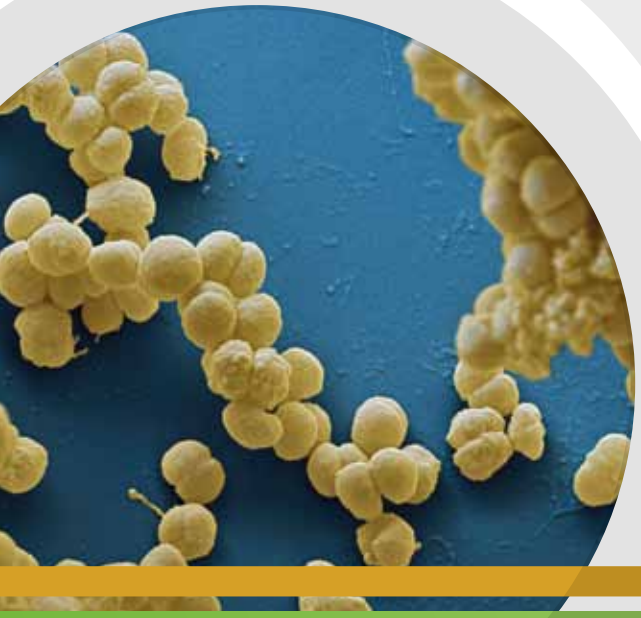

TECHNICAL REPORT

External quality assessment scheme 2011 for *Neisseria meningitidis*

as part of the IBD-Labnet surveillance network

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This report was commissioned by the European Centre of Disease Prevention and Control (ECDC), coordinated by Dr Adoración Navarro Torné 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 ECDC.2273).

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Contents

Abbreviations

cc	Clonal complex of multilocus sequence types
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
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
DSN	Dedicated surveillance network
ECDC	European Centre for Disease Prevention and Control
EMGM	European Monitoring Group on Meningococci
EQA	External Quality Assurance
EU	European Union
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)
HPA	Health Protection Agency (UK)
I	Intermediate
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRU	HPA 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
PHLS	The Public Health Laboratory Service (UK)
PorA	Porin A protein, (variable regions VR1 and VR2 used for sequence typing)
QMS	Quality management system
R	Resistant
RIF	Rifampicin
S	Susceptible
ST	Sequence type of Multilocus sequence typing
SU	Sulphonamide
TESSy	The European Surveillance System (ECDC)
UK	United Kingdom
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 cause of meningitis and rapidly fatal sepsis in healthy individuals worldwide. It is the only agent among the major bacterial agents causing meningitis that may give rise to epidemic as well as endemic disease. The meningococcus is carried in the human nasopharynx asymptomatically by 5–10% of adults in non-epidemic periods but may be greater than 30% for first-year university students. *N. meningitidis* is responsible for morbidity and mortality among the cases and may result in sequelae. In addition, it may be responsible for more unusual presentations, such as arthritis, osteomyelitis and cellulitis. The risk of meningococcal disease is higher among those with complementary deficiencies, asplenia and other underlying conditions.

In Europe, 3 814 (0.75 per 100 000) cases were reported to ECDC in 2011.

Meningococcal disease surveillance is paramount and has a variety of 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 and to act in outbreaks), surveillance for vaccination purposes and the estimation of the burden of meningococcal disease. Meningococcal surveillance systems are partially based on laboratory diagnoses and therefore there is a need for accuracy and proficiency in surveillance laboratory performance.

In general, External Quality Assessment (EQA) schemes enable laboratory performance to be assessed against reference methods and compared to other peer laboratories. They provide 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 and confirm a specific pathogen or novel resistance patterns.

Meningococci are characterised using serologic typing systems based on structural differences of the polysaccharide capsule (serogroup), major outer membrane protein porin B (serotype), major outer membrane protein porin A (sero-subtype) and lipooligosaccharide (immunotype). Molecular-based typing of meningococci has revealed that genetically related strains, described as clonal complexes (cc), cause most disease. Some of these complexes, such as cc ST-11, show particular epidemiological features – e.g. relatively low carriage, rapid transmissibility and raised case-fatality ratio.

ECDC promotes the performance of EQA schemes under which laboratories send simulated clinical specimens or bacterial isolates to be tested by routine and/or reference laboratory methods, to improve the quality and amount of relevant epidemiological information collected in the European Union (EU). This information is used, in turn, to provide standardised case demographic and infecting organism characterisation data to populate fields in TESSy, the ECDC database. ECDC has sponsored the IBD-labnet EQA for *N. meningitidis* in order to encourage the use of molecular detection and characterisation, allowing individual participants to compare their results to the consensus, review any anomalies and to facilitate an overall assessment of EU reference laboratory typing capacity.

