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TECHNICAL REPORT

External quality assessment scheme for *Neisseria meningitidis*

2014

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External quality assessment scheme for *Neisseria meningitidis* – 2014

As part of the IBD-labnet laboratory surveillance network

This report was commissioned by the European Centre of Disease Prevention and Control (ECDC), coordinated by Assimoula Economopoulou, and produced and written by Steve Gray (Public Health England, Meningococcal Reference Unit, Manchester, UK) and Vivienne James (UK NEQAS for Microbiology, hosted by Public Health England, Colindale, London) on behalf of the IBD-labnet consortium (referring to specific contract No. 4ECDC.4857).

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Abbreviations

CC	Clonal complex of multilocus sequence types
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
CSF	Cerebrospinal fluid
CT	Cycle threshold (the number of rounds of PCR [cycles] required to reach the positive cut-off value using real-time PCR assays)
CTX	Cefotaxime
DG SANCO	Directorate-General of Health and Consumers
EMGM	European Monitoring Group on *Meningococci*
EQA	External quality assessment
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU-IBIS Network	European Invasive Bacterial Infections Surveillance
FetA	Iron-binding protein (variable region used for *FetA* sequence typing)
I	Intermediate
IBD-labnet	Invasive bacterial diseases laboratory network
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRU	PHE Meningococcal Reference Unit
NIBSC	National Institute of Biological Standards and Control, supplier of monoclonal antibodies for phenotyping (South Mimms, UK)
PEN	Penicillin
PCR	Polymerase chain reaction
PHE	Public Health England (UK)
PHLS	The Public Health Laboratory Service (UK)
PorA	Porin A protein (variable regions VR1 and VR2 used for sequence typing)
R	Resistant
RIF	Rifampicin
S	Susceptible
ST	Sequence type of multilocus sequence typing
SU	Sulphonamide
TESSy	The European Surveillance System (ECDC)
UK NEQAS	United Kingdom National External Quality Assessment Service
VR	Variable region (of protein used for sequence typing)

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 may cause epidemic as well as endemic disease.

Meningococcal disease surveillance is paramount in meeting the following public health objectives: 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), surveillance for vaccination purposes, and the estimation of the burden of meningococcal disease. Meningococcal surveillance systems are partially based on laboratory diagnosis (including serogroup determination), therefore, there is a need for accuracy and proficiency in surveillance laboratory performance.

ECDC promotes the performance of external quality assessment (EQA) schemes, under 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 (e.g. serogroup) and typing methods as well as antimicrobial susceptibility testing, and the sensitivity of the methods in place to detect and confirm a specific pathogen or novel resistance patterns. This means that quality assessment enables laboratory performance to be assessed in comparison to reference methods and to other peer laboratories.

In July 2014, a panel of two viable isolates of *N. meningitidis* of the major disease-causing serogroups with three simulated CSF (non-culture) samples for molecular studies (one of which was negative for *N. meningitidis* DNA), was sent by UK NEQAS to 30 participating reference laboratories in the IBD-labnet surveillance network for quality assessment testing. The laboratories were asked to perform:

- phenotypic characterisation of viable isolates (serogroup and antimicrobial susceptibility testing (gradient diffusion MIC results))
- molecular characterisation by *porA* typing, *fetA* typing and MLST.

Genogroup characterisation of isolates was also requested where used routinely. Non-culture simulated septicaemia samples were characterised by molecular testing only: PCR species confirmation, genogroup, *porA* typing, *fetA* typing and MLST. The targets were specifically selected to reflect the meningococcal characterisation data most commonly reported to TESSy.

Overall, the EQA performance has shown that European *meningococcus* reference laboratories differ in their capacities and capabilities of the distributed *N. meningitidis* material, but that there have been improvements since the first ECDC IBD-labnet distribution.

The correct serogroup characterisation of viable isolates reported in 2014 was lower than the 90% reported in 2012, with correct reports received from 24–26 (80–87%) of the participating laboratories, depending on the sample.

The few incorrect phenotypic serogrouping reports (4/36) are most likely due to the participant's limited resources or reactivity of the reagents. This was similar to what was observed in the 2009, 2011 and 2012 EQA distributions.

The comparison of minimum inhibitory concentration (MIC) between laboratories requires a standard methodology such as that recommended by EMGM: gradient diffusion methodology (such as Etest) and a standardised agar plate medium (Müller-Hinton plus blood). Due to difficulties encountered in interpreting the 2009 EQA, laboratories were asked to report only the MIC values obtained. The EQA project manager then interpreted the reported MIC values in accordance with the EUCAST guidelines. Using EUCAST breakpoints, both isolates were susceptible to penicillin, ceftriaxone, cefotaxime, rifampicin and ciprofloxacin. MIC determinations in the 2014 distribution were in excellent agreement. Only 2/109 incorrect reports in the EUCAST resistant level and 11/109 in the EUCAST intermediate level were received.

There was a slight increase in the absolute number of participants that correctly reported the complete isolate 'fine types' in 2014 compared with 2012 (18/30 compared to 16/29). In contrast, there was slight reduction in the non-culture characterisations, most noticeable with MLST ST. In 2014, the ST consensus was correctly reported by only 1–2 (3–7%) of the participating laboratories, compared with 6/29 (21%) in 2012. This may reflect difficulties with the specific samples or changes in practice in the laboratories. Given the more exacting demands of the 2014 non-culture MLST analysis, there was excellent agreement (100%) for the few (5–7/30, 17–23%) reporting CC.

In conclusion, the results of the IBD-labnet EQA exercise 2014 suggest that regular EQA distributions for reference laboratories are required in order to further evaluate the integration of molecular typing methods, particularly with regard to more exacting samples (non-culture samples).

The majority of laboratories were able to report the serogroup (or genogroup) for isolates and non-culture samples in a standardised report form as captured within the UK NEQAS website (see main findings below). Non-culture detection was achieved by most laboratories too. Standardised reporting of sequence-based fine typing of the isolates was accomplished by over half the laboratories but was poor for the non-culture samples. This would suggest that network laboratories provide good-quality information on culture-proven cases but that at present (2014) detection is limited to genogroup level for non-culture samples.

Targeted training and support might be required to assist laboratories that have problems with organism characterisation and in particular the establishment of robust molecular typing techniques. In a number of countries that do not report data on molecular typing and detection, financial resources to purchase reagents and equipment are limited. Another obstacle to improved laboratory performance may be the small number of locally available case samples (isolates or non-culture samples).

Table A. Main findings from the 2014 *N. meningitidis* EQA

Main findings	Future direction	Possible actions
Excellent response to EQA distribution (30 responses) but not all laboratories provided results to all targets.	Need to determine the barriers preventing laboratories from completing characterisations. Why are some laboratories persistent non-responders?	• Targeted questionnaire • Regular EQA distributions • Support partnership with other participants. • Supportive visits to laboratories.
Phenotypic serogroup determination was successfully achieved by 80–87% (24–26/30) of laboratories.	Need to achieve agreement on a methodology and reagents to reduce 'auto-agglutinable' reports. Availability of reagents to discriminate serogroups Y and W.	• Targeted training • Regular EQA distribution • Encourage genogroup methodology for Y and W confirmation.
Standard methodology for MIC testing greatly improved quality of comparisons. Relatively few (and mainly minor) differences were observed.	Continue with EQAs. MIC data capture and analysis could be simplified through only reporting certain antibiotic MICs on certain organisms. Promote standardised methodology.	• Only accept EUCAST MIC values • Promote standardised methodology.
Genogroup is not tested for or reported routinely for isolates by many participants (11–12; 37–40%)	Encourage or expressly request that genogroup should be tested for.	Targeted training. Obtain agreement that genogroup testing is mandatory.
83% of laboratories were able to detect *N. meningitidis* in non-culture samples; 63–73% confirmed genogroup	Support laboratories with advice and training to establish molecular assays for non-culture *N. meningitidis* samples and genogroup confirmation.	• Targeted training and support • Recommend effective methodologies.
A minimum of 57% and 4% of laboratories were able to report 'fine type' of isolates and non-culture samples, respectively. All laboratories reporting 'fine type' results were in excellent agreement for isolates but far fewer participants reported complete non-culture characterisations.	• Support laboratories with training to establish assays. Increase the number of laboratories performing MLST for both isolates and non-culture samples. • Determine if laboratories routinely determine sequence types on all case isolates (and/or clinical samples).	• Targeted training and support (both sequencing and software) • Recommend effective methodologies.
Incomplete assessment of methods, reagents and processes used for molecular testing.	• If it is considered necessary to assess or compare reagents and protocols, a detailed questionnaire is required. • Consider distribution of more exacting non-culture material or a (commercial) DNA standard.	Targeted questionnaire to assess participants' routine testing for molecular detection and typing to determine if single-gene sequencing will be continued or if there is a move toward whole-genome sequencing of isolates. Is non-culture MLST a routine laboratory procedure at this laboratory or only conducted for EQAs?

