dr Ruta Pabērza	Laboratory of the State Agency Infectology Center of Latvia Bacteriology Department 3 Linezera street Riga, LV 1006, Latvia

Country	Contact person	Institution
Liechtenstein (represented by Switzerland)	Dr Béatrice Ninet	Centre National des Méningocoques Hôpitaux Universitaires de Géneva Laboratoire Central de Bactériologie Rue Micheli-du-Crest 24 1211 Genève 14, Switzerland
Lithuania	Dr Snieguole Dauksiene	Microbiological Department National Public Health Surveillance Laboratory Zolyno str. 36 10210 Vilnius, Lithuania
Luxembourg	Dr Jos Even	Director, Laboratoire National de Santé 42 rue du Laboratoire L-1911 Luxembourg, Luxembourg
Malta*	Dr Jackie Maistre Melillo	Disease Surveillance Unit Department of Public Health 37-39 Rue D'Argens Msida MSD 05, Malta
Netherlands	Dr Arie van der Ende	Reference Laboratory for Bacterial Meningitis Department of Medical Microbiology Academic Medical Canter L-1-Z. Meibergdreef 15 1105 AZ Amsterdam, The Netherlands
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Poland	Dr Alicja Kuch/ Dr Anna Skoczynska	National Reference Centre for Bacterial Meningitis Department of Epidemiology and Clinical Microbiology National Medicines Institute Chelmska Street 30/34 00-725 Warsaw, Poland
Portugal	Dr Maria João Simões	Departamento de Doenças Infecciosas Laboratorio Nacional de Referência de *Neisseria meningitidis* Instituto Nacional de Saúde Dr Ricardo Jorge Avenida Padre Cruz 1649-016 Lisboa, Portugal
Romania	Dr Marina Pana	Cantacuzino Institute Bacterial Respiratory Infections 102 Splaiul Independentei, Sector 5 C.P.1-525 Bucharest, Romania
Scotland	Dr Edwards Giles	Scottish Meningococcus and Pneumococcus Ref. Lab Stobhill Hospital Balornock Road Glasgow G21 3UW, UK
Slovakia	Dr Alena Vaculiková	Head, National Reference Centre for Meningococci Public Health Authority of the Slovak Republic Trnavská 52 826 45 Bratislava, Slovakia
Slovenia	Dr Metka Paragi	Head of Laboratory for Immunology and Molecular Diagnostics Institute of Public Health Slovenia Grablovičeva 44 1000 Ljubljana, Slovenia
Spain	Dr Julio Vázquez	Centro Nacional de Microbiología Instituto de Salud Carlos III, Ctra Majadahonda-Pozuelo Km 2 28220 Madrid, Spain
Sweden	Prof em. Per Olcén	National Reference Laboratory for Pathogenic Neisseria Department of Laboratory Medicine, Microbiology, SE-701 85 Örebro, Sweden

Note: Malta did not participate in the EQA exercise. The typing of the strains for Malta was done by UK reference laboratory.

Annex 2: Cover letter

This letter was sent to all the laboratories participating in the EQA exercise for requesting the agreement of the participants to the terms and conditions of the EQA distribution.

Dear XXXX,

In the next few weeks your institute will be taking part in EQA schemes for *Haemophilus influenzae* and/or *Neisseria meningitidis* as part of the *Laboratory surveillance and External Quality Assurance (EQA) of invasive bacterial diseases in EU* project. The samples for the EQA will be sent to you from the Health Protection Agency (HPA) by agreement of the University of Würzburg.

Before the HPA can send the samples we need you to sign and return the attached conditions of participation agreement. Please obtain signature on behalf of your institute and fax the document back to me on +44 XXXXXXX and send the original in the post to:

Business Development Department
Health Protection Agency
Centre for Infections
61 Colindale Avenue
London NW9 5EQ
England

Thank you for your co-operation.

Yours sincerely,

Annex 3: Terms and conditions of participation

Health Protection Agency ("HPA")

N. meningitidis EQA Scheme and or *H.influenzae* EQA Scheme ("Scheme")

1. Samples distributed as part of the Scheme may contain microbiological pathogens of Hazard Groups 1 and 2 as defined by the Advisory Committee on Dangerous Pathogens (The Approved List of Biological Agents, HMSO, 2004) ("Samples"). Participants must ensure and warrant that their laboratory facilities and expertise are adequate to ensure the safe handling of the Samples during their participation in the Scheme and any IQ Use.

2. The Samples shall be used for the purpose of participation in the Scheme only. In addition the Participant may use the Samples or derivatives thereof ("Materials" which expression shall include constructs, strains, derivatives, portions, progeny or improvements obtained from or as a result of the use of the Materials) for other internal quality use by the Participant outside of the Scheme ("IQ Use"). The Materials shall not be passed on to any other party.

3. Participants will process the quality assessment Samples in the same way as their routine samples. This is necessary to achieve the primary purpose of the Scheme, which is to allow participants an insight into their levels of performance in routine work.

4. Each participant laboratory will be registered under a unique code number.

5. All reports, and the data they contain, issued by the HPA are Copyright and may not be published in any form without prior permission of the HPA.

6. Participants in the Scheme have entire responsibility for all Samples distributed to them under the Scheme and all activities carried out by them or any third party in relation to the Samples from the time of receipt of the Samples.

7. HPA warrants that all work carried out by it in relation to the Scheme will be carried out using all reasonable care and skill. All conditions, terms and warranties implied by common law, statute or otherwise are, to the extent permitted by law, hereby excluded.

8. The total liability of the HPA to the participant resulting from or in connection with the provision of any or all of the Samples or Materials provided by the HPA to the Participant, or the provision of the Scheme by the HPA to the participant or IQ Use by the Participant shall be for death and personal injury resulting from HPA's negligence or in any other circumstances where liability may not be so limited under any applicable law in England and Wales.

9. HPA shall not be liable in any circumstances for indirect or consequential loss howsoever caused, including, without limitation, loss of anticipated profits, goodwill, reputation, business receipts or contracts, or losses or expenses resulting from third party claims.

10. If the Recipient wishes to submit for publication results from IQ Use of the Materials, the Recipient shall provide HPA with a copy of the final proposed publication at least sixty (60) days prior to its submission. HPA shall within thirty (30) days of receipt provide in writing any reasonable objections it has to the proposed publication and the Recipient shall give due regard to any amendments required by HPA and shall refrain from publication of any information in respect of the Materials which in HPA's reasonable opinion is damaging to its interests

11. The Recipient agrees to inform HPA of any intellectual property or product arising from use of the Materials and, prior to any commercial exploitation of such intellectual property or product, to negotiate with HPA terms properly reflecting the contribution of the Materials.

12. (a) These conditions and any dispute or claim arising out of or in connection with them or their subject matter or formation (including non-contractual disputes or claims) shall be governed by and construed in accordance with the law of England and Wales.

(b) The parties irrevocably agree that the courts of England and Wales shall have exclusive jurisdiction to settle any dispute or claim that arises out of or in connection with these conditions or their subject matter or formation (including non-contractual disputes or claims).

13. The recipient will inform HPA of receipt of the Samples within 5 working days.

14. If you agree to the above conditions, please sign, date and return a copy of these conditions to Business Development Department, HPA Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, England.

We hereby acknowledge receipt and accept the conditions outlined above.

Signed .....

Name .....

For and on behalf of

Name of Recipient Organisation .....

Address .....

.....

.....

Date .....