The distribution and testing (characterisation) of *N. meningitidis* isolates and simulated non-culture samples was designed to allow the reference laboratories to compare their results (anonymously) to those from other EU reference laboratories testing *N. meningitidis*. The ECDC's intention was to improve the quality of testing, moving toward molecular characterisation and away from limiting serological assays, where the use of molecular typing (DNA sequence based) was applicable to both isolates and non-culture positive (DNA) samples. Similarly, the aim was to demonstrate the utility and applicability of molecular (PCR) detection and characterisation assays. The application of these assays would improve countries' capacity to confirm cases and determine the new standard, sequence-based typing ('fine type').

In February 2011, a collection of four viable isolates of *N. meningitidis* of the major disease-causing serogroup (A, B, C and Y) together with four simulated blood (non-culture) samples for molecular studies was sent by UK NEQAS to 31 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 (i.e. serogroup and antimicrobial susceptibility testing by gradient diffusion minimum inhibitory concentration (MIC) results)
- Molecular characterisation by PorA typing, FetA typing and MLST.

Genogroup 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 characterisation targets were specifically selected to populate TESSy, the ECDC database, which is used for EU level surveillance data notification.

Thirty reports were recorded by UK NEQAS.

Twenty-nine (97%) laboratories reported the consensus isolate serogroup A and C, 93% (28 laboratories) reported serogroup B but only 80% (24 laboratories) identified serogroup Y.

A range of 22 to 23 (73–77%) laboratories reported the consensus non-culture detection and 16 to 19 laboratories (53–63%) reported the consensus non-culture group confirmation.

Correct 'fine type' molecular characterisation (group: porAVR1, porAVR2: fetA: MLST CC) were reported for isolates by 16 (53%) and for non-culture by six (20%) of the 30 laboratories.

The phenotypic characterisation of viable isolates was quite successful with serogrouping reports received from 29 (97%) participating laboratories for each sample. Serogroup A and C were confirmed by 97% of the laboratories. However, the phenotypic serogrouping reports demonstrated some limited discrepancies or errors for serogroup B (93%) and Y (82%). This may have been due to the limited resources or reactivity of the reagents. A similar phenomenon was observed in the 2009 EQA exercise.

The comparison of MIC between laboratories requires a standard methodology such as that recommended by the European Monitoring Group on Meningococci: gradient diffusion methodology (by E-test or similar) and a standardised agar plate medium (Mueller Hinton plus blood). Previous difficulties of interpretation in the 2009 EQA suggested that from the epidemiological point of view, it would be advisable to collect MIC values (if determined by standard or compatible methods) and then interpret them according to only one guideline (EUCAST for consistency) which could be achieved using the ECDC TESSy database. Converting the submitted MICs to EUCAST values (and allowing (+) or (-) one dilution) showed that even though some MICs were different to the mode, the EUCAST interpretation was consistent.

The MLST analysis of non-culture samples revealed slightly more problems than the MLST of viable isolates. MLST was achieved by 47–50% (15–16) of laboratories for the isolates but only 20% (six laboratories) for the non-culture samples; cc was reported by 53% (16) of laboratories for the isolates but only increased to 23% (seven laboratories) for the non-culture samples.

Overall, the EQA performance has shown that European Reference Laboratories for Meningococci differ in their capacities and level of characterisation of the distributed *N. meningitidis* material, but that there have been improvements since the first ECDC IBD-labnet distribution.

In conclusion, the results of the IBD-labnet EQA exercise proved that a regular EQA scheme for the reference laboratories is required in order to maintain the movement towards improved quality of epidemiological reports, specifically, the encouragement to adopt molecular typing techniques in addition to molecular detection. It was also concluded that targeted training and support might be requested to assist laboratories that have problems with organism characterisation and, in particular, the establishment of robust molecular typing techniques depending on their particular needs. This is necessary if all EU countries are to provide accurate *N. meningitidis* molecular typing data to TESSy to cover their own populations and therefore provide representative European epidemiology of this important disease.