Introduction

The European Centre for Disease Prevention and Control (ECDC) is a European Union agency with a mandate to operate dedicated surveillance networks 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 assessment schemes.' (Article 5.3, EC 851/2004[1]).

External quality assessment (EQA) is part of quality management and evaluates the performance of laboratories through an outside agency on material that is supplied specifically for this purpose. ECDC's disease-specific networks organise a series of EQA for EU/EEA countries. In some of these networks, non-EU/EEA countries are also involved in the EQA activities organised by ECDC. The aim of external quality assessments is to identify needs of improvement in laboratory diagnostic capacities relevant to the surveillance of diseases listed in 'Decision No 1082/2013/EU of the European Parliament and of the Council of 22 October 2013 on serious cross-border threats to health' and to ensure comparability of results in laboratories from all EU/EEA countries.

The main purposes of external quality assessment schemes are as follows:

- 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
- identification of needs for training activities.

N. meningitidis, meningococcal disease and epidemiology

N. meningitidis is a selective commensal and pathogen of humans. Up to 10% of the general population are carriers of meningococcus. 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. Meningococcal disease can be particularly severe in children: the attack rate and case-fatality ratio can be 20 times that of the adult population.

In outbreaks, meningococcal disease affects mostly older children, adolescents and adults. The epidemiology of the disease varies in different countries. In general, there is a pattern of endemicity interspersed with outbreaks. Many surface structures, e.g. capsule, lipopoly(oligo)saccharide, pili, are major contributors to the virulence of *N. meningitidis*.

The development of serological typing of meningococci was the basis of serogrouping of meningococci. Of the 13 recognised serogroups, five serogroups (A, B, C, Y, and W-135) are most commonly associated with disease, although instances of disease caused by serogroup X and 29E may be reported.

The geographical distribution of the serogroups shows that serogroup A strains cause most epidemics in the so-called meningitis belt (the Sahel region of sub-Saharan Africa) and Asia, but more localised epidemics of serogroup C may also occur. In the Americas, Europe and Australasia, meningococcal disease follows a seasonal pattern, is characterised by lower rates, and serogroups C and especially B are the most common serogroups. Serogroup Y infections have emerged as a significant cause of morbidity in the USA in recent years. A small but observable increase in serogroup Y cases (from a low base) has been noted in a number of European countries.

An increasing number of non-culture cases is being confirmed by local and reference laboratories within Europe. The application of PCR-based techniques has led to an increase in laboratory confirmations, and some countries now report up to 50% of cases as laboratory confirmed.

Molecular detection and typing techniques enable accurate and discriminatory typing and comparison of genetically and pathogenically distinct meningococci. The use of these techniques has provided, and will continue to provide, an improved understanding of the epidemiology of meningococcal disease.

Whole-genome sequencing, which is on the rise, is compatible with the sequence typing methods described above and can therefore be used to generate the same characterisations.

[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

European surveillance, ECDC programme and IBD-labnet

The European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) undertook the successful surveillance of invasive diseases caused by Neisseria meningitidis and Haemophilus influenzae. EU-IBIS was coordinated by the Public Health Laboratory Service in London, UK, between 1999 and September 2006; the network was funded by the European Commission (Directorate-General for Health and Consumers, DG SANCO).

In 2006, the network was integrated into ECDC and the implementation of laboratory surveillance methods was outsourced to a consortium of experts that constitute the IBD-labnet. The IBD-labnet consortium has achieved a consensus for the laboratory methods and variables to be used for the characterisation and discrimination of circulating meningococcal strains.

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.

Based on 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(VR):clonal complex (MLST), where the clonal complex may be determined even if the full ST designation was not possible.

This scheme provides the highest resolution with the lowest sequencing efforts and costs and was therefore recommended for inclusion in the TESSy database. Consensus was also achieved on antimicrobial susceptibility testing for the surveillance of antimicrobial susceptibility. The minimum inhibitory concentrations (MICs) for rifampicin (RIF), penicillin (PEN), ciprofloxacin (CIP), cefotaxime (CTX) and ceftriaxone (CRO) were recommended as the laboratory variables for meningococci to be determined, recorded and collated by ECDC.

EQA role and aims

In order to support the Member States and further build capacity, ECDC has conducted a series of EQA exercises to ensure that European laboratory surveillance delivers high-quality, standardised results and that training needs can be properly assessed. The ECDC-funded IBD-labnet EQA allows reference laboratories to compare test results so that they can achieve the same level of characterisation for both culture and non-culture-confirmed (PCR only) cases of meningococcal disease.

It was acknowledged that some countries were not able to provide their own molecular typing data for local and European surveillance due to economic reasons. Some countries have the capacity to process large numbers of samples and have additional spare capacity for molecular typing and therefore offered their help to countries that have not yet implemented molecular typing methods. This has further improved European surveillance and molecular typing of *N. meningitidis*.

This report describes the fourth ECDC-funded EQA. Previous EQA rounds took place in in 2009, 2011 and 2012. All reports are available from: http://ecdc.europa.eu/en/publications/.

1 Materials and methods

1.1 Objectives

The objectives of the 2014 EQA exercise were to:

- improve data quality, assist in the standardisation of techniques, and facilitate consistent epidemiological data for submission to the ECDC TESSy database.
- support the move towards molecular detection, confirmation and accurate characterisation of *N. meningitidis*

It was envisaged that the reference laboratories would store the viable cultures and retain any unused non-culture 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.

1.2 Study design

The number of isolates and non-culture samples distributed in 2014 was reduced in response to feedback from previous EQA schemes: participants claimed that large panels of isolates (and non-culture samples) were unduly costly to process and caused a very high workload for some of the laboratories.

The 2014 EQA scheme therefore featured only a small panel of material comprising viable *Neisseria meningitidis* isolates and non-viable simulated clinical samples for phenotypic and genotypic characterisation (where possible), which was distributed to all EU Member States and candidate countries with suitable reference facilities.

UK NEQAS produced an anonymised summary which showed the submitted results of the participating laboratory, the consensus result, and the number of laboratories for each submitted result. The assumption was made that the consensus result was most likely the correct result.

The EQA also allowed for the collection of additional supportive information relating to the gene (molecular) targets used for detection and serogroup designation, including the option to report the techniques used for nucleic acid extraction, amplification and detection. Also included was a short anonymous survey on laboratory facilities and equipment. In addition, methodological information was collected to assess how certain techniques influence laboratory performance.

The participating reference laboratories were then asked to compare their submitted results to the consensus results to determine differences. Laboratories could then investigate differences such as molecular typing designation difference (*porA*, *fetA* or MLST) to study the quality of the chromatogram and base-calling or even the clerical process. Phenotypic serogroup or MICs could be repeated by the laboratory to resolve discrepancies.

Table 1 indicates the procedures and test results required for reporting via the UK NEQAS website.

Table 1. Tests requested of the participating laboratories

Procedure	Isolates	Non-culture (simulated CSF)	Technique name
Phenotype	Serogroup	-	Serology (agglutination, co-agglutination, latex or ELISA)
	MICs: PEN, CTX, CRO, RIF, CIP	-	Gradient diffusion
Genotype	-	Species DNA detection	PCR or real-time PCR
	Genogroup	Genogroup	PCR or real-time PCR
	porA (VR1 and VR2)	*porA* (VR1 and VR2)	DNA sequencing
	fetA VR	*fetA* VR	DNA sequencing
	MLST (cc and ST)	MLST (cc and ST)	DNA sequencing

1.3 Participants

Thirty European meningococcal reference laboratories participated in the 2014 IBD-labnet EQA distribution.

The participant countries were: Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom.

1.4 The 2014 EQA panel – expected results

1.4.1 Isolates

The 2014 *N. meningitidis* IBD-labnet EQA panel (UK NEQAS distribution 3630) consisted of two viable isolates of *N. meningitidis*: serogroup C (sample id 2502) and W135 (2503), as described in Table 2.

The isolates were fully sequenced as identified within the Meningitis Research Foundation – Meningococcal Genome Library, http://www.meningitis.org/research/genome, Table 3.

1.4.2 Non-culture samples

The non-culture samples were designed to simulate CSF and comprised heat-killed suspensions of meningococci diluted by UK NEQAS in CSF matrix (sucrose-albumin solution). The two positive samples 2504 (group Y) and 2505 (group B) were diluted to simulate a 'medium level' and 'low level–weak' positive as indicated by the PHE MRU real-time (ABI Taqman) PCR assay; 2504 and 2505 were fully characterised as noted in Tables 2 and 3; the estimated viable concentrations are given in Table 4.

Sample 2506 was a Negative non-culture sample. It did not contain any bacterial DNA: Nm-, Spn- and Hi-.