Annex 4: Interim consensus report

UK NEQAS Microbiology Quality Assessment **Distribution No. 2452**

Special survey for *Neisseria meningitidis* identification and typing

Date: 30.03.2009

Part 1. Results for *Neisseria meningitidis* strain characterisation

NT = Not typable/serosubtypable

Specimen number	Serogroup	Serotype	Serosubtype		
			VR1	VR2	VR3
9199	W135	15	P1.5	NT	NT
9200	Y	2c	P1.5	P1.2	NT
9201	B	14	P1.7	P1.16	NT
9202	C	2a	P1.5	NT	NT
9203	B	1	NT	NT	P1.6
9204	A	21	NT	P1.9	NT

Part 2. Antimicrobial Susceptibility Testing

Specimen number	Antimicrobial agent:	Result
9199	Ciprofloxacin	Resistant
	Ceftriaxone	Susceptible
	Cefotaxime	Susceptible
	Penicillin	Susceptible
	Rifampicin	Susceptible
	Sulphonamide	Resistant
9200	Ciprofloxacin	Susceptible
	Ceftriaxone	Susceptible
	Cefotaxime	Susceptible
	Penicillin	Susceptible
	Rifampicin	Susceptible
	Sulphonamide	Susceptible
9201	Ciprofloxacin	Susceptible
	Ceftriaxone	Susceptible
	Cefotaxime	Susceptible
	Penicillin	Susceptible
	Rifampicin	Resistant
	Sulphonamide	Resistant
9202	Ciprofloxacin	Susceptible
	Ceftriaxone	Susceptible
	Cefotaxime	Susceptible
	Penicillin	Intermediate
	Rifampicin	Susceptible
	Sulphonamide	Resistant
9203	Ciprofloxacin	Susceptible
	Ceftriaxone	Susceptible
	Cefotaxime	Susceptible
	Penicillin	Intermediate
	Rifampicin	Susceptible
	Sulphonamide	Resistant
9204	Ciprofloxacin	Resistant
	Ceftriaxone	Susceptible
	Cefotaxime	Susceptible
	Penicillin	Susceptible
	Rifampicin	Susceptible
	Sulphonamide	Resistant

UK NEQAS Microbiology Quality Assessment **Distribution No. 2452**

Special survey for *Neisseria meningitidis* identification and typing

Date: 30.03.2009

Part 3. Results for *Neisseria meningitidis* strain genotyping.
Specimens 9205 to 9210 contained killed organisms and were included as non-culture samples for molecular testing only.
ND = Not Done

Specimen number	Result (pos/neg)	Genogroup	Genotype	*por*AVR1	*por*AVR2	*por*AVR3
9199	Positive	W135	3-291	5-3	10-65	37-1
9200	Positive	Y	2-55	5-1	2-2	36-2
9201	Positive	B	3-36	7	16	35
9202	Positive	C	2-2	5	2-1	36-2
9203	Positive	B	3-82	18	25	38-1
9204	Positive	A	3-47	20	9	35-1
9205	Positive	B	ND	22	9	35-1
9206	Positive	A	ND	5-2	10	37-1
9207	Negative					
9208	Positive	X	ND	5-1	10-1	36-2
9209	Positive	C	ND	7-4	14-6	35-1
9210	Positive	B	ND	19-2	13	36

Specimen number	MLST	MLST CC	*fet*A	*pen*A
9199	6361	174	1-7	1
9200	23	23	1-7	22
9201	32	32	3-3	3
9202	11	11	5-5	9
9203	414	41/44	1-5	15
9204	4789	5	3-1	4
9205	1195	269	5-1	27
9206	75	1	3-5	83
9207				
9208	751	Unassigned	4-23	57
9209	1031	334	3-9	22
9210	461	461	1-45	9

Annex 5: Final summary report

UK National External Quality Assessment Service for Microbiology

Neisseria meningitidis	Laboratory :
Distribution : **2452**	Page 1 of 21
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Intended Result			Your Report	Your Score
Specimen 9199				
	Serogroup	W135	W135	Not scored
	Serotype	Not typable/serosubtypable	1.15	Not scored
	Serosubtype VR1	P1.5	P1.5	Not scored
	Serosubtype VR2	Not typable/serosubtypable	Not done	Not scored
	Serosubtype VR3	Not typable/serosubtypable	Not done	Not scored
Specimen 9200				
	Serogroup	Y	Y	Not scored
	Serotype	Not typable/serosubtypable	4	Not scored
	Serosubtype VR1	P1.5	P1.5	Not scored
	Serosubtype VR2	P1.2	P1.2	Not scored
	Serosubtype VR3	Not typable/serosubtypable	Not done	Not scored
Specimen 9201				
	Serogroup	B	B	Not scored
	Serotype	14	14	Not scored
	Serosubtype VR1	P1.7	P1.7	Not scored
	Serosubtype VR2	P1.16	Not done	Not scored
	Serosubtype VR3	Not typable/serosubtypable	Not done	Not scored
Specimen 9202				
	Serogroup	C	C	Not scored
	Serotype	2a	2a	Not scored
	Serosubtype VR1	P1.5	P1.5	Not scored
	Serosubtype VR2	Not typable/serosubtypable	Not done	Not scored
	Serosubtype VR3	Not typable/serosubtypable	Not done	Not scored
Specimen 9203				
	Serogroup	B	B	Not scored
	Serotype	1	1	Not scored
	Serosubtype VR1	Not typable/serosubtypable	Not done	Not scored
	Serosubtype VR2	Not typable/serosubtypable	P1.16	Not scored
	Serosubtype VR3	P1.6	P1.6	Not scored
Specimen 9204				
	Serogroup	A	A	Not scored
	Serotype	21	4	Not scored
	Serosubtype VR1	Not typable/serosubtypable	Not done	Not scored
	Serosubtype VR2	P1.9	P1.9	Not scored
	Serosubtype VR3	Not typable/serosubtypable	Not done	Not scored

Comments
This distribution was sent to laboratories in 30 countries. Due to import restrictions only 29 laboratories received the specimens in time to test and report their results.
Intended results are based on the consensus.
On the histograms in this report your result is indicated by an arrow.
Serotyping results have been presented as reported by the participating laboratory. This is to assist with discussions aimed at standardising naming conventions.

For the genotyping results the approach has been different. In order to present the data in a unified manner some responses have been interpreted by the organising laboratory (UK NEQAS). For example some participants narrowed the MLST to two or three possibilities; this has been interpreted as unassigned. It you do not agree with the way we have interpreted your result please contact us so that a more appropriate response can be entered.

Antimicrobial susceptibility data are presented on pages 8 and 9. MIC results have been presented showing the range and mode. For data that included an operator (> or <) the operator has been removed. Although the data is not then strictly accurate this was the only way such diverse information could be presented.
Antimicrobial susceptibility testing was performed by 27 participants with 19 reporting the use of CLSI guidelines, 1 BSAC, 1 EUCAST, 1 SRGA, 1 a combination and 4 Other. Listed below are observations for where the guideline MIC is known and participants reported discrepant results.
Specimen 9199
Ciprofloxacin: 2 reports of S and 3 reports of R were misinterpretations of the MIC for the guideline stated.
Penicillin: 3 reports of I were attributed to 'Other' guideline.
Specimen 9200
Penicillin: 4 of 7 CLSI reports for I should have been interpreted as S. One laboratory reported intermediate where this category does not exist for their guideline.
Sulphonamide: 1 report of I should have been interpreted as R
Specimen 9201
Penicillin: 1 report of I should have been S and another R.
Specimen 9202
Penicillin: 2 of the 5 reports of R should have been interpreted as I.
Specimen 9203
Penicillin: 2 of the 19 reports for I should have been interpreted as S.
Specimen 9204
Ciprofloxacin: the two S reports were misclassified, 1 should have been I and the other R. One of the reports of I should have been R.
Penicillin: 2 CLSI users reporting I should have classified their result as S.