Main findings from 2011 *N. meningitidis* EQA

Main findings	Future direction	Possible actions
Excellent response to EQA distribution (30 responses) but not all laboratories could provide results for all targets.	Need to determine the barriers preventing laboratories from completing the range of characterisation data (in order of importance): 1. Accurate serogroup or genogroup determination 2. The ability to perform non-culture detection and genogroup 3. 'Fine type' molecular characterisation of isolate 4. 'Fine-type' molecular characterisation of non-culture samples.	Targeted questionnaire. Regular EQA distribution.
Phenotypic serogroup determination was successfully achieved by 29/30 (97%) laboratories.	Need to achieve accurate methodology to confirm serogroup Y and A for all laboratories.	Targeted training. Regular EQA distribution.
• Utilising standard methodology for MIC testing greatly improved comparisons. • Converting the submitted MICs to EUCAST values and allowing for (+) or (-) one dilution differences resolved the mainly minor differences observed. • EUCAST interpretation showed consistency.	Maintain EQA. Reduce MIC data capture and analysis by only reporting specific antibiotic MICs on specific organisms.	
Genogroup is not tested or reported routinely for isolates.	Encourage ability to confirm genogroup.	Targeted training
23/30 (77%) of laboratories were able to detect *N. meningitidis* in simulated clinical (non-culture) samples.	Support laboratories with training to establish standard molecular assays for non-culture *N. meningitidis* and genogroup confirmation.	Targeted training and support Recommendation of effective methodologies.
16/30 (53%) and 6/30 (20%) of laboratories were able to perform 'fine type' on isolates and non-culture samples respectively. All those reporting the 'fine type' results were in agreement.	Support laboratories with training to establish standard assays Ascertain whether laboratories are routinely determining sequence types for all case isolates (and/or clinical samples) Assess whether the laboratories using molecular tests will generate sufficient data for TESSy.	Targeted training and support (both sequencing a software). Recommendation of effective methodologies.
Incomplete assessment of methods, reagents and processes used for molecular testing.	If it is deemed 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 requesting details of participants' routine processing (testing) for molecular detection and typing.

Introduction

Background

The European Centre for Disease Prevention and Control (ECDC) is a European Union 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. As part of 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 assessment (EQA) is a part of the quality management system (QMS). It evaluates the 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 EQAs 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 for improvement in laboratory diagnostic capacities relevant to the surveillance of diseases 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 assessment 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
- Identification of needs for training activities.

N. meningitidis, meningococcal disease and epidemiology

N. meningitidis is a selective commensal and pathogen of humans. The meningococcus is carried in the human nasopharynx asymptomatically by 5–10% 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. Many surface structures, e.g. capsule, lipopoly(oligo)saccharide and 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 there are instances of disease caused by serogroup X and 29E 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 the 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 and at lower rates: where serogroup C and especially B are the most common. 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.

Increasing numbers of non-culture-confirmed cases are being seen reported by local and reference laboratories within Europe. The application of PCR-based techniques is such that for some countries up to 50% of cases are now laboratory-confirmed and reported (e.g. UK).

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

[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 and IBD-labnet

The European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) was responsible for the surveillance of invasive diseases caused by *Neisseria meningitidis* and *Haemophilus influenzae* between 1999 and 2006. EU-IBIS was coordinated by Public Health England (PHE), formerly the Public Health Laboratory Service (PHLS) in London, UK and funded by the European Commission (DG SANCO).

From October 2006 until October 2007, the network was funded by ECDC after which the epidemiological and laboratory surveillance was integrated into the activities of ECDC. The network worked closely with the European Monitoring Group on Meningococci to integrate the 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 to characterise circulating meningococcal strains.

In 2009, the consortium concluded that laboratory surveillance should rely only on molecular typing data, with the exception of the serogroup. Molecular typing schemes have proved superior (increased discrimination) when compared to serological typing. Based on previous published recommendations of the European Monitoring Group on Meningococci, the IBD-labnet consortium agreed on the following molecular typing scheme for *N. meningitidis* serogroup: PorA(VR1):PorA(VR2):FetA(VR): clonal complex (MLST): Where the cc may be determined even if the full ST designation were not possible.

This scheme provides highest resolution with lowest sequencing efforts and costs and hence, it was recommended as the laboratory variable to be included 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

To support the Member States, ECDC has promoted the performance of EQA exercises to ensure that high-quality, standardised results can be reported as part of the European laboratory surveillance and to assess the training needs for capacity building. It is hoped that the ECDC-funded IBD-labnet EQA will allow reference laboratories to compare the results of testing, in order to achieve the same level of characterisation for both culture and non-culture (PCR only) confirmed cases of meningococcal disease.

It was accepted that for economic reasons some countries might not be able to provide their own molecular typing data for local and European surveillance. Some countries that are processing larger numbers of samples and have spare capacity and availability of molecular methods have offered to help other countries that are not yet able to implement the molecular typing methods, with the aim of providing accurate molecular typing data on *N. meningitidis* for EU surveillance.

This report describes the second ECDC-funded EQA following on from the EQA 2009 and subsequent technical report *External quality assurance scheme for Neisseria meningitidis 2009*, published in Stockholm, March 2011[2].

[2] ISBN 978-92-9193-238-2, doi 10.2900/38021. Available at http://ecdc.europa.eu/en/publications/Publications/1103_EQA_NMening_2009.pdf

1. Materials and methods

1.1 Objectives

The objectives of the 2011 EQA 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 phenotypic and genotypic characterisation (where possible) to all EU Member States and candidate countries with suitable reference facilities.
- To distribute the panel safely for testing by all participant reference laboratories.
- To receive electronic test reports from the participants for analysis.
- To compile the consensus report for participant comparison and review.
- To improve quality of data, assisting in the standardisation of techniques and facilitating consistent epidemiological data for submission to the ECDC TESSy database.