Table 2. Expected characterisation results for isolates and non-culture samples in 2014 EQA panel 3630

Sample ID	Sample type	Species	Phenotype	Serogroup/ genogroup	AST					*porA* VR1	*porA* VR2	*fetA*	MLST	
					PEN	CRO	CTX	RIF	CIP				ST	CC
2502	Isolate	*Nm*	**C**: 14:P1.19,15	**C**	**S**	**S**	**S**	**S**	**S**	19–1	15–11	1–7	467	269
2503	Isolate	*Nm*	**W**: 2a:P1.5,2	**W**	**S (I)**[1]	**S**	**S**	**S**	**S**	5	2	1–146	11	11
2504	Non-culture	*Nm*	**Y**: NT:P1.5	**Y**	-	-	-	-	-	5–1	10–4	4–1	1655	23
2505	Non-culture	*Nm*	**B**: 4:P1.4	**B**	-	-	-	-	-	7–2	4	1–5	41	41/44
2506	Non-culture	Negative	-	-	-	-	-	-	-	-	-	-	-	-

[1] *Penicillin MIC at EUCAST susceptible cut-off*

Table 3. Isolate and non-culture samples defined by whole-genome sequencing within the Meningitis Research Foundation – Meningococcal Genome Library[1]

Nm sample	MRF MGL id[1]	PHE MRU laboratory number	EQA sample type	*N. meningitidis* group
2502	20031	10-240631	culture	C
2503	28142	13-240114	culture	W135
2504	28228	13-240265	Non-culture	Y
2505	28012	13-240234	Non-culture	B
2506[2]	-	-	Non-culture	Negative (*Nm*-, *Spn*-, *Hi*-)[2]

[1] http://www.meningitis.org/research/genome

[2] *Negative 2506 was only supplied with the Nm panel, 3630*

Table 4. Estimated viable concentration of non-culture samples

Non-culture samples	Estimated viable orgs/mL	Expected real-time PCR CT[1]	Comment
2504	3.0×10^4	~ 32–33	+ positive
2505	2.2×10^3	~ 33–35	low positive

[1] *Using Qiagen QIAamp Media MDx extraction and ABI TaqMan PHE MRU PCR assays*

1.4.3 Receipt, testing and reporting of the *N. meningitidis* EQA panel

The participating laboratories were advised to reconstitute and handle the EQA material in a safe manner and test the samples with the methods available to them to confirm the identity of the samples and characterise them. Participants were encouraged to use the same methods they routinely use but were not discouraged from using techniques or reagents they usually do not apply to routine samples.

Results were to be returned to UK NEQAS by 13 August 2014 (17:00 GMT) via the UK NEQAS website (https://results.ukneqas.org.uk) or transmitted by fax; the reporting form was included with the EQA samples.

1.4.4 Standards and accreditation

There was no requirement for participant laboratories to be operating in accordance with ISO standards although it was assumed that national accreditation requires evidence of participation in relevant EQA schemes.

UK NEQAS is an accredited organisation whose schemes are accredited by Clinical Pathology Accreditation UK Ltd under Centre Reference Number 0001. The PHE MRU is accredited within the Manchester Medical Microbiology Partnership by Clinical Pathology Accreditation UK Ltd under Centre Reference Number 0635.

1.4.5 Website result submission

Participants were able to enter characterisation results to the website at any time until the closing date (deadline). Corrections could be entered by resubmitting the corrected results, thus overwriting earlier submissions. It was also possible to print out submissions at any time.

1.4.6 Assessment of performance

The EQA was designed to collect characterisation data from participants to determine the consensus value or result. Reports were sent to participants showing their own results compared to the consensus for the characterisation targets. For the MICs, all submitted values were shown and the mode indicated.

Anonymity was maintained as individual participants could not determine the identity or results of other participants.

Participants were not scored on their results or performance but actively encouraged to compare their results to the consensus and determine if they did or did not achieve the consensus result. It was assumed that participants would be able to resolve issues themselves; in addition, there were opportunities to discuss results at the annual IBD-labnet meeting, either during the plenum or informally with other participants or the EQA co-ordinator.

2 Results and discussion

EQA panels were distributed to 30 countries, and 30 results reports were returned to UK NEQAS. A draft report was produced and made available to all the participating reference laboratories via the UK NEQAS website (https://results.ukneqas.org.uk). Access was only possible with the laboratories' unique code.

2.1 Characterisation of viable isolates

The phenotypic characterisation of the two viable isolates (samples 2502 and 2503) was very successful, with serogroup reports returned from 29/30 (97%). Only one laboratory did not confirm (report) the *N. meningitidis* isolate group by serology but preferred genogrouping (PCR) alone.

The consensus mode MIC reports are shown in Table 5. The reported MIC values were converted to the equivalent EUCAST MIC when necessary (some commercial gradient MIC strips use a different MIC dilution series). Participants were not requested to report susceptibility interpretation by EUCAST (or any other guidelines).

A summary of the tests for which each laboratory submitted results is given in Table 6.

Table 5. Consensus MIC reports. EUCAST MIC dilution

	Sample 2502		Sample 2503	
	Mode MIC (mg/L)	EUCAST interpretation	Mode MIC	EUCAST interpretation
CIP	0.008	S	0.008	S
CRO	<0.002	S	0.002, <0.016[2]	S
CTX	0.004, <0,016[2]	S	0.008	S
PEN	0.06	S	0.06	S (I)[1]
RIF	0.03, 0.016[2]	S	0.008	S

Note: Converted from reported MIC to equivalent EUCAST dilution

[1] *Penicillin MIC at EUCAST cut-off values*

[2] *Bi-modal MIC as some laboratories used high-range strips with the lowest MIC value at 0.0016mg/L*

Table 6. Isolate characterisation; summary of tests for which each laboratory submitted results

	Phenotypic characterisation		Genotypic characterisation					
	Serogroup	AST	Genogroup	*porA* VR1	*porA* VR2	*fetA*	MLST	
							CC	ST
NM20	+	+	+	+	+	+	+	+
NM21	+	+	+	+	+	+	+	+
NM22	+!	+	+	+	+	+	+	+
NM23	+	+	+	+	+	-	+	+
NM24	+	+	+	+	+	+	+	+
NM26	+	+	+	+	+	+	-	-
NM27	+	+	+	+	+	+	+	+
NM28	+	+	+	+	+	+	+	+
NM29	+	+	-	+	+	+	+	+
NM30	+	+	-	+	+	+	+	-
NM31	+	+	-	+	+	+	+	+
NM32	-	+	+	+	+	+	+	+
NM33	+	-	-	+	+	+	+	+
NM34	+	+	+	+	+	+	+	+
NM35	+	+	-	+	+	+	+	+
NM36	+	+	-	-	-	-	-	-
NM37	+	+	+	+	+	+	+	+
NM38	+!	+	-	-	-	-	-	-
NM39	+	+	-/+	+	+	+	+	+
NM40	+	+	-	-	-	-	-	-
NM41	+	+	+	+	+	+	+	+
NM42	+	+	+	+	+	+	+	+
NM43	+!	+	+	-	-	-	-	-
NM45	+!	+	+	-	-	-	-	-
NM47	+	+	+	+	+	+	-	-
NM48	+!	+	-	-	-	-	-	-

	Phenotypic characterisation		Genotypic characterisation					
	Serogroup	AST	Genogroup	*porA* VR1	*porA* VR2	*fetA*	MLST	
							CC	ST
NM49	**+!**	**+**	**+**	**-**	**-**	**-**	**-**	**-**
NM51	**+!**	**+**	**+**	**-**	**-**	**-**	**-**	**-**
NM52	**+!**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
NM54	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**-**

+: Test method performed

-: tTest method not performed

-/+: test method was performed only on one of the two samples.

! Non-consensus result for one sample

2.1.1 Serogroup/genogroup of isolates

Phenotypic and genotypic group

The maximum number of reports for phenotypic (serological) serogroup determination was 29/30 (97%).

2502: 26/30 (87%) laboratories sent correct reports for serogroup C; 3/30 (10%) submitted incorrect reports. Of the incorrect reports, two stated that the isolate was auto-agglutinable and one that it was not serogroupable. Eighteen (18/30; 60%) laboratories reported genogroup C, and 12/30 (40%) laboratories did not report a genogroup on the isolate presumably as serogrouping was the routine testing procedure. One laboratory (NM32) reported genogroup results only.

Combining the correct serogroup and genogroup results resulted in 28 out of 30 laboratories (93%) that determined the correct serogroup, i.e. C.

Sixty-seven per cent (20/30) of the laboratories confirmed both *porA* VR1 19-1 and VR2 15-11, with 10/30 (33%) laboratories not reporting *porA* VRs (NM36, NM38, NM40, NM43, NM45, NM48, NM49, NM51, NM52, and NM54).