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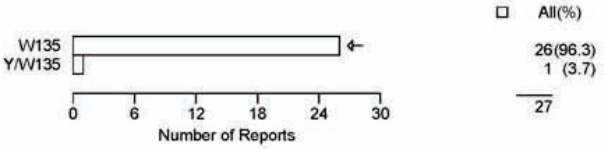
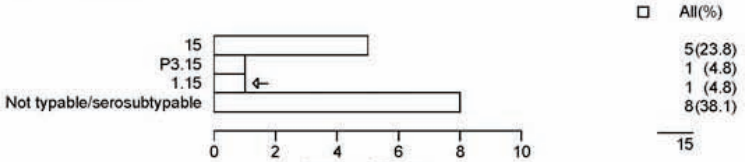
PART 1

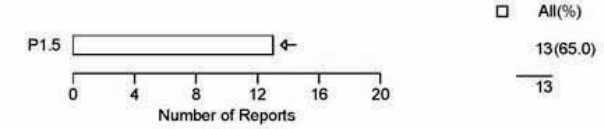
Specimen : 9199 (Serogroup)



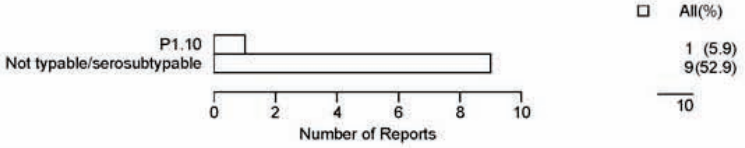
Specimen : 9199 (Serotype)



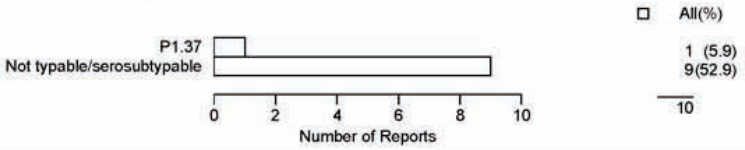
Specimen : 9199 (Serosubtype VR1)



Specimen : 9199 (Serosubtype VR2)



Specimen : 9199 (Serosubtype VR3)



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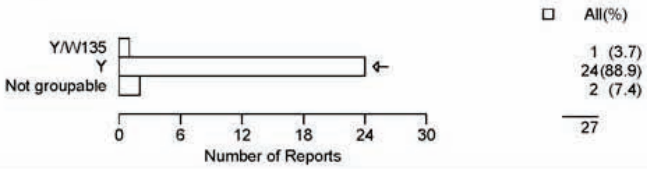
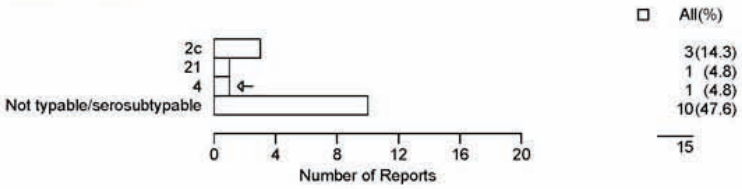
PART 1

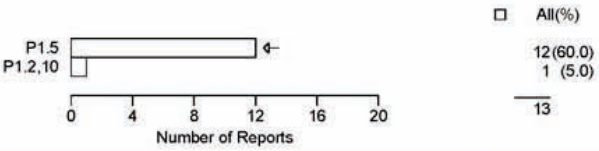
Specimen : 9200 (Serogroup)



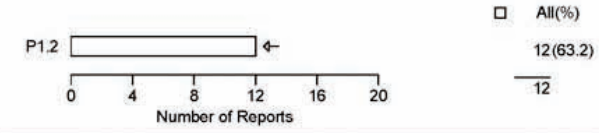
Specimen : 9200 (Serotype)



Specimen : 9200 (Serosubtype VR1)



Specimen : 9200 (Serosubtype VR2)



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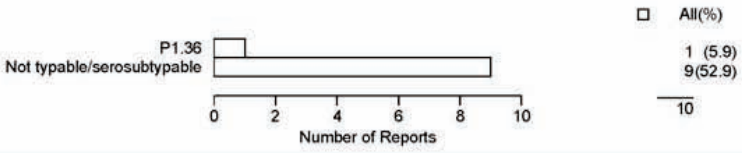

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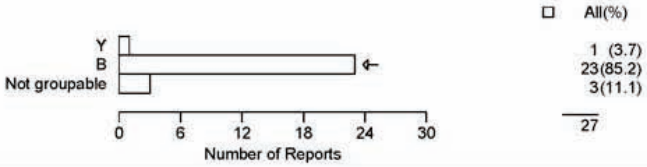
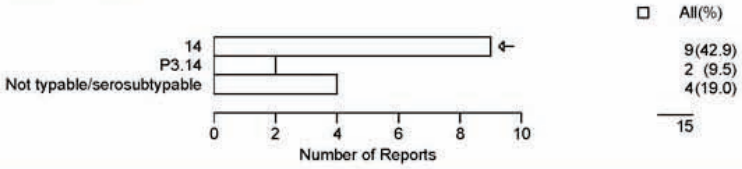
PART 1

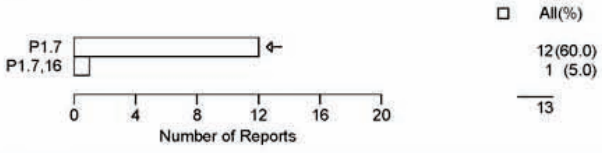
Specimen : 9201 (Serogroup)



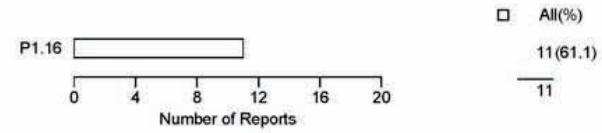
Specimen : 9201 (Serotype)



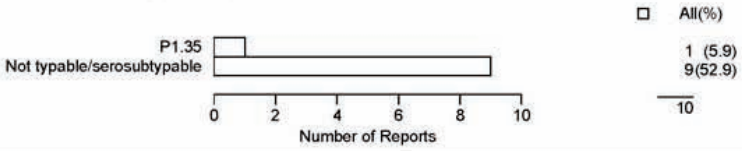
Specimen : 9201 (Serosubtype VR1)



Specimen : 9201 (Serosubtype VR2)



Specimen : 9201 (Serosubtype VR3)



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Neisseria meningitidis	Laboratory :
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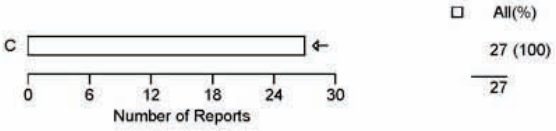
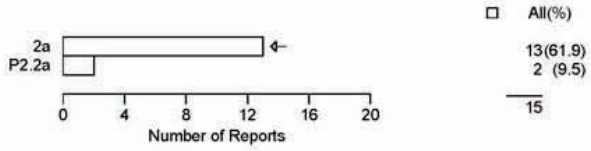
PART 1

Specimen : 9202 (Serogroup)



Specimen : 9202 (Serotype)



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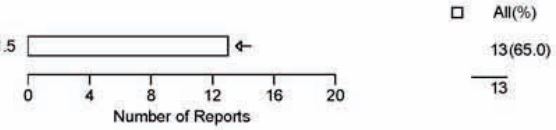
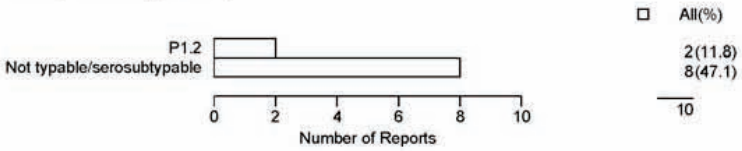


Specimen : 9202 (Serosubtype VR2)



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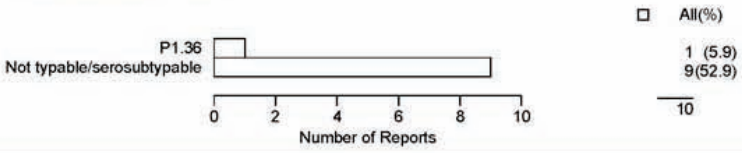