Specifically support the move towards molecular detection, confirmation and accurate characterisation of *N. meningitidis.*

1.2 Study design

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 (Manchester, UK), with the expert knowledge of Dr Vivienne James (UK NEQAS) and facilities at the external Quality Assurance Department, Colindale, London. It should be noted that UK NEQAS for Microbiology has undertaken several international EQA schemes for other organisms that require freeze-drying, distribution, results analysis and web-based reporting.

The design of the project allowed individual reference laboratories to test the EQA panel using their routinely available techniques in order to complete some or all of the characterisation fields (results) within a specified time period. By limiting the result (report) fields to specific criteria and data format acceptance the participants were obliged to use standard methods and nomenclature.

The 2009 EQA study suggested that the phenotypic (serological) characterisation of serogroup A isolates (cultures) may be problematic for some laboratories. The variable nature of slide agglutination and availability of standard antisera was partially addressed at a training workshop in Würzburg, Germany, in June 2010. Commercially available antisera were demonstrated for use applying a standard technique within the confines of a microbiological safety cabinet.

Following feedback from the 2009 EQA that large panels of isolates (and non-culture samples) may confer a disproportionate workload on some reference laboratories, it was decided to reduce the number of isolates and non-viable samples from six to four of each.

The EQA was received and tested by the participant laboratories to determine the phenotypic and genotypic results (see Table 1). Results were then either entered and reported via the UK NEQAS website or copies faxed to UK NEQAS using the unique identifier for each laboratory.

An anonymous summary was produced by UK NEQAS showing the submitted results for the participant laboratory, the consensus result and the number of laboratories with each submitted result. The assumption was made that the consensus result was most likely the correct result.

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

The participant reference laboratories were then asked to compare their own submitted results to the consensus results to determine differences, if any. Participant 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.

It was hoped that the laboratories would have sufficient time to review their results prior to the annual IBD-labnet meeting in 2011, Slovenia.

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

Table 1. Tests requested from the participating laboratories

Procedure	Isolates	Non-culture (simulated septicaemia)	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 similar
	Genogroup	Genogroup	PCR or similar
	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-one European meningococcal reference laboratories participated in the 2011 IBD-labnet EQA distribution.

The participant countries were: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein (Switzerland), Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden and UK (England & Wales and Scotland).

The list of the participating reference laboratories with full contact and address details can be found in Annex 1.

All participants were contacted prior to the IBD-labnet EQA distribution in 2011 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. In essence, this confirmed the recipient's details and their responsibility for safe handling of the material. Clauses relating to the retention and further use of the material, with specific restrictions on third-party distribution and the necessity for review of any publications relating to the EQA material, were also included.

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

1.4 Timelines

Table 2. Timelines for the EQA exercise

Event	Date
Design and preparation of EQA (isolates and simulated non-culture samples). Includes MIC selection	October 2010
Assessment of prepared, simulated septicaemic material	November–December 2010
Transfer to UK NEQAS from HPA MRU	December 2010
Preparation of simulated septicaemia samples and freeze-drying of panel	January 2010
Pre-despatch checks of freeze-dried EQA panel at HPA MRU	January - February 2011
Re-design 2009 web report to accept TESSy data fields	January 2011
Distribution of EQA panel (14-02-2011)	February 2011
Testing and report – (four weeks) – return date 25-03-11	March 2011
Analysis of returned reports	April 2011
Distribution of individual reports email (with consensus results) to participants, by 20-04-2011. Results on UK NEQAS website (https://results.ukneqas.org.uk), dist. 2801 via unique participants' code	April - May 2011
EQA Summary presentation in IBD-labnet workshop (at EMGM 2011, 18-05-11)	May 2011
Technical summary report of ECDC IBD-labnet EQA and recommendations –	September 2011

1.5 The EQA panel material

1.5.1 Isolates

The 2011 IBD-labnet EQA panel (UK NEQAS distribution 2801) consisted of four viable isolates of *N. meningitidis*, selected to be representative of major disease-causing serogroups (A, B, C and Y). Ideally, serogroup W135 would have been included too but it was necessary to reduce the number of samples to four isolates. All the isolates used in the panel were from cases confirmed in England and Wales over the period 2005–2010 (Table 3).

Selection was made based on a combination of characterisation factors, but primarily the MIC values as determined at the HPA MRU using gradient diffusion methodology (E-test, bioMerieux). The aim was to select meningococci that yielded raised MICs to antibiotics other than just penicillin or rifampicin. Organisms were selected that demonstrated unusual MIC levels to more than one antibiotic – a difficult task. The antibiotics initially reviewed were: penicillin (PEN), cefotaxime (CTX), rifampicin (RIF) and ciprofloxacin (CIP). Ceftriaxone (CRO) was not routinely tested at HPA MRU.