Other results:

- 19/30 (63%) laboratories confirmed the *fetA* VR F1-7, with 11 (37%) laboratories not reporting *fetA* VR (NM23, NM36, NM38, NM40, NM43, NM45, NM48, NM49, NM51, NM52, and NM54).
- 18/30 (60%) laboratories confirmed MLST CC 269, and 17 (57%) MLST ST467; 12/30 (40%) and 13/30 (43%) laboratories did not report MLST CC or MLST ST, respectively. NM30 stated that they had only achieved partial MLST characterisation (6/7 loci) sufficient for MLST CC but not ST.
- 2503: 24/30 (80%) of serogroup W135 report were correct, one was incorrect (not serogroupable); 4/30 (13%) laboratories reported serogroup Y/W135. One laboratory reported genogroup results only, and did so correctly.
- 19/30 (63%) laboratories reported genogroup W135 and 11/30 (37%) laboratories did not report a genogroup on the isolate, presumably because serogrouping was the routine testing procedure. Therefore 29/30 (97%) could confirm the correct W135 report.
- 20/30 (67%) laboratories confirmed both *porA* VR1 5 and VR2 2, with 10 (33%) laboratories not reporting *porA* VRs (NM36, NM38, NM40, NM43, NM45, NM48, NM49, NM51, NM52, and NM54).
- 19/30 (63%) laboratories confirmed the *fetA* VR F1-7, with 11/30 (40%) laboratories not reporting *fetA* VR (NM23, NM36, NM38, NM40, NM43, NM45, NM48, NM49, NM51, NM52, and NM54).
- 18/30 (60%) laboratories confirmed MLST CC 11 and 17 (57%) also confirmed MLST ST11; 12/30 (40%) and 13/30 (43%) laboratories did not report MLST CC or MLST ST, respectively. NM30 stated that they had only achieved partial MLST characterisation (6/7 loci), sufficient for MLST CC but not ST.

2.1.2 Antimicrobial susceptibility – MIC results

MIC reports were submitted by up to 29/30 (97%) laboratories, depending on the antibiotic (see Table 7). The following number of reports were received: 29 (97%) for penicillin, 28 (93%) for ciprofloxacin, 26 (87%) for rifampicin, 24 (80%) for cefotaxime and 22 (73%) for ceftriaxone.

Laboratories were requested to report the MIC value and not the corresponding EUCAST dilution value; EUCAST interpretation was not requested.

Table 7. Isolate characterisation; gradient diffusion MIC values reported by the laboratories

	2502 MIC (mg/L)					2503 MIC (mg/L)				
	PEN	CRO	CTX	RIF	CIP	PEN	CRO	CTX	RIF	CIP
NM20	0.032	NE	0.002	0.016	0.006	0.064	NE	0.006	0.008	0.006
NM21	0.032	NE	0.003	0.064	0.004	0.032	NE	0.006	0.016	0.004
NM22	0.047	<0.002	NE	0.094	0.016	0.125	0.004	NE	0.047	0.012
NM23	0.032	<0.002	0.002	0.023	0.008	0.047	<0.002	0.006	0.125	0.012
NM24	0.125	<0.002	0.002	0.016	0.008	0.25	<0.002	0.002	0.008	0.016
NM26	0.064	<0.002	NE	0.032	0.012	0.094	0.002	NE	0.023	0.012
NM27	0.064	NE	0.006	0.023	0.016	0.094	NE	0.008	0.012	0.023
NM28	0.047	NE	<0.016	0.032	0.003	0.125	NE	<00.016	0.008	0.004
NM29	0.047	NE	0.003	0.032	0.006	0.064	NE	0.006	0.023	0.008
NM30	0.047	<0.002	0.004	0.023	0.006	0.064	<0.002	0.004	0.016	0.006
NM31	0.047	<0.002	0.003	0.016	0.006	0.064	0.002	0.006	0.023	0.006
NM32	0.064	<0.016	NE	0.016	0.006	0.094	<0.016	NE	0.008	0.006
NM33	NE[1]	NE	NE	NE	NE	NE	NE	NE	NE	NE
NM34	0.023	<0.002	0.004	0.032	0.004	0.047	<0.002	0.006	0.016	0.006
NM35	0.03	NE	NE	NE	NE	0.06	NE	NE	NE	NE
NM36	0.012	<0.002	<0.002	0.023	0.002	0.047	<0.002	0.002	0.008	0.004
NM37	0.064	0.002	<0.016	0.023	0.006	0.094	0.004	0.023	0.023	0.006
NM38	0.023	0.016	0.016	0.004	0.004	0.064	0.016	0.016	0.002	0.004
NM39	0.064	0.002	0.016	0.032	0.008	0.064	0.002	0.016	0.032	0.008
NM40	0.06	0.016	0.016	NE	0.012	0.094	0.016	0.016	NE	0.012
NM41	0.047	<0.016	<0.016	0.016	0.004	0.094	<0.016	<0.016	0.008	0.008
NM42	0.047	NE	0.004	0.023	0.006	0.064	NE	0.006	0.008	0.008
NM43	0.047	<0.002	0.004	0.032	0.006	0.064	0.002	0.008	0.012	0.008
NM45	0.032	0.02	0.02	0.02	0.02	0.032	0.04	0.04	0.02	0.02
NM47	0.047	<0.016	<0.016	0.008	0.003	0.047	<0.016	<0.016	0.032	0.004
NM48	0.032	1.0	NE	0.008	1.0	0.023	<0.016	NE	0.008	0.003
NM49	0.012	0.002	0.002	0.016	0.004	0.023	0.002	0.008	0.016	0.004
NM51	0.047	<0.016	<0.016	0.047	0.006	0.064	<0.016	<0.016	0.023	0.006
NM52	0.032	<0.002	0.003	NE	0.003	0.064	0.002	0.006	NE	0.003
NM54	0.064	<0.016	0.004	0.032	0.004	0.125	<0.016	0.006	0.012	0.006

[1]*NE: Not evaluated. Laboratories recorded 'NE' or left report field blank; assumed gradient diffusion MIC not tested for that antibiotic.*

Laboratory NM33 did not report (or test) any antibiotic MICs. All other laboratories tested and reported at least three antibiotic MICs including PEN. RIF was not reported by NM35, NM40 and NM52.

Using EUCAST breakpoints, it was determined that both isolates (2502 and 2503) were susceptible to penicillin, ceftriaxone, cefotaxime, rifampicin and ciprofloxacin; see Table 5. It should be noted that for 2503 the penicillin MIC = 0.06, which is EUCAST cut-off.

EUCAST susceptibility interpretation was not required for this EQA; reporting the obtained MIC value was sufficient. Participants were encouraged to compare their reported MICs to the mode and range as stated in their individual reports from UK NEQAS.

For sample 2502, discrepant results (MICs not interpreted as EUCAST susceptible) were ciprofloxacin (MIC 1.0 mg/L = resistant) and ceftriaxone (MIC 1.0 mg/L = resistant) by NM48. All reported penicillin MICs were susceptible except for intermediate values of 0.125 mg/L by NM24. All reported cefotaxime and rifampicin MICs were susceptible.

For sample 2503, all reported MICs for ciprofloxacin, ceftriaxone, cefotaxime and rifampicin were susceptible. A number of laboratories reported MICs in the EUCAST intermediate levels: 0.094 mg/L by NM26, NM27, NM32, NM37, NM40 and NM41; 0.125mg/L by NM22, NM28 and NM54; and 0.25 mg/L by NM24.

Most laboratories used commercial gradient diffusion strips started at 0.002 mg/L (low-level range strips) for all antibiotics, but several laboratories used a higher starting dilution of 0.016 mg/L. This meant that some results were recorded as <0.016 mg/L or 0.016 mg/L and it was therefore impossible to assign agreement to the consensus in most instances. This has no bearing on interpretation of EUCAST susceptibility as all breakpoints are > 0.016 mg/L. The low-range strips offer the ability to more accurately monitor minor susceptibility changes in the meningococcal population and also compare laboratories' performance.

2.1.3 MIC materials and methodology

A small set of questions was included with the EQA panel requesting participants to record: the gradient diffusion method, the commercial strip manufacturer (supplier), the agar plate medium and the manufacturer (supplier).

One laboratory, NM33, did not report MIC results. Therefore, the denominator was adjusted to 29 from the maximum 30 for the analysis of the MIC materials and methodology used.

Ninety-three percent (27/29) of the laboratories reporting MICs stated Etest methodology and 3% (1/29) 'other method' in response to the gradient MIC question. One participant left the field blank but reported Liofilchem.

A maximum of 28/29 responses regarding the commercial gradient strip manufacturer were made but dependent upon the antibiotic. For penicillin, where there were 28/29 responses, the breakdown was: AB biodisk-bioMerieux 19 (66%), Liofilchem 6 (21%), Oxoid 1 (3%), and other 1 (3%).

There was no apparent association of 'outlier' MIC reports with medium composition, supplier or commercial strip supplier. However, it was interesting to note that four laboratories (NM37, NM48, NM37 and NM48) did not indicate the exact methodology or strip manufacturer; NM37 and NM48 were already mentioned in section 2.1.2 because of discrepant MICs.

2.1.4 MIC conclusion

The MIC testing of the two susceptible isolates demonstrated that there were no issues affecting MIC determination other than availability of reagents (MIC gradient strips) and the different usage of the MIC ranges. The only clinically relevant discrepancies were by NM48 with regard to ceftriaxone and ciprofloxacin.