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Neisseria meningitidis	Laboratory :
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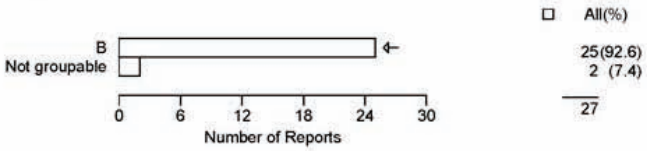
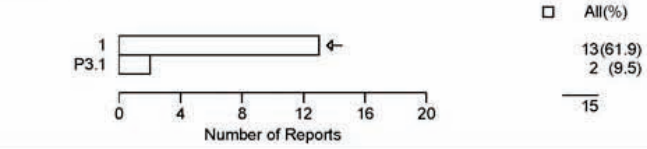
PART 1

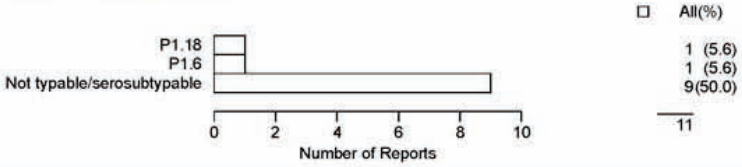
Specimen : 9203 (Serogroup)



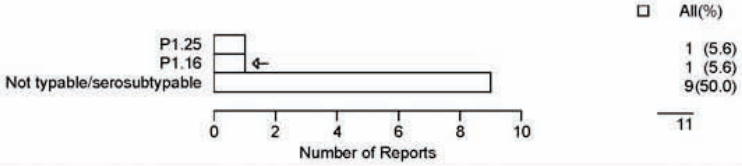
Specimen : 9203 (Serotype)



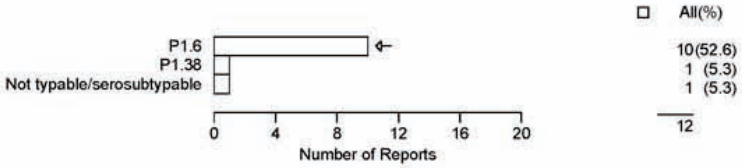
Specimen : 9203 (Serosubtype VR1)



Specimen : 9203 (Serosubtype VR2)



Specimen : 9203 (Serosubtype VR3)



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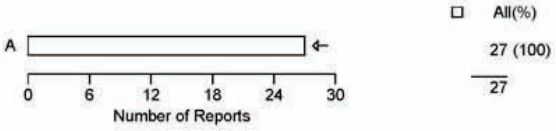
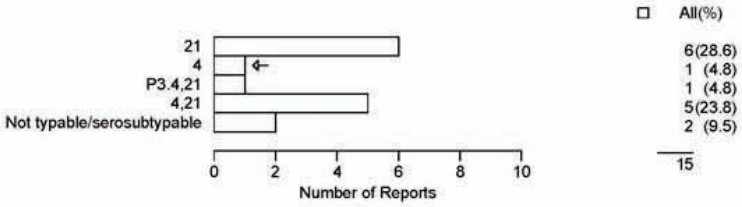
PART 1

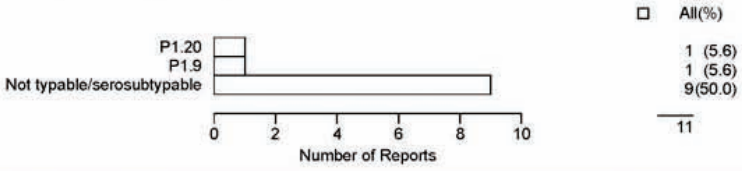
Specimen : 9204 (Serogroup)



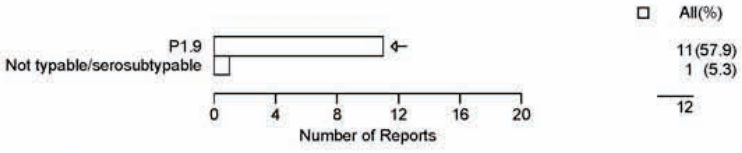
Specimen : 9204 (Serotype)



Specimen : 9204 (Serosubtype VR1)



Specimen : 9204 (Serosubtype VR2)



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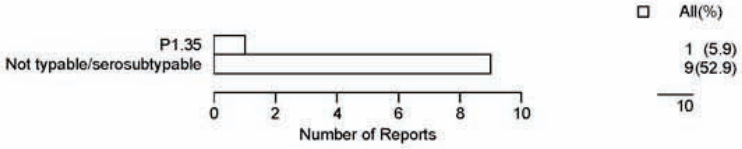

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Neisseria meningitidis	Laboratory :
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Dispatch Date : 30-Mar-2009	

PART 2

Specimen : 9199

Antimicrobial agent	Correct result	Your result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
ciprofloxacin	resistant	intermediate	2	7	15	62.5
ceftriaxone	susceptible	susceptible	14	0	0	100
cefotaxime	susceptible	susceptible	20	0	0	100
penicillin	susceptible	susceptible	21	4	0	80.8
rifampicin	susceptible	susceptible	23	0	0	100
sulphonamide	resistant	Not examined	0	1	16	94.1

Specimen : 9200

Antimicrobial agent	Correct result	Your result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
ciprofloxacin	susceptible	susceptible	24	0	0	100
ceftriaxone	susceptible	susceptible	14	0	0	100
cefotaxime	susceptible	susceptible	20	0	0	100
penicillin	susceptible	susceptible	13	12	0	50.0
rifampicin	susceptible	susceptible	23	0	0	100
sulphonamide	susceptible	Not examined	12	3	2	70.6

Specimen : 9201

Antimicrobial agent	Correct result	Your result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
ciprofloxacin	susceptible	susceptible	24	0	0	100
ceftriaxone	susceptible	susceptible	14	0	0	100
cefotaxime	susceptible	susceptible	20	0	0	100
penicillin	susceptible	susceptible	22	3	0	84.6
rifampicin	resistant	resistant	1	0	22	95.7
sulphonamide	resistant	Not examined	0	0	17	100

Specimen : 9202

Antimicrobial agent	Correct result	Your result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
ciprofloxacin	susceptible	susceptible	24	0	0	100
ceftriaxone	susceptible	susceptible	14	0	0	100
cefotaxime	susceptible	susceptible	18	2	0	90.0
penicillin	intermediate	intermediate	0	20	5	76.9
rifampicin	susceptible	susceptible	23	0	0	100
sulphonamide	resistant	Not examined	0	0	17	100

Specimen : 9203

Antimicrobial agent	Correct result	Your result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
ciprofloxacin	susceptible	susceptible	24	0	0	100
ceftriaxone	susceptible	susceptible	14	0	0	100
cefotaxime	susceptible	susceptible	19	1	0	95.0
penicillin	intermediate	intermediate	5	19	1	73.1
rifampicin	susceptible	susceptible	23	0	0	100
sulphonamide	resistant	Not examined	1	0	16	94.1

Specimen : 9204

Antimicrobial agent	Correct result	Your result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
ciprofloxacin	resistant	intermediate	2	6	16	66.7
ceftriaxone	susceptible	susceptible	14	0	0	100
cefotaxime	susceptible	susceptible	20	0	0	100
penicillin	susceptible	susceptible	22	3	0	84.6
rifampicin	susceptible	susceptible	23	0	0	100
sulphonamide	resistant	Not examined	0	0	17	100