Once the HPA MRU MIC levels were reviewed and a diversity of values confirmed (particularly some organisms with raised MICs to different antibiotics) it was important to ensure that a variety of *PorA* VR1, *PorA* VR2 and cc and ST results would also be determined. The panel therefore did not reflect the most commonly characterised meningococci in England and Wales or Europe but provided for a diversity of typing results. It should be noted that only a small proportion of case isolates within the extensive archive of the HPA MRU were routinely characterised by MLST and therefore selection was mainly based upon phenotype (including MICs) and *PorA* variants.

Pure preparations of the meningococcal cultures were transported to UKNEQAS for lyophilisation and subsequent distribution to participants where they were to be re-constituted and manipulated within microbiological safety cabinets as directed.

Sample 0814: A serogroup B isolate was selected as representative of the predominant meningococcal infections in Europe. Serogroup B accounts for over 80% of laboratory-confirmed cases in England and Wales. The specific serogroup B case isolate (from CSF, England and Wales, 2007) was selected for the raised MIC to penicillin (PEN, 0.5mg/L = resistant by EUCAST guidelines), with the more unusual phenotype B: 4:P1.7, P1.9 and PorA VR1= 7, PorA VR2= 9 and FetA VR = F4-1. The MLST results were not available at the HPA MRU.

Sample 0815: A serogroup C isolate was included as a representative of the second most common serogroup causing meningococcal infections in Europe. The serogroup C case isolate (from synovial fluid, England and Wales, 2010) was selected for the unusual phenotype C: 14:P1.19, P1.15 with *PorA* VR1= 19-1, *PorA* VR2= 15-11 and MIC slightly raised to rifampicin (RIF, 0.032 mg/L). FetA VR and MLST were not available within the HPA MRU but the expectation was that the serogroup C case isolate was unlikely to be cc11 and probably cc269. This demonstrated the potential of serogroup C to be associated with cc, seen more often with other serogroups in Europe in recent years. A serogroup C, cc11 isolate, was included in the previous distribution 2452 (2009), sample 9202.

Sample 0816: A serogroup Y case isolate (from blood, England and Wales, 2006) was selected as an example of serogroup Y with raised MIC to penicillin (PEN = 0.5 mg/L = resistant by EUCAST guidelines). Although the base was very low, the number of serogroup Y cases has appeared to be increasing in several European countries in recent years. The isolate was phenotypically characterised as Y: 2c: P1.5, P1.2 at the HPA MRU. PorA VR1, PorA VR2, FetA VR and MLST were not available within the HPA MRU, although the phenotype suggested the organism would be the common cc23.

Sample 0817: A serogroup A case isolate (from blood, England and Wales, 2008); selected as representative of the rare case isolates of serogroup A in England and Wales. Phenotypically characterised as A: 4,21:P1.9 (where serotype reactions were determined with both serotype 4 and 21) and with raised MICs to ciprofloxacin (CIP = 0.38 mg/L) and rifampicin (RIF = 0.5 mg/L). Both the CIP and RIF MICs would be regarded as resistant according to EUCAST guidelines.

1.5.2 Non-culture-simulated septicaemia samples

The non-culture samples were designed to simulate clinical septicaemia and comprised heat-killed suspensions of meningococci diluted in sterile horse serum. The intention was to produce four samples, one of which would not contain any meningococci (negative). The dilution of meningococci (positive samples) was designed to mimic levels detected by PCR assays upon clinical samples (serum, EDTA blood, etc.). The three positive samples were to simulate a weak, medium and strong positive result, as indicated by HPA MRU realtime (ABI Taqman™) PCR assays. The samples were designed for safe nucleic acid (DNA) extraction and subsequent testing by PCR-based assays. Depending on participants' assay availability the molecular testing strategy could include conventional PCR followed by gel electrophoresis, real-time PCR or DNA sequencing.

It was thought unlikely that sufficient organisms were available for detection of *N .meningitidis* and determination of serogroup by commercial latex antigen kits or other serological methods. Serological assessment of the samples was not made pre- or post-distribution.

Sample 0818: A heat-killed dilution of a serogroup W135 case isolate (from blood, England and Wales, 2009) was selected. The organism was phenotypically characterised as W135: NT:NT. The organism was confirmed genotypically as PorA VR1= 18-7, PorA VR2= 9-5 and ST-184 and cc22, a representative of W135, (distinct from those associated with the Haji outbreaks of 2000 and 2001) but less common than the PorA VR1= 18-1 and PorA VR2= 30 strains circulating in England and Wales. It is a more unusual organism, selected to increase the diversity of molecular typing results within the EQA panel.

The dilution of 0818 aimed to produce a reasonably good and clearly positive PCR reaction, at a level similar to many clinical samples equating to CT30 using the HPA MRU *ctrA* PCR Realtime Taqman™ assay.