The use of high range-gradient diffusion strips for CRO and CTX is acceptable clinically but does not allow for accurate surveillance of trends at lower (susceptible) dilutions.

The use of standard (control) organisms to allow for local checking of MIC methodology is recommended as is the storage of the EQA panels for regular re-testing and review.

2.2 Simulated non-culture samples

2.2.1 Species detection and *N. meningitidis* genogroup confirmation

Three simulated CSF samples (2504, 2505 and 2506) were distributed. The freeze-dried sera were re-constituted in sterile pharmacy (or molecular) grade water and the nucleic acids extracted by the routinely available local methods.

Two samples contained *N. meningitidis* DNA: 2504 a 'medium-level positive' group Y and 2505 'a low-level' group B, as characterised in Tables 2 and 4.

Sample 2506 contained no bacterial DNA (negative *for N. meningitidis*).

A summary of the test for which each laboratory submitted results is given in Table 8.

Table 8. Simulated non-culture samples characterisation; summary of tests for which each laboratory submitted results

	Detection	Genogroup	*porA* VR1	*porA* VR2	*fetA*	MLST	
						CC	ST
NM20	+	+	+	-	-	-	-
NM21	+	+	+	+	+	+	-
NM22	+	+	-	-	-	-	-
NM23	+	+	+	+	+	-	-
NM24	+	+!	+/-	+/-	+	+	-
NM26	+!	+!	-	-	-	-	-
NM27	+	+	+/-	+/-	+	-	-
NM28	+	+	-	-	-	-	-
NM29	+	+	+	+	+	-	-
NM30	+	+	+	+	+	-	-
NM31	+	+	+	+	+	+	+
NM32	+	+	+	+	+	-	-
NM33	-	-	-	-	-	-	-
NM34	+	+	+	+	+	+	+!
NM35	+	+	-	-	-	-	-
NM36	-	-	-	-	-	-	-

	Detection	Genogroup	*porA* VR1	*porA* VR2	*fetA*	MLST	
						CC	ST
NM37	+/-	+/-	+!/-	-	+!/-	+/-	-
NM38	+	-	-	-	-	-	-
NM39	+	+	+	+	+	-	-
NM40	+	-	-	-	-	-	-
NM41	+	+!	+	+	+/-	+/-	-
NM42	+	+	-	-	-	-	-
NM43	+	+	-	-	-	-	-
NM45	+	+	-	-	-	-	-
NM47	+	+	+	+	+	-	-
NM48	+	-	-	-	-	-	-
NM49	+	+	+	-	-	-	-
NM51	+	+!	-	-	-	-	-
NM52	-	-	-	-	-	-	-
NM54	-	-	-	-	-	-	-

+: Test method performed

-: Test method not performed

-/+: Test method performed only on one of the two samples

! Non-consensus result for one sample

- 2504: 25/30 (83%) participants detected *N. meningitidis* DNA; 4/30 (13%) did not report any data (NM33, NM36, NM52 and NM54); only one laboratory (NM26) reported an incorrect, negative result.
- 19/30 (63%) laboratories confirmed group Y; 7/30 (23%) laboratories (NM33, NM36, NM38, NM40, NM48, NM52 and NM54) did not report a genogroup, and 4/30 (13%) laboratories provided incorrect reports. The incorrect reports included two group C (NM24 and NM51) and two reports of 'Not A, B, C, Y or W135' (NM26 and NM41).
- 11/30 (37%) laboratories submitted correct *porA* VR1 5-1 reports; one incorrect report (of 7-1) came from NM37; 18/30 (60%) participants did not report *porA* VR1.
- Correct *porA* VR2 10-4 reports were made by 10/30 (33%) laboratories, with 20 (67%) not reporting *porA* VR2.
- 12/30 (40%) laboratories submitted correct *fetA* VR F4-1 reports; one incorrect (F5-1) report was sent by NM37; 17/30 (57%) participants did not report *fetA* VR.
- 7/30 (23%) participants determined the correct MLST CC 23; only two of the seven confirmed MLST ST 1655 (NM31 and NM34). 23/30 (77%) laboratories did therefore not report MLST CC, and 28/30 (93%) failed to report MLST ST. Three participants (NM24, NM30 and NM41) reported evidence of partial MLST characterisation; NM24 and NM41 reported MLST CC only.
- 2505: 25 (83%) participants detected *N. meningitidis* DNA and 5/30 (17%) did not report anything (NM33, NM36, NM37, NM52 and NM54).
- 22/30 (73%) laboratories confirmed group B; 8/30 (27%) laboratories (NM33, NM36, NM37, NM38, NM40, NM48, NM52 and NM54) did not report a genogroup. There were no incorrect reports for genogroup.
- 13/30 (43%) laboratories submitted correct *porA* VR1 7-2 reports; 17/30 (57%) participants did not report *porA* VR1.
- Correct *porA* VR2 4 reports were made by 12/30 (40%) laboratories with 18/30 (60%) not reporting *porA* VR2.
- 11/30 (37%) correct *fetA* VR F1-5 reports were made; 19/30 participants (63%) did not report *fetA* VR.
- 5/30 (17%) participants determined the correct MLST CC 41/44, but only one laboratory (NM31) confirmed MLST ST 41. One laboratory (NM34) reported MLST ST 4922 (which did not match the expected result from whole-genome sequencing. See Annex 1 for comparison of ST 41 and ST4922). Partial MLST characterisation was reported by NM24, NM30 and NM41, which is insufficient for MLST CC designation. 25/30 (83%) laboratories therefore did not report MLST CC, and 29 did not determine (94%) MLST ST.
- 2505 was designed as a 'low-level' positive, which may have contributed to the difficulties in determining MLST. This process requires optimal DNA extraction and, at times, a first round PCR assay 'mis-priming'.
- 2506, negative: The negative result was confirmed by 22/30 (73%) participants, but incorrect reports were made by 4/30 (13%) laboratories that filed a positive report: 'meningococcal DNA detected' (NM24, NM28, NM45 and NM49). Four laboratories did not report at all: NM33, NM36, NM52 and NM54.
- Three of the laboratories reporting 'false positive' compounded the error by confirming genogroups C (NM24), W135 (NM28) and Y (NM45). NM49 reported 'Not A, B, C, Y or W135', which was correct.

2.2.2 Non-culture detection and genogroup summary

A minimum of 83% (25/30) participant laboratories (see Table 9) could detect non-culture *N. meningitidis* in 2014 compared with 62% (18/29) in 2012 and 76% in 2011.

Genogroup confirmation is more challenging as the assays appear to be less sensitive; at the same time, the more diluted simulated CSF and septicaemia samples are more challenging. More than 50% of the participating laboratories determined the genogroups.

The use of more diluted (but still detectable) positive samples is required to broaden the range of genogroups and simulate the processing of samples at genuine clinical levels.

Table 9. Proportion of participating (30) laboratories agreeing with the consensus species detection and genogroup

Sample	Species detection	Genogroup
2504	25/30 (83%)	19/30 (63%)
2505	25/30 (83%)	22/30 (73%)
2506	22/30 (73%)	N/A[1]

[1] *N/A = Not applicable, negative sample*

2.3 Summary of genotyping consensus reporting

Results on consensus genotyping from the 30 participants are summarised in Table 10. Twenty of the 30 participating laboratories (67%) completed *porA* sequence typing (VR1 and VR2) of isolates 2502 and 2503; for the non-culture samples 2504 and 2505 the completion range dropped to 33–40%. Similarly, the range of *fetA* consensus agreements for isolates (2502 and 2503) was 63%, but dropped to 37–40% for the non-culture samples (2504 and 2505). ST consensus was 57% for the isolates (2502 and 2503) but only 3–7% for the non-culture samples (2504 and 2505). The CC consensus report was 60% for the isolates (2502 and 2503) and 17–23% for the non-culture samples (2504 and 2505).

Table 10. Summary of molecular typing results for isolates and non-culture samples

	Detection	Group	*porA*	*fetA*	MLST	
					ST	CC
Isolates	N/A	28–29/30 (93–97%[1)]	20/30 (67%)	19/30 (63%)	17/30 (57%)	18/30 (60%)
Non-culture	25/30 (83%)	19–22/30 (63–73%)	10–12/30 (33–40%)	11–12/30 (37–40%)	1–2/30 (3–7%)	5–7/30 (17–23%)

[1] *Combination of serogroup and genogroup as a number of labs do not routinely determine genogroup for isolates*

2.3.1 Molecular 'fine type' for isolates and non-culture samples

Characterisation of the isolates and non-culture samples by group, *porA* VR1 and VR2, *fetA* VR and MLST CC is presented as Table 11 for both 2014 sample types compared to that reported in 2012. Where an increased number of laboratories reported complete isolate 'fine types' in 2014 than in 2012 (18/30 compared to 16/29) but there was a slight decrease in the number of laboratories reporting complete non-culture 'fine types' in 2014 than 2012 (5-7/30 laboratories compared to 6-7/29).Moreover, the designation of MLST ST for the non-culture samples was particularly poor, only 1 – 2 laboratories submitted reports, Table 12. This indicates, that sample 2505 may have been too exacting, a low level positive.