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		ciprofloxacin	ceftriaxone	cefotaxime	penicillin	rifampicin	sulphonamide
9199	n	24	7	16	26	21	12
	range	0.06 - 0.19	0.002 - 0.016	0.002 - 0.08	0.023 - 0.125	0.003 - 0.125	0.25 - 1024
	mode	0.125	0.002	0.003	0.023	0.008	96.000
9200	n	24	8	16	26	22	16
	range	0.002 - 0.008	0.002 - 0.016	0.003 - 0.047	0.032 - 0.125	0.016 - 0.19	0.032 - 1024
	mode	0.002	0.002	0.003	0.032	0.047	3.000
9201	n	24	7	16	26	4	14
	range	0.002 - 0.008	0.002 - 0.016	0.002 - 0.032	0.006 - 0.125	0.32 - 32	0.75 - 512
	mode	0.003	0.002	0.008	0.016	32.000	96.000
9202	n	24	9	16	26	22	6
	range	0.002 - 0.012	0.002 - 0.016	0.006 - 0.064	0.19 - 0.75	0.012 - 0.094	1.0 - 1024
	mode	0.002	0.002	0.008	0.190	0.023	1.500
9203	n	24	8	16	26	21	15
	range	0.002 - 0.008	0.002 - 0.016	0.002 - 0.064	0.03 - 0.38	0.003 - 0.032	0.38 - 384
	mode	0.002	0.002	0.006	0.094	0.012	32.000
9204	n	24	7	15	26	22	13
	range	0.06 - 0.25	0.002 - 0.016	0.002 - 0.032	0.015 - 0.094	0.064 - 0.38	0.38 - 1024
	mode	0.125	0.002	0.016	0.032	0.094	48.000

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Intended Result		Your Report	Your Score
Specimen 9199			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	W135	W135	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	5-3	5-3	Not scored
porAVR2	10-65	10-65	Not scored
porAVR3	37-1	37-1	Not scored
Specimen 9200			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	Y	Y	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	5-1	5-1	Not scored
porAVR2	2-2	2-2	Not scored
porAVR3	36-2	36-2	Not scored
Specimen 9201			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	B	B	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	7	7	Not scored
porAVR2	16	16	Not scored
porAVR3	35	35	Not scored
Specimen 9202			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	C	C	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	5	5	Not scored
porAVR2	2-1	2-1	Not scored
porAVR3	36-2	36-2	Not scored
Specimen 9203			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	B	B	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	18	18	Not scored
porAVR2	25	25,25-7	Not scored
porAVR3	38-1	38-1	Not scored
Specimen 9204			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	A	A	Not scored
Target gene(s) (genogrp)	siaD	sacB	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	20	20	Not scored
porAVR2	9	9	Not scored
porAVR3	35-1	35-1	Not scored

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Intended Result		Your Report	Your Score
Specimen 9205			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	B	B	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	Not done	22	Not scored
porAVR2	9	9	Not scored
porAVR3	35-1	35-1	Not scored
Specimen 9206			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	A	A	Not scored
Target gene(s) (genogrp)	siaD	sacB	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	5-2	5-2	Not scored
porAVR2	10	10	Not scored
porAVR3	37-1	37-1	Not scored
Specimen 9207			
Result for genotyping	Negative	Negative	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	Not done	Negative	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	Not done	Negative	Not scored
porAVR2	Not done	Negative	Not scored
porAVR3	Not done	Negative	Not scored
Specimen 9208			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	X	Not groupable	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	5-1	5-1	Not scored
porAVR2	10-1	10-1	Not scored
porAVR3	36-2	36-2	Not scored
Specimen 9209			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	C	C	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	7-4	7-4	Not scored
porAVR2	14-6	14-6	Not scored
porAVR3	35-1	35-1	Not scored
Specimen 9210			
Result for genotyping	Positive	Positive	Not scored
Target gene(s) (ID)	ctrA	crgA	Not scored
Genogroup	B	B	Not scored
Target gene(s) (genogrp)	siaD	siaD	Not scored
Genotype porB	Not done	Not done	Not scored
porAVR1	19-2	19-2	Not scored
porAVR2	13	13	Not scored
porAVR3	36	36	Not scored

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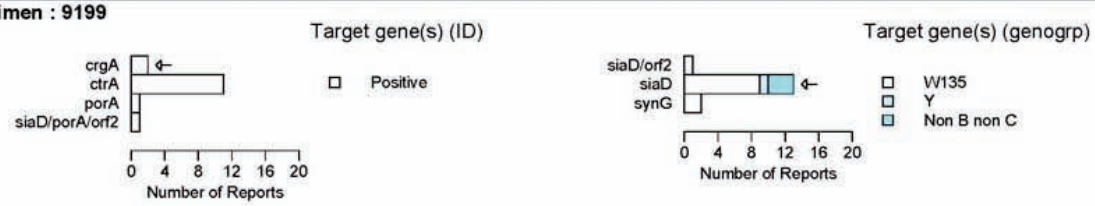
Specimen : 9199



Specimen : 9200

Specimen : 9201

Specimen : 9202

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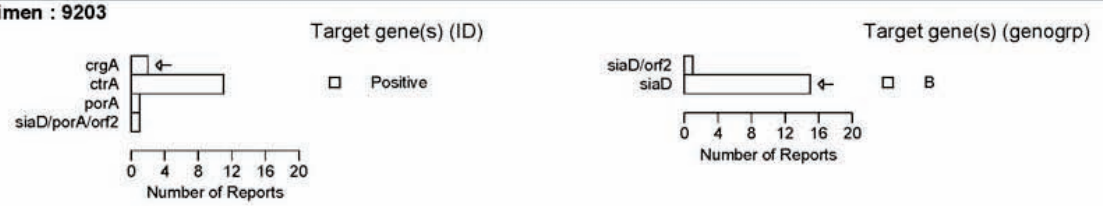
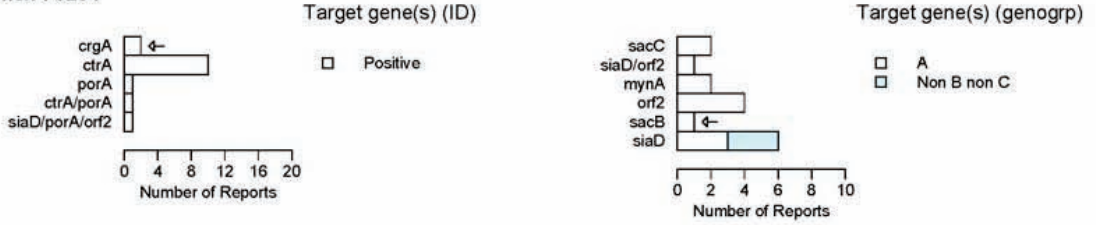
Specimen : 9203



Specimen : 9204



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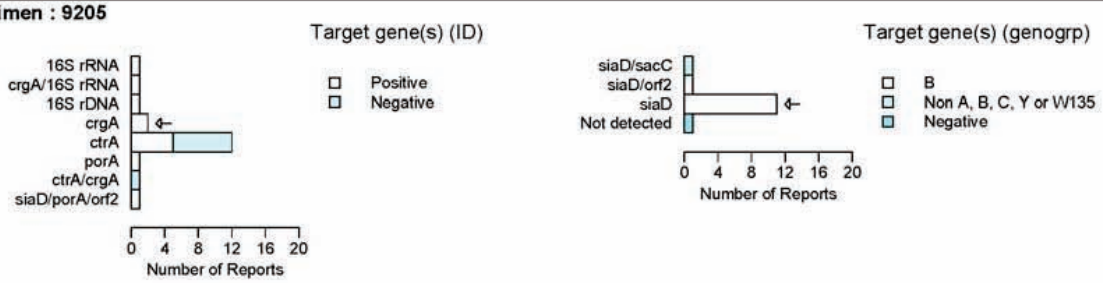
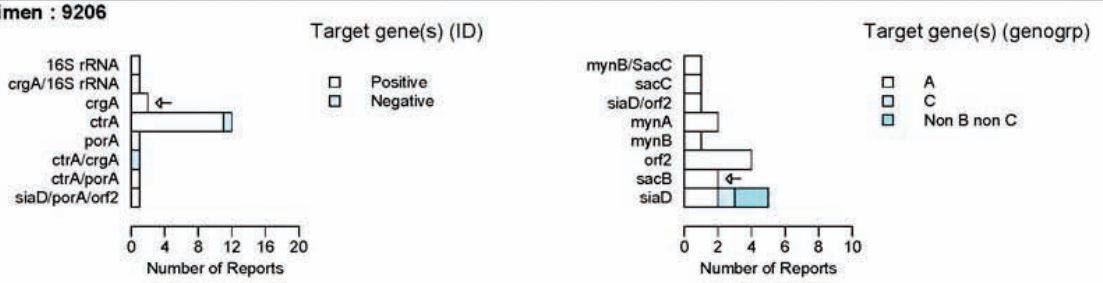