Sample 0819: A heat-killed dilution of a serogroup B case isolate (from blood, England and Wales, 2005) was selected; phenotypically described as B:NT:NT (and therefore not reactive with the widely available NIBSC monoclonal antibody panel for serotype and sero-subtype characterisation). Genotypically, PorA VR1= 17, PorA VR2= 16-30 and ST-136 and 41/44: Where serogroup B cc41/44 meningococci are commonly confirmed in many European states. The FetA VR was not known.

The dilution of 0819 aimed to produce a weak, low-level positive PCR reaction, a level similar to clinical samples equating to CT34 using the HPA MRU ctrA PCR realtime Taqman™ assay. (Approximately 30% of samples received and tested at the HPA MRU may be confirmed at similar CTs or higher; where the negative cut-off CT>45 is used).

Sample 0820: Negative; the sample did not include *N. meningitidis* or any other bacteria and comprised only of freeze-dried diluent (sterile horse serum).

Sample 0821: A heat-killed dilution of a serogroup C case isolate (from blood, England and Wales, 2010) was selected; phenotypically described as C:NT:NT (and therefore not reactive with the widely available NIBSC monoclonal antibody panel for serotype and sero-subtype characterisation). Genotypically, PorA VR1= 18-1, PorA VR2= 3. The FetA and MLST results were not available at the HPA MRU but the expectation was that the serogroup C isolate was unlikely to be cc11: thereby demonstrating that not all serogroup C infections are due to cc11 and may associate with PorA variants seen more often in recent years with W135 (in England and Wales).

Table 3. Summary of N. meningitidis selected for the 2011 EQA panel based on results initially available at HPA MRU

2010 Dist. 2801	HPA MRU Lab No.		Clinical site	Serogroup	Serotype	*PorA*		*FetA*VR	MLST	
						VR1	VR2		ST	cc
0814	M07	0240790	CSF	B	4	7	9	4-1		
0815	M10	0240631	Synovial fluid	C	14	19-1	15-11			
0816	M06	0241360	Blood	Y	2c					
0817	M08	0240233	Blood	A	4 /21	20	9	3-1		
0818	M09	0240033	Blood	W135	nt	18-7	9-5		184	22
0819	M05	0240795	Blood	B	nt	17	16-30		136	41/44
0820	NEGATIVE (sterile horse serum diluent only - no bacteria)									
0821	M10	0240607	Blood	C	nt	18-1	3			

1.5.3 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 suspension of meningococci in a protein matrix or diluent, ideally, one that was suitable for freeze-drying and acceptable for import into all Member States. For this reason sterile horse (equine) serum rather than bovine was used. During the extensive assessment of suitable positive dilutions for the 2009 EQA (distribution 2452) it was observed that 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 may be used but more reproducible results were obtained using horse serum as the diluent.

On receipt of the freeze-dried samples, it was necessary for the laboratories to re-constitute the material with 1mL of sterile water within a microbiological safety cabinet before commencing the local nucleic acid extraction

procedure. 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:

- Viable cell count was estimated by Miles & Misera
- The stock was estimated to contain~10^7–10^8 viable orgs/mL
- The stock suspension was heat-killed (100°C for 10 mins)
- HPA MRU *ctrA* and *siaD* realtime PCR (ABI, Taqman™) assays were used to assess suitable dilutions for the EQA panel simulating typical clinical samples as estimated from *ctrA* CT values
- Frozen stock suspensions were transported to UK NEQAS for MRU-specified dilution (10^3–10^4 viable orgs/mL) in sterile horse serum
- Freeze-drying and international distribution.

Packaging and transport of EQA samples

The 2011 *N. meningitidis* EQA panel (UK NEQAS distribution 2801) was packaged and transported under UN3373 transport conditions. UK NEQAS ensured that the appropriate customs, import and safety documentation accompanied the EQA samples. Instructions for use and safe re-constitution of the freeze-dried material were included.

The EQA distribution was carried out by UK NEQAS on 14 February 2011.

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

Upon receipt, the participating laboratories were advised to reconstitute and handle the EQA material in a safe manner: testing the samples using the methods available to confirm the identity and characterisation of the samples. Participants were encouraged to use their routinely available methods but were not discouraged from using additional techniques or reagents (that they may not use on routine samples submitted to their laboratories).

Results were to be returned to UK NEQAS by 25 March 2011, preferably via the UK NEQAS website (https://results.ukneqas.org.uk) or faxed using the copy of the results report included with the EQA samples.

The request for sulphonamide MIC was an error, Sulphonamides are not used for therapy or prophylaxis and have not been used as an epidemiological marker for a number of years and are therefore not required for TESSy reporting.

An extensive questionnaire was not included but information was requested on the following:

Part 1 Serogroup for isolates (samples 0814–0817)

Comments on serogroup determination; specifically which reagents were used (tested) if the result recorded was 'Not Determined'.

Part 2 MIC for isolates (samples 0814–0817)

The manufacturer of the commercial gradient diffusion MIC strips.

The plate agar medium used for MIC.