Table 11. Comparison of 'fine type' (serogroup: *porA*: *fetA*: cc) reports in 2014 and 2012

		Isolates		Non-culture	
		2014	2012	2014	2012
3 samples		-	15/29 (52%)	-	3/29 (10%)
2 samples		18/30 (60%)	16/29 (55%)	5/30 (16%)	6/29 (21%)
1 sample		18/30 (60%)	16/29 (55%)	7/30 (23%)	7/29 (24%)
No report		12/30 (40%)	13/29 (45%)	23/30 (77%)	22/29 (76%)
Change 2012 to 2014		Isolates ↑		Non-culture ↓	

[1]*Only three samples in 2012*

Table 12. Number and proportion of correct MLST CC and ST reports for isolates and non-culture samples in 2014

	Isolates		Non-culture	
	2502	**2503**	**2504**	**2505**
CC	18/30 (60%)	18/30 (60%)	7/30 (23%)	5/30 (17%)
ST	17/30 (57%)	17/30 (57%)	2/30 (7%)	1/30 (3%)

2.3.2 Trends in 'fine type' reporting in EQAs 2009–2014

An indication of the capability of European laboratories to characterise *N. meningitidis* by molecular (DNA-sequence) typing is given by a review of the trend over the past four IBD-labnet EQAs (2009–2014) as shown in Table 13a and b.

The tables below should only be seen as an indication of molecular typing ability because the samples (isolates and non-culture) were not the same in each panel. The number of participants that correctly reported 'fine type' of isolates has increased from 8/29 (28%) in 2009 to 17/30 (57%) (in 2014). Improvements in non-culture typing were less significant, reflecting the difficulties associated with low-level positives that would also be encountered in actual clinical samples.

Up to 6/29 (21%) participants correctly reported the complete 'fine types' for two non-culture samples in 2012; in 2014, this number sank to 2/30 (7%) (see Table 13b).

Table 13a. Summary of correct molecular typing results for isolates and non-culture samples from participants in *N. meningitidis* EQAs 2009–2014

	2009		2011		2012		2014	
	Culture	**Non-culture**	**Culture**	**Non-culture**	**Culture**	**Non-culture**	**Culture**	**Non-culture**
Geno–group	NA	8–19/29 (26–66%)	NA	16–19/30 (53–63%)	NA	15–18/29 (52–62%)	NA	19–22/30 (63–73%)
***porA* VR1**	16–17/29 (55–59%)	5–11/29 (17–38%)	21–22/30 (70–73%)	13–16/30 (43–53%)	20/29 (69%)	11–13/29 (38–44%)	20/30 (67%)	10–12/30 (33–40%)
***porA* VR2**	17–18/29 (59–62%)	4–12/29 (14–41%)	21–22/30 (70–73%)	13–16/30 (43–53%)	20/29 (69%)	13–14/29 (45–48%)	20/30 (67%)	10–12/30 (33–40%)
fetA	9/29 (31%)	3–6/29 (10–21%)	20–21 (67–70%)	9–10/30 (30–33%)	20/29 (69%)	12–13/29 (41–45%)	19/30 (66%)	11–12/30 (37–40%)
MLST CC	12/29 (42%)	4–6/29 (14–21%)	16/30 (53%)	7/30 (23%)	16/29 (55%)	6–7/29 (21–24%)	18/30 (60%)	5–7/30 (17–23%)
MLST ST	8/29 (28%)	2/29 (7%)	14–15/30 (47–50%)	6/30 (20%)	10–16/29 (34–55%)	6/29 (21%)	17/30 (57%)	1–2/30 (3–7%)

Table 13b. Maximum of correctly reported complete 'fine types' (serogroup: *porA*: *fetA*: cc: ST) for isolates and non-culture samples from participants in *N. meningitidis* EQAs, 2009–2014

Year	Total (N)	'Fine type'	
		Isolates	Non-culture
2009	**29**	8 (28%)	2 (7%)
2011	**30**	15 (53%)	6 (20%)
2012	**29**	16 (55%)	6 (21%)
2014	**30**	17 (57%)	2 (7%)

2.3.3 Summary of laboratories not returning isolate and non-culture typing reports

The number of laboratory submissions needs to be increased so that all characterisation targets can be met and 'fine typing' capacity can be improved. The current level is unacceptably low.

Tables 14 and 15 indicate the number of laboratories that did not report molecular typing characteristics for isolates and non-culture samples, respectively. With regard to both isolate and non-culture characterisation, it is apparent that *porA* and *fetA* are more likely to get characterised. Interestingly, non-culture *porA* VR2 proved more difficult (i.e. fewer responses were received) in the 2014 EQA than in earlier distributions.

On a more positive note, only five laboratories skipped non-culture sample species detection, and only eight did not report the genogroup. See Table 15.

Table 14. Isolate typing characteristics not returned by participants

	Number of laboratories	Lab ID
fetA VR	11 (37%)	NM23, NM36, NM38, NM40, NM43, NM45, NM48, NM49, NM51, NM52, NM54
porA VR1 and VR2	10 (33%)	NM36, NM38, NM40, NM43, NM45, NM48, NM49, NM51, NM52, NM54
MLST CC	12 (40%)	NM26, NM36, NM38, NM40, NM43, NM45, NM47, NM48, NM49, NM51, NM52, NM54
MLST ST	13 (43%)	NM26, NM30, NM36, NM38, NM40, NM43, NM45, NM47, NM48, NM49, NM51, NM52, NM54

Table 15. Non-culture typing characteristics not returned by participants

	Number of laboratories	Lab ID
Detection	5 (17%)	NM33, NM36, NM37[1], NM52, NM54
Genogroup	8 (27%)	NM33, NM36, NM37[1], NM38, NM40, NM48, NM52, NM54
fetA VR	19 (63%)	NM20, NM22, NM26, NM28, NM33, NM35, NM36, NM37[1], NM38, NM40, NM41[1], NM42, NM43, NM45, NM48, NM49, NM51, NM52, NM54
porA VR1	18 (60%)	NM22, NM24[1], NM26, NM27[1], NM28, NM33, NM35, NM36, NM37[1], NM38, NM40, NM42, NM43, NM45, NM48, NM51, NM52, NM54
porA VR2	20 (67%)	NM20, NM22, NM24[1], NM26, NM27[1], NM28, NM33, NM35,NM36, NM37, NM38, NM40, NM42, NM43, NM45, NM48, NM49, NM51, NM52, NM54
MLST CC	26 (87%)	NM20, NM22, NM23, NM26, NM27, NM28, NM29, NM30, NM32, NM33, NM35, NM36, NM37[1], NM38, NM39, NM40, NM41[1], NM42, NM43, NM45, NM47, NM48, NM49, NM51,NM52, NM54
MLST ST	28 (93%)	NM20, NM21, NM22, NM23, NM24, NM26, NM27, NM28, NM29, NM30, NM32, NM33, NM35, NM36, NM37, NM38, NM39, NM40, NM41, NM42, NM43, NM45, NM47, NM48, NM49, NM51, NM52, NM54

[1] *Laboratory characterised only one of two isolates*

2.3.4 Non-consensus reporting by participants in EQAs 2009, 2011, 2012 and 2014

Some laboratories have found difficulties with the molecular characterisation of isolates on more than one occasion (see Table 16). Likewise, a number of laboratories have demonstrated problems with the non-culture samples (see Table 17).

Table 16. Non-consensus isolate characterisations reported by participants in four EQAs distributions, 2009–2014

	2009 (6 isolates)	**2011** (4 isolates)	**2012** (3 isolates)	**2014** (2 isolates)
Serogroup	**NM36** (2)[1], **NM38**, **NM40**, **NM45**, **NM40**, **NM48**	**NM22**, **NM38**, **NM45**	**NM45**	**NM22**, **NM38**, **NM43**, **NM45**, **NM48** **NM49**, **NM51**, **NM52**
Genogroup	**NM32**	NM23	**NM45**	
porA	**NM22** (2), **NM23**, **NM26**, **NM27**, **NM28**, **NM28**, **NM32**, **NM34**, **NM37**, **NM41**	**NM37**		
fetA	-	-	-	-
MLST CC		**NM37** **NM37**		

[1] *Brackets indicate the number of non-consensus samples for that participant in the EQA panel.*

Table 17. Non-consensus non-culture characterisation reporting by participants in four EQA distributions, 2009–2014

	2009 (6 samples*)	2011 (4 samples*)	2012 (4 samples*)	2014 (3 samples*)
Detection	NM22 (5)[1], **NM25**, **NM31**, **NM35**, NM37 (2), NM38 (3), **NM39**, **NM43**, **NM44**	-	NM20(2), NM22, **NM28**, **NM35**, **NM37**, NM45	NM26
Genogroup	**NM31**, **NM49**	NM23, **NM37**	**NM21**, NM23, **NM28**, **NM32** (2), **NM35**, **NM37**, NM45	**NM24**, NM26, **NM41**, **NM51**
porA	**NM26**, **NM32**	**NM24**, **NM37**	**NM24**, **NM27**, **NM32** (5)	**NM37**
fetA	-	-	NM23, **NM26**	**NM37**
MLST	NM20 (2), **NM34A**, **NM37** (3), **NM41** (2)	**NM21**	**NM37**	-
CC	**NM25** (2)	-	**NM39**	-

[1] *Brackets indicate the number of non-consensus samples for that participant in the EQA panel.*

** Including one negative* N. meningitidis *sample.*

2.4 Methodology review

It was accepted that most participants would use conventional slide agglutination or other serological techniques to establish the serogroup and that MIC investigation was specifically targeted to gradient diffusion strips. Information on the MIC gradient strip manufacturer/supplier was requested, and a review of the responses in association with MIC results can be found in Section 2.1.3.