Specimen : 9206



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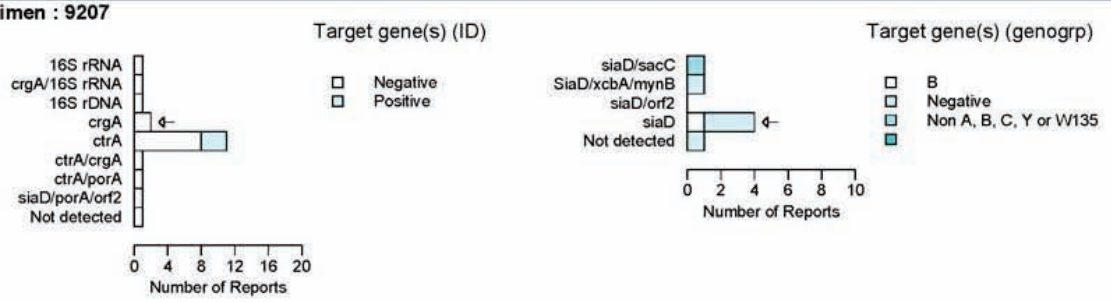
Specimen : 9207



Specimen : 9208

Specimen : 9209

Specimen : 9210

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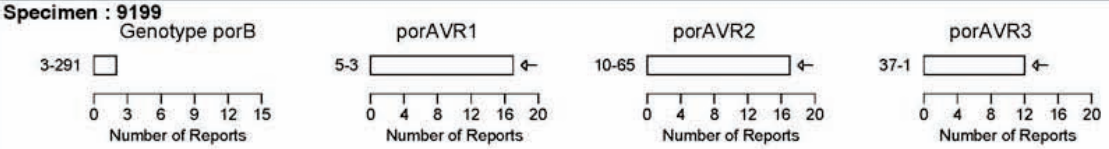
Specimen : 9199



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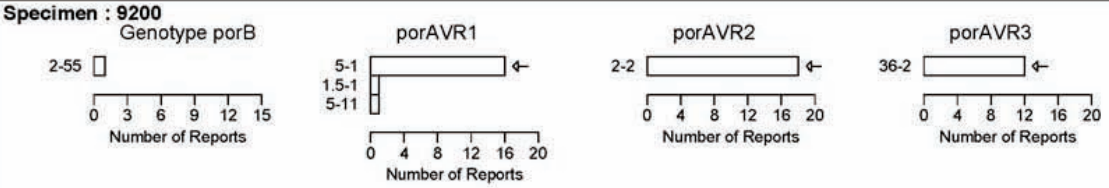
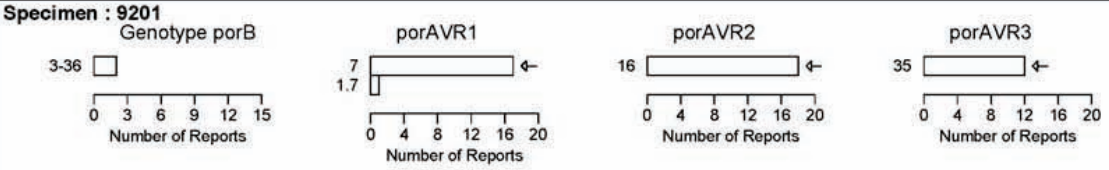
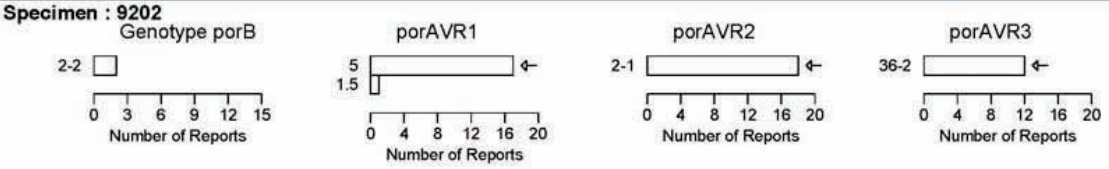


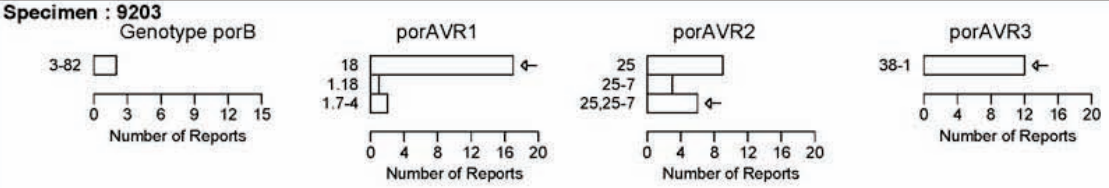
Specimen : 9201



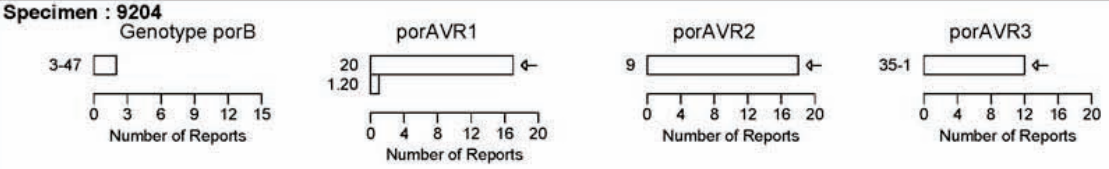
Specimen : 9202



Specimen : 9203



Specimen : 9204



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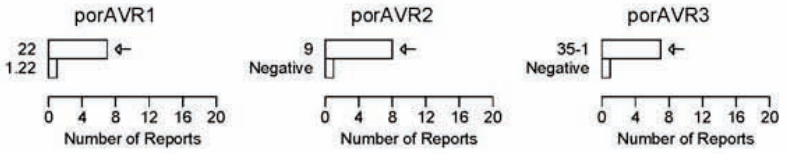
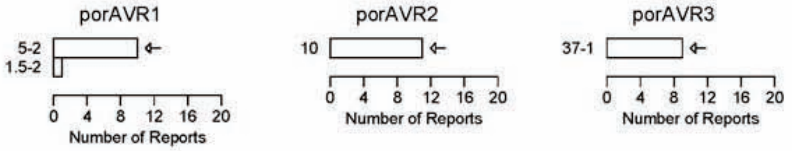
PART 3