Part 3 molecular typing results

The extraction, amplification and detection methods for both culture (0814–0817) and non-culture (0818–0821) samples.

A space was allocated for general comments on the molecular typing.

Standards and accreditation

Designation and interpretation of *N. meningitidis* phenotypic characterisations are not known to be standardised although the genotypic designations are quite strictly controlled through the PubMLST website where the MLST, PorA and FetA databases are hosted and managed by Dr K Jolley, Department of Zoology at the University of Oxford, UK.

Previous *N. meningitidis* EQA distributions highlighted the problem of antibiotic susceptibility interpretation and which guidelines to follow and report. For the purposes of the TESSy database and this EQA distribution it was decided to report the MIC (mg/L) values only without the local interpretation. If desired, ECDC may then interpret using EUCAST guidelines for European surveillance.

There was no requirement for participant laboratories to be operating to ISO standards although there was perhaps an assumption that local (national) accreditation would require 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 HPA MRU is accredited under the Manchester Medical Microbiology Partnership by Clinical Pathology Accreditation UK Ltd under Centre Reference Number 0635.

Website result submission problem

Due to a server problem around the submission date of 25 March and over the following weekend it was felt appropriate to extend submissions for a further week. Although this unfortunately reduced the time available for analysis and review, a report was released to participants in good time (via the website) prior to the annual IBD-labnet meeting.

Assessment of performance and statistics

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 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 whether or not they achieved the 'correct' result. It was hoped that participants would be able to resolve issues themselves (locally) but opportunities were also available to discuss their results at the annual IBD-labnet meeting, either openly or informally with other participants or the co-ordinator. Dr S Gray was also available via email steve.gray@hpa.org.uk.

2. Results and discussion

EQA panels were distributed to 31 countries (see Section 1.2 participants) and 30 reports of results were returned to UK NEQAS which was considered an excellent response.

UK NEQAS collated the results and produced a draft report that was reviewed by Dr S. Gray. An email reminder was sent to all the participating reference laboratories indicating that they could access their individual report via the UK NEQAS website (https://results.ukneqas.org.uk) from 15 April 2011 using a unique code.

The summary report was comprehensive, indicating the individual laboratory's results compared to all other submitted results. When reviewed in detail minor errors were observed in the assignment of the ccs and this required a revision which was released on 28 April 2011.

2.1 Characterisation of viable isolates

The phenotypic characterisation of the four viable isolates (Nos. 0814–0817) was generally quite successful with the serogroup reported by 29 laboratories for each sample.

The consensus isolate phenotype (serogroup and MIC) results are shown in Table 4, as compiled from the complete final report. The modes were determined from the actual MIC values submitted.

Table 4. Consensus isolate phenotypic characterisations, serogroup and mode MIC recorded

Sample	Serotype	MIC (mg/L)				
		CIP	CRO	CTX	PEN	RIF
0814	B	0.004	0.003	0.016	0.5	0.008
0815	C	0.003	<0.002	0.002/0.003[1]	0.032	0.016
0816	Y	0.003/0.004[2]	0.003	0.016	0.5	0.004-0.008[3]
0817	A	0.19	<0.002	0.002/0.004[4]	0.047	0.125

[1]0815 CTX bimodal distribution was observed

[2]0816 CIP bimodal distribution was observed

[3]0816 RIF bimodal distribution was observed

[4]0817 CTX bimodal distribution was observed.

2.1.1 Serogroup

Phenotypic serogroup

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

All 29 laboratories confirmed serogroup C for sample 0815 and serogroup A for 0817. Sample 0814, was confirmed as serogroup B by 28 laboratories (representing 93% of participants) with one laboratory recording it as not possible to identify the serogroup (Table 4).

Sample 0816 proved more problematic with 24/29 (82%) of submitted serogroup results confirming serogroup Y and one other laboratory recording either Y or W135 (Y/W135). Therefore, by inference, correct results were recorded by 25 (83%, 25/30) of all the participant laboratories. Two laboratories recorded incorrectly; one recorded serogroup W135 and the other neither A, C, Y or W135.

Genogroup

0814: 17 laboratories submitted genogroup results; all correctly identifying serogroup B. Although all 17 laboratories stated the use of *siaD*-based PCR assays a number of synonyms were reported.

0815: 17 laboratories submitted genogroup results; all correctly identifying serogroup C. The majority of laboratories (16) stated the use of *siaD* assays, one used *ctrA*.

0816: 17 laboratories submitted genogroup results but only 15 correctly identified serogroup Y (all using *siaD* assays). One laboratory incorrectly identified the meningococcus as serogroup C using a *siaD* assay.

0817: 17 laboratories submitted genogroup results but only 15 specifically identified serogroup A. Two laboratories using either *siaD* or *ctrA* assays recorded the sample as 'neither B nor C' which was also correct.