An EQA web reporting form was set up to capture basic information regarding the molecular typing methods used for isolates and simulated septicaemia samples.

The methods used for the genotyping of isolates are presented in Table 18. Simple heated (boiled) suspensions of meningococci are confirmed as suitable for genotyping even though capture columns predominate, with a number of laboratories using conventional PCR and gel detection to determine results.

DNA sequencing was only reported by three laboratories when *porA*, *fetA* and MLST all required sequencing, which was achieved by more than 33% of all laboratories (10/30). This suggests the question should be specific to genogroup compared to the sequence typing assays.

Table 18. Methods used for genotyping of isolates, samples 2502 and 2503

	Extraction	Amplification	Detection
	Capture column[1] (12)	PCR-conventional (16)	Gel electrophoresis (13)
	Boil (9)	Real-time PCR (10)	Real-time PCR (10)
	Magnetic beads (3)		Sequencing (3)
	Other (2)		
Total	**26**	**26**	**26**

[1] *Eleven laboratories used capture column with centrifugation; one used it without centrifugation.*

Similar responses were reported for the non-culture samples 2504 and 2505 (Table 19). More exacting DNA extraction (and concentration) techniques were required for the simulated septicaemia samples, with the predominant use of spin columns. Real-time PCR was noted, presumably for the species detection and genogroup confirmation. Two laboratories reported 'sequencing', which could refer to the DNA sequencing of PCR products to confirm species/genogroup or the other molecular typing assays. It is not clear why increased reports of extraction and amplification methods were made for the non-culture samples when it is apparent that fewer sample reports were made.

In hindsight, the questions regarding molecular processes and techniques were not specific enough (as previously noted in the 2011 and 2012 distributions). The fact that that most (nearly all) laboratories that applied molecular methods achieved the consensus result (apart from MLST) is sufficient to confirm their utility. The non-culture samples had been designed to be more challenging (weaker positives) than in 2012.

Also, the method responses could have been targeted to a specific sample or samples.

If it is considered necessary to determine which reagents and method a laboratory uses; this issue may be best addressed with a specific questionnaire, separate and additional to EQA panel distribution.

Table 19. Methods used for genotyping of non-culture, samples 2504 and 2505

	Extraction	Amplification	Detection
	Capture column[1] (16)	PCR-conventional (7)	Gel electrophoresis (7)
	Magnetic beads (5)	Real-time PCR (19)	Real-time PCR (18)
	Boom (1)		Sequencing (1)
	Salt precipitation (1)		
	Boil (1)		
	Other (2)		
Total	**26**	**26**	**26**

[1] *Fourteen laboratories used capture column with centrifugation; two used it without centrifugation.*

2.5 Review of participants' non-consensus trends

Although it was expected that participants would review their own results following the designation and distribution of the consensus results, it is possible to review submissions over the four IBD-labnet EQAs 2009, 2011, 2012 and 2014 (see Tables 13a and b). Clearly, it is important to increase the submissions to all characterisations for as many laboratories as possible (Section 2.3.4) but the quality of the reports is the reason for regular EQA panel distributions. Consideration should be given to the fact that non-consensus results are relatively few, as detailed in the sample-specific results.

Tables 16 and 17 indicate that a small number of laboratories did not match the consensus in two or more of the distributions for specific characterisations. It may be seen that some laboratories are observed more than once in a distribution and occasionally in more than one distribution.

Laboratory NM37, noted for previous non-consensus isolate typing reports, performed well in 2014. Non-consensus serogroup reports were reported by NM22, NM38 and NM45 as had been the case in at least one previous EQA distribution.

2.6 Summary of IBD-labnet *N. meningitidis* EQA 2014 compared to previous distributions

The fourth IBD-labnet EQA panel 2014 was distributed to 30 countries (31 countries in 2011, 30 in 2009, and 29 in 2012). There were relatively few problems with the typing of isolates in any of the panels although it should be noted that the number of samples was reduced from 6 isolates in 2009 to 4 in 2011, 3 in 2010, and finally 2 in 2014. To expand the pool of EQA samples it was necessary to use 'new' isolates not previously distributed. For isolates (Table 13b), the consensus 'fine type' characterisation improved consistently over the four EQAs: 28% (2009), 53% (2011), 55% (2012) and 57% (2014). Results were less encouraging with regard to the non-culture 'fine type' reports: 7% (2009), 20% (2011), 21% (2012) and 7% (2014), as shown in Table 13b.

The evaluation of MICs results in 2014 was facilitated by conversion of the submitted MIC values (dilutions) to the EUCAST doubling dilution series. Differences were observed but they appeared to be minor with very few outliers in 2014. EUCAST interpretation demonstrated that the 2014 MIC reports and the few 'reduced susceptibility' reports would have been unlikely to have any clinical consequences.

Website reporting and feedback for participants still needs to be improved. In 2012, participants for the first time mentioned the need for better explanations and user guidance on how to enter and save results on the UK NEQAS website. It should be emphasised that it is possible to amend, update and print laboratory reports until the closing date.

The 'drop-down' option for reporting appeared to cause less confusion and reduced the number of clerical errors compared to previous distributions, but the website needs further refinement.

Like in previous distributions, molecular typing of isolates showed good agreement, with only a few laboratories indicating problems.

The usage of *fetA* characterisation has been observed since 2011 although it is apparent that a number of laboratories do not have the capability for molecular typing.

The non-culture samples again proved more difficult than the isolates as they were more demanding in 2014. Apart from the MLST ST and CC investigations, the participating laboratories which tested the non-culture material were generally very accurate. Failure to confirm ST and CC through MLST was possibly due to the 'weak' simulated CSFs.

There was an increase in the number of laboratories reporting molecular detection results. Up to 83% of laboratories did so in 2014 (2012: 69%); where there had been a previous improvement from 69% in 2009 to 77% in 2011. It was disappointing that 4 (13%) laboratories reported 'false positives' for the negative sample (2506); three laboratories compounded the error with a genogroup report. Self-review may reveal simple laboratory or transcription errors that could have been avoided by process controls or methodological changes.

There was a marked improvement in 'fine-type' ascertainment in 2014 as more laboratories demonstrated *fetA* typing. This was most noticeable with the isolates and reflects general problems with non-culture samples. With regard to *fetA* typing this may be caused by the lack of a designated non-culture protocol with defined nested and sequencing primer sets.

In 2014, the reduction in laboratories reporting non-culture MLST CC, and particularly ST, may reveal the exact nature of 7 loci sequencing following specific nested PCR amplifications. It is also possible that participants do not routinely perform non-culture MLST and may have only attempted MLST for the EQA.

Some laboratories may be reluctant to pursue MLST in favour of single-antigen gene sequencing not included in the 'fine type', for example antigens included in the MenB vaccine Bexsero.

Similarly, recent advances in sequencing technology such as whole-genome sequencing for isolates may impact the timeliness of molecular characterisations.

3 Conclusions

All in all, the 2014 fourth *N. meningitidis* IBD-labnet EQA, with its increase in the number of laboratories reporting isolate 'fine types', can be considered a success. The EQA demonstrated an increased competence and capability to characterise samples. Problems were encountered with the non-culture samples: compared with previous distributions, fewer non-culture MLST reports were received, as were a few 'false positive' identifications. Some improvements were noted in the number and quality of responses to the requested detection and characterisation targets. The reduction in requested information and restricted options for website reporting greatly facilitated the review of the results. But even after reducing the number of isolates from 4 in 2011 to 2 in 2014, the EQA panel was a considerable amount of work for some participants.

The EQA managed to assess the ability of European reference laboratories to produce serogroup (and genogroup) results, sequence-based characterisations and MIC values for disease surveillance. It also showed that non-culture detection is improving, while non-culture characterisation is lagging behind.

Serogrouping was again identified as problematic for some laboratories, notably with regard to serogroup C isolates and 'auto-agglutination' or 'not-serogroupable' reports. The local availability of specific serogroup Y and W antisera or monoclonal antibodies would improve serogroup W confirmation. Differentiation of Y and W135 may be regarded as essential for a reference laboratory in terms of accurate surveillance but would not influence case management because the same quadrivalent vaccines would be used.