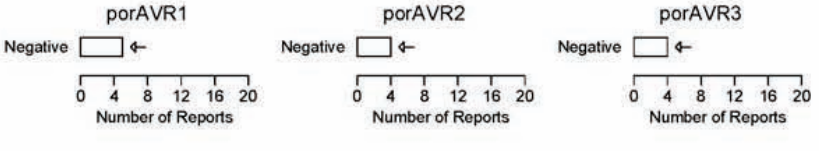
Specimen : 9205
Genotype porB



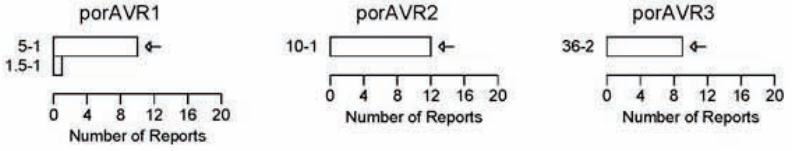
Specimen : 9206
Genotype porB



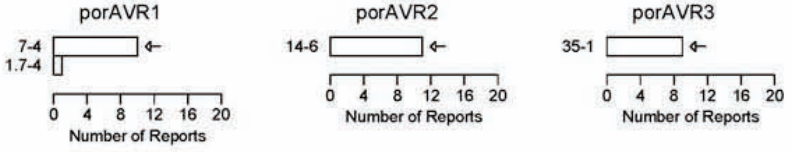
Specimen : 9207
Genotype porB



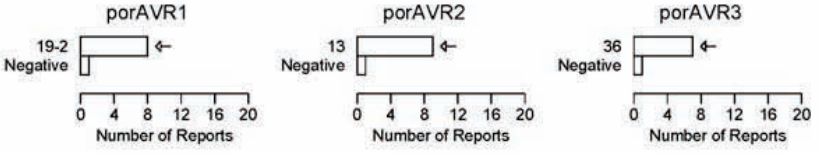
Specimen : 9208
Genotype porB



Specimen : 9209
Genotype porB



Specimen : 9210
Genotype porB



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Intended Result			Your Report	Your Score
Specimen 9199				
	MLST	6361	6361	Not scored
	MLST CC	174	174	Not scored
	fetA	1-7	1-7	Not scored
	penA	Not done	Not done	Not scored
Specimen 9200				
	MLST	23	23	Not scored
	MLST CC	23	23	Not scored
	fetA	1-7	1-7	Not scored
	penA	Not done	Not done	Not scored
Specimen 9201				
	MLST	32	32	Not scored
	MLST CC	32	32	Not scored
	fetA	3-3	3-3	Not scored
	penA	Not done	Not done	Not scored
Specimen 9202				
	MLST	11	11	Not scored
	MLST CC	11	11	Not scored
	fetA	5-5	5-5	Not scored
	penA	Not done	Not done	Not scored
Specimen 9203				
	MLST	414	414	Not scored
	MLST CC	41/44	41/44	Not scored
	fetA	Not done	1-5	Not scored
	penA	Not done	Not done	Not scored
Specimen 9204				
	MLST	Not done	4789	Not scored
	MLST CC	5	5	Not scored
	fetA	3-1	3-1	Not scored
	penA	Not done	Not done	Not scored

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Intended Result			Your Report	Your Score
Specimen 9205				
	MLST	Not done	Not done	Not scored
	MLST CC	Not done	Not done	Not scored
	fetA	Not done	5-1	Not scored
	penA	Not done	Not done	Not scored
Specimen 9206				
	MLST	Not done	Not done	Not scored
	MLST CC	Not done	Not done	Not scored
	fetA	Not done	3-5	Not scored
	penA	Not done	Not done	Not scored
Specimen 9207				
	MLST	Not done	Not done	Not scored
	MLST CC	Not done	Not done	Not scored
	fetA	Not done	Not done	Not scored
	penA	Not done	Not done	Not scored
Specimen 9208				
	MLST	Unassigned	Not done	Not scored
	MLST CC	Not done	Not done	Not scored
	fetA	Not done	4-23	Not scored
	penA	Not done	Not done	Not scored
Specimen 9209				
	MLST	Not done	Not done	Not scored
	MLST CC	Not done	Not done	Not scored
	fetA	Not done	3-9	Not scored
	penA	Not done	Not done	Not scored
Specimen 9210				
	MLST	Not done	Not done	Not scored
	MLST CC	Not done	Not done	Not scored
	fetA	Not done	1-45	Not scored
	penA	Not done	Not done	Not scored

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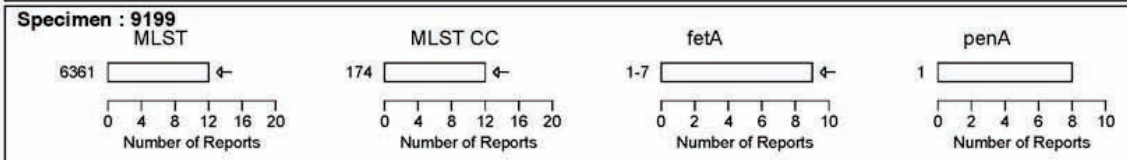
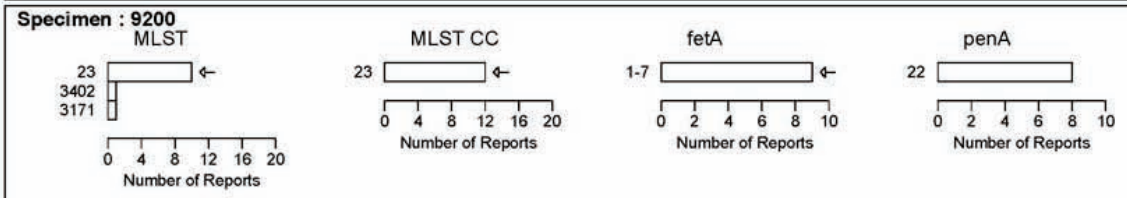
PART 3

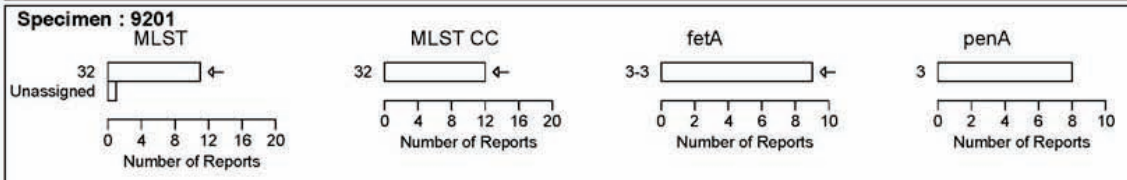
Specimen : 9199



Specimen : 9200



Specimen : 9201



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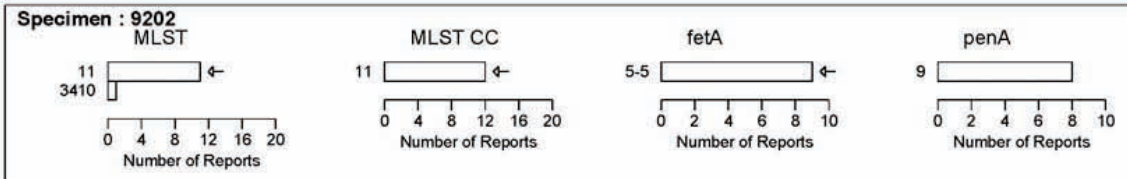

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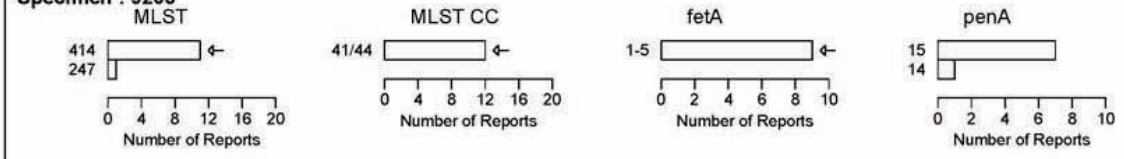

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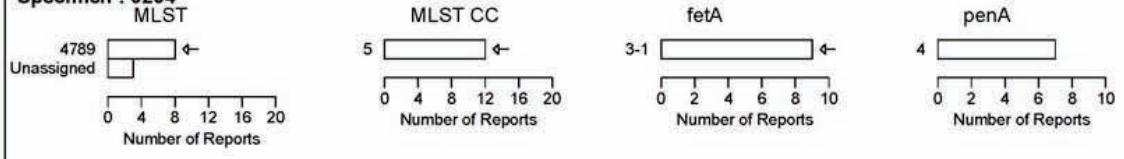

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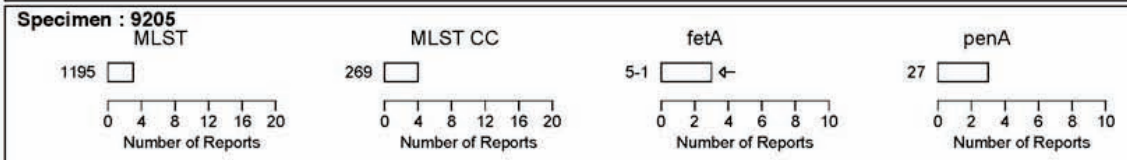
Specimen : 9205



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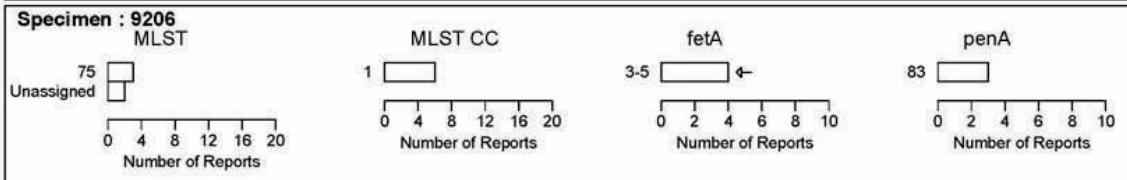

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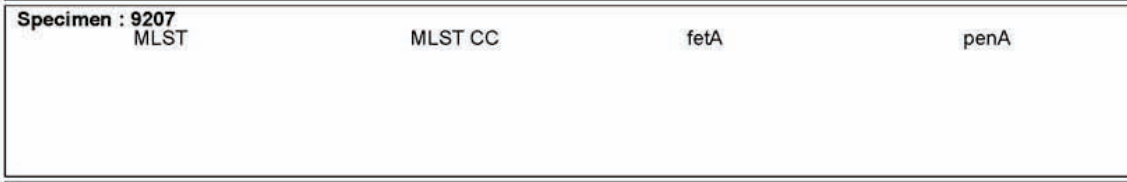

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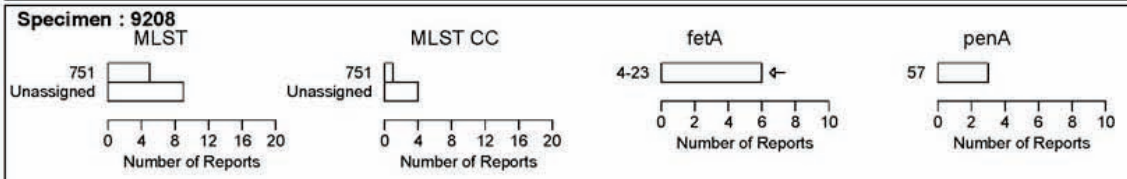

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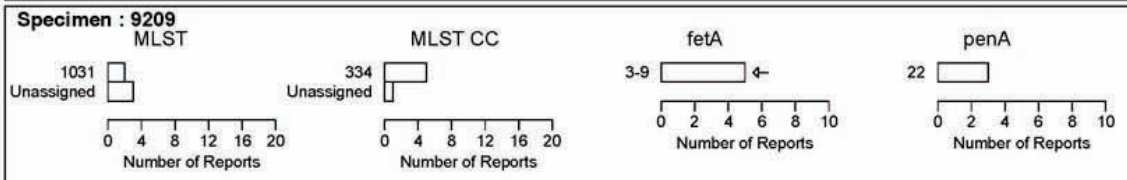
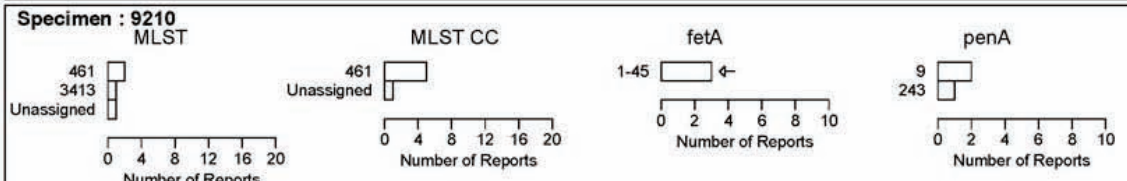


Specimen : 9210



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Neisseria meningitidis	Laboratory :
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Specimen type		Extraction method used	Amplification method	Detection method	Combination count
Organisms	ID	Boil	PCR - conventional	Gel electrophoresis	4
Organisms	ID	Boil	Real-time PCR	Real-time Taqman probes	2
Organisms	ID	Boil	Real-time PCR	Real-time fluorescence	3
Organisms	ID	Magnetic bead	Real-time PCR	Real-time Taqman probes	1
Organisms	ID	Spin column	PCR - conventional	Gel electrophoresis	3
Organisms	ID	Spin column	Real-time PCR	Real-time Taqman probes	2
Organisms	ID	Other	PCR - conventional	Gel electrophoresis	1
Organisms	ID	Other	Real-time PCR	Real-time Taqman probes	2
Organisms	ID	Other	Real-time PCR	Real-time fluorescence	1
Organisms	Genogroup	Boil	PCR - conventional	Gel electrophoresis	4
Organisms	Genogroup	Boil	PCR - conventional	Sequencing	1
Organisms	Genogroup	Boil	Real-time PCR	Real-time fluorescence	2
Organisms	Genogroup	Magnetic bead	Real-time PCR	Real-time Taqman probes	1
Organisms	Genogroup	Spin column	PCR - conventional	Gel electrophoresis	5
Organisms	Genogroup	Spin column	Real-time PCR	Real-time Taqman probes	2
Organisms	Genogroup	Other	PCR - conventional	Gel electrophoresis	3
Organisms	Genogroup	Other	Real-time PCR	Real-time Taqman probes	1
Organisms	Genogroup	Other	Real-time PCR	Real-time fluorescence	1
Blood	ID	Magnetic bead	Real-time PCR	Real-time Taqman probes	2
Blood	ID	Spin column	PCR - Conventional	Gel electrophoresis	5
Blood	ID	Spin column	PCR - Conventional	Sequencing	2
Blood	ID	Spin column	Real-time PCR	Real-time Taqman probes	3
Blood	ID	Spin column	Real-time PCR	Real-time fluorescence	3
Blood	ID	Other	Real-time PCR	Real-time Taqman probes	4
Blood	ID	Other	Real-time PCR	Real-time fluorescence	1
Blood	Genogroup	Magnetic bead	Real-time PCR	Real-time Taqman probes	1
Blood	Genogroup	Magnetic bead	Real-time PCR	Real-time fluorescence	1
Blood	Genogroup	Spin column	PCR - conventional	Gel electrophoresis	8
Blood	Genogroup	Spin column	Real-time PCR	Real-time Taqman probes	2
Blood	Genogroup	Spin column	Real-time PCR	Real-time fluorescence	2
Blood	Genogroup	Other	Real-time PCR	Real-time Taqman probes	2
Blood	Genogroup	Other	Real-time PCR	Real-time fluorescence	2
Serum	ID	Magnetic bead	Real-time PCR	Real-time Taqman probes	2
Serum	ID	Spin column	PCR - conventional	Gel electrophoresis	5
Serum	ID	Spin column	PCR - conventional	Sequencing	1
Serum	ID	Spin column	Real-time PCR	Real-time Taqman probes	4
Serum	ID	Spin column	Real-time PCR	Real-time fluorescence	3
Serum	ID	Other	Real-time PCR	Real-time Taqman probes	4
Serum	ID	Other	Real-time PCR	Real-time fluorescence	1
Serum	Genogroup	Salt precipitation	PCR - conventional	Gel electrophoresis	1
Serum	Genogroup	Magnetic bead	PCR - conventional	Gel electrophoresis	1
Serum	Genogroup	Magnetic bead	Real-time PCR	Real-time Taqman probes	1
Serum	Genogroup	Magnetic bead	Real-time PCR	Real-time fluorescence	1
Serum	Genogroup	Spin column	PCR - conventional	Gel electrophoresis	7
Serum	Genogroup	Spin column	Real-time PCR	Real-time Taqman probes	2
Serum	Genogroup	Spin column	Real-time PCR	Real-time fluorescence	2
Serum	Genogroup	Other	Real-time PCR	Real-time Taqman probes	3
Serum	Genogroup	Other	Real-time PCR	Real-time fluorescence	1

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