The utility of genogrouping for the laboratories using it has been highlighted by the non-culture samples, and it is possible that widespread adoption of PCR-based genogrouping could also become important for the typing of isolates. However, that is different from knowing if a capsule is being expressed and whether a polysaccharide vaccine would actually be an effective intervention.

Participation in the four ECDC IBD-labnet EQAs (2009, 2011, 2012 and 2014) was on the understanding of participant anonymity and therefore laboratories (countries) were indicated by their codes (NM'XX'). It was agreed that much of the EQA evaluation and review of procedures would be carried out by the laboratories themselves on receipt of their individual reports, comparing their results to the consensus and repeating or re-evaluating their results as required. It is too easy to dwell on the reported results and the relatively minor errors observed than the fact that nearly all submitted genotyping data were in agreement. Laboratories testing and submitting results for genotyping of the isolates – and particularly the non-culture samples – are to be encouraged. This report has drawn attention to (anonymous) laboratory performance as requested by ECDC; notably, the laboratories that have not submitted specific characterisation reports, those reporting non-consensus (over the three EQAs) and those capable of 'fine-type' determination. The achievement of participants that reported complete isolate 'fine types' and non-culture 'fine types' should not be understated.

It can be safely assumed that laboratories which were not in a position to test the material did not submit any data. The resources and technical procedures required to molecularly characterise material by all the requested assays should not be underestimated. It was, however, encouraging to see that a significant proportion of the participants not only tested the material but achieved the consensus. The submission of EQA results may not necessarily imply that a laboratory (country), although capable of accurate characterisation (e.g. 'fine type'), is in a position to characterise all routine samples and submit the data to TESSy. To determine the overall capacity for routine testing and reporting to ECDC, it would be more appropriate to use a questionnaire than this EQA process.

Opportunities were given to participants submit their comments on the EQA panel or specific results in person at the annual IBD-labnet meeting in Frankfurt, Germany, in 2014 or by email to steve.gray@phe.gov.uk but to date there have been very few comments other than appreciation of the EQA and ECDC's support.

Interestingly, one laboratory discussed their non-culture MLST report (ST4922) for sample 2505. The expected result for 2505 was ST41, as determined by Illumina whole-genome sequencing and confirmed by only one participant (NM31); as a result, there was no consensus within the EQA distribution. According to the authors previous EQA experiences, 'weak' non-culture positive samples may on rare occasions allow for mis-priming in the initial round of PCR amplification. The product after two rounds of PCR is sequenced correctly but it is not the sequence of the 'infecting' organism.

As in 2012, there are a number of laboratories (countries) unable to detect *N. meningitidis* in non-culture samples and then apply the more exacting molecular typing methods. It is not possible to determine from the EQA if it this is the result of a lack of resources or expert knowledge but one may speculate that it is more likely to be the former. Therefore, one may assume that countries not reporting non-culture detection in the EQA are unable to confirm non-culture cases and as such underreport meningococcal cases.

The IBD-labnet training workshop (Würzburg, Germany, 2010) did not address the practical or technical issues of molecular typing and only superficially demonstrated molecular detection. *In-silico* analysis and use of the typing database website was demonstrated but not the intensive 'hands-on' training one would need to generate the DNA

sequences. Similarly, to set up routine non-culture detection service, a laboratory would require considerably more training with the equipment a laboratory would have local access to. To address the issues it would be appropriate to send out a short but directed questionnaire to ascertain which laboratories were/are unable to complete all the requested typing targets and then to set up specific training courses to meet their needs. Some effort has been made in this direction with laboratory placements in 2011 and 2012, but none in 2014.

There are also problems comparing the EQA distributions when the samples are not identical. This is compounded with meningococci as there innumerable strains that could be used – although only relatively few clonal complexes exist that are responsible for disease in Europe. Selecting only two isolates limits the scope of serogroup assessment as the more unusual organisms (serogroups X and 29E) may escape testing in favour of serogroups B, C, Y, W135 and A. Similarly, it was not the intention to distribute non-culture samples that were too difficult to detect and determine molecular types. In reality, there is a wide variety of meningococci that does not cause disease but may be required to be assessed as part of potential case investigations, and there are certainly many confirmed cases with very low positivity with respect to PCR detection (that may not allow for serogroup determination or molecular typing).

The EQA distributions are an essential part of quality assessment for both the participants and ECDC in order to validate the quality of the data ECDC aims to collect from Member States. The support of ECDC IBD-labnet is valued by the participants, as reflected in the high level of participation and compliance.

Annex. Comparison of MLST ST41 and ST4922

A detailed review of reports for sample 2505 MLST ST showed that the two reported designations ST41 and ST4922 (both ST-41/44 clonal complex) differed by only the *aro*E locus. When *aro*E 9 (ST41) and *aro*E 1 (ST4922) were compared (using the PubMLST Neisseria 'allele sequence comparison'), it was apparent that there were 45 nucleotide differences between the two alleles.

It could be speculated that as the differences were spread throughout the locus it could be due to sub-optimal sequence data rather than simple mispriming. Another explanation could be a technical mix-up of samples.

Re-testing of the sample by the laboratory (from the original material or DNA extract) may reveal the possible cause of the observed aroE sequence differences.

It is possible that sequence editing was required to accommodate dye problems but there could be other reasons that the participant could consider. Previous experience has shown that on rare occasions 'weak' non-culture positive samples may allow for mis-priming in the initial round of PCR amplification. The product after two rounds of PCR is sequenced correctly but it is not the sequence of the 'infecting' organism.

It is worth re-stating that only two laboratories determined the MLST ST and that the sample was therefore proven to be exacting. To match 6/7 alleles was a good result and highlights one of the issues with ST: that all 7 loci are required.

Table A1. Comparison of ST4922 (reported by NM34) compared to the expected ST41 result

ST	*abcZ*	*adk*	*aroE*	*fumC*	*gdh*	*pdhC*	*pgm*	Clonal complex
41	3	6	9	5	9	6	9	ST-41/44 complex/lineage 3
4922	3	6	1	5	9	6	9	ST-41/44 complex/lineage 3

Table A2. Comparing *aro*E 9 (ST41) and *aro*E 1 (ST4922) base differences using PubMLST 'allele sequence comparison'

Nucleotide differences between *aro*E: 9 and *aro*E: 1
Identity: 90.82 %

```
      10   20   30   40   50   60   70   80   90   100
  9
TATCGGTTTGGCCAACGACATCACGCAGGTTAAAAACATTGCCATCGAGGGCAAAACTATTTTACTTTTGGGCGCGGGCGGTGCGGT
GCGCGGCGTGATT
  1 ..............................C.................A........C..C..G.................C...................

     110   120   130   140   150   160   170   180   190   200
  9
CCTGTTTTGAAAGAACACCGTCCTGCCCGTATCGTCATTGCCAACCGTACCCATGCCAAAGCCGAGGAATTGGCGCAGCTTTTCGGCA
TTGAAGCCGTCC
  1 ................................................C.....C............A..........G.....................

     210   220   230   240   250   260   270   280   290   300
  9
CGATGGCGGATGTGAACGGCGGTTTTGATATCATCATCAACGGCACATCCGGCGGTTTGAGCGGTCAGCTTCCGGCCGTCAATCCTG
AAATTTTCCGCGA
  1 ................................................G........C.................T.......G..............T..G

     310   320   330   340   350   360    370   380   390
  9 CTGCCGCCTTGCCTACGATATGGTGTACGGCGAAGCGGCAAAACCGTTTTTGGATTTTGCCCG--
GCAATCGGGCGCGAAACAAACCGCCGACGGACTGG
  1 ........................T........C.....GC.GGA.......A.C.......AAA....C--..T...GCCG..GTTT.A..........

  400   410   420   430   440   450   460   470   480   490
  9
GTATGCTGGTCGGTCAGGCGGCGGCTTCCTACGCCCTCTGGCGCGGATTTACGCCCAATATCCGCCCTGTTATCGAATACATGAAAG
CCATG
  1 ................A.....................................G.....................................
```

Nucleotide differences between *aroE*: 9 and *aroE*: 1

Identity: 90.82 %
Differences: 45

31: T → C
49: G → A
58: T → C
61: T → C
64: A → G
82: T → C
148: T → C
154: T → C
166: G → A
177: A → G
247: A → G
256: T → C
274: G → T
282: A → G
297: G → T
300: A → G
325: G → T
334: A → C
340: A → G
341: A → C
343: A → G
344: C → G
345: C → A
353: G → A
355: T → C
363: G → A
364: G → A
365: C → A
366: A → G
367: A → C
368: T → A
369: C → A
370: G → C
373: C → T
377: A → G
378: A → C
379: A → C
380: C → G
383: A → G
384: C → T
385: C → T
386: G → T
388: C → A
415: G → A
455: A → G