Serogroup A was confirmed by laboratories recording the following PCR assays: *siaD* (1), *sacB* (5), *orf2* (3), *mynA* (1), *siaD/orf2* (3), *siaD/xcbA/mynB* (1), and *siaD/sacC* (1). Whilst it is unlikely that *siaD* was actually used to confirm serogroup A, the other combinations reflect synonyms used for genes and the full range of assays that may have been applied to determine the serogroup A result.

2.1.2 Antimicrobial susceptibility – MIC results

Note: Incorrect mode MICs initially reported via the UK NEQAS website. Unfortunately errors were made in the initial report released to the UK NEQAS website, most notably 0816 PEN MIC mode was recorded as 1.5 mg/L but was actually 0.5 mg/L. This was corrected for the IBD-labnet EQA workshop 2011 in Ljubljana, Slovenia. Further incorrect modes were identified later. The corrected and erroneous modes are presented for comparison in Annex 2.

We apologise for any inconvenience caused to participants in their reviews and analysis.

All laboratories reporting MICs tested PEN MICs 29/30 (97%). CIP was reported by 28/30 (93%) laboratories and RIF by 24/30 (80%) but considerably fewer reported (tested) CRO and CTX 19/30 (63%) (Table 5).

Most laboratories used commercial gradient diffusion strips that started at 0.002 mg/L for all antibiotics but at least four laboratories used a higher starting dilution of 0.016 mg/L. This meant that some results were recorded as <0.016 mg/L and were therefore impossible to assign agreement to the consensus in most instances.

The analysis of the actual submitted MIC values reported for each sample and the calculation of frequencies are presented in Annex 3; where the consensus (mode) values and the distribution of the reported MIC values are indicated.

Table 5. Gradient diffusion MIC reports from laboratories

	Number of labs reporting MIC	% labs reporting MIC[2]
CIP	28	93
CRO[1]	19	63
CTX	19	63
PEN	29	97
RIF	24	80

[1]CRO: a maximum of 19 reports were made for 0814 but only 18 for 0815, 0816 and 0817

[2]The denominator was 30 laboratories even though MIC reports were only received from a total of 29 laboratories.

Table 6. Number and proportion% of laboratories in agreement with MIC mode (consensus) using the submitted MIC values

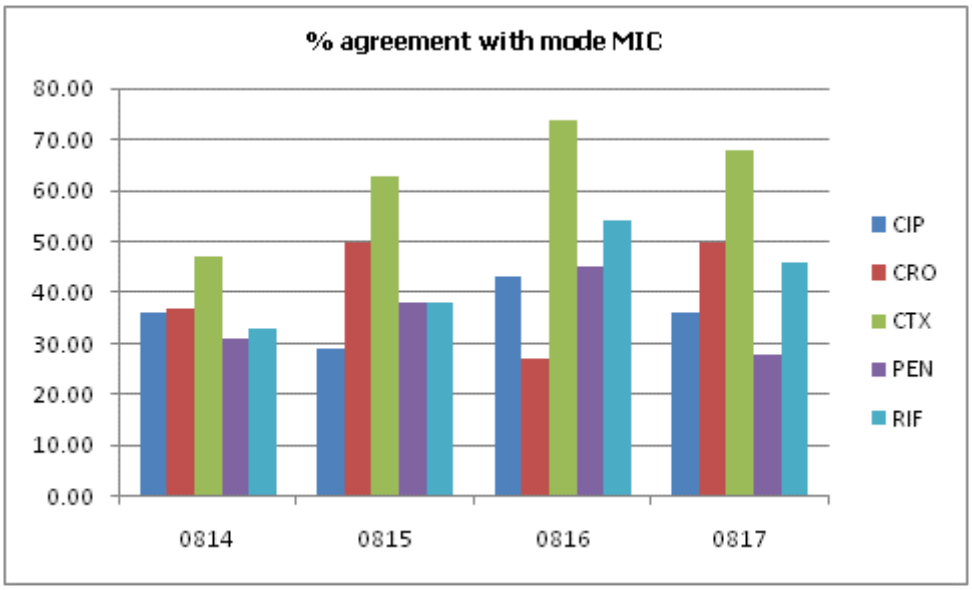
Antibiotic	No. (%) of labs reporting the consensus EQA number				Total reports
	0814	0815	0816	0817	
CIP	10 (36)	8 (29)	12 (43)	12 (43)	28
CRO[1]	7 (37)	9 (50)	5 (27)	5 (27)	19
CTX	9 (47)	12 (63)	14 (74)	14 (74)	19
PEN	9 (31)	11 (38)	13 (45)	13 (45)	29
RIF	8 (33)	9 (38)	13 (54)[1]	13 (54)[1]	24

[1] Range used 0.004–0.008 to calculate mode (consensus) for 0816 RIF

Table 6 demonstrated that the consensus (mode) result was attributed to a relatively small number (proportion) of laboratories when the actual submitted MIC values were analysed. For example, the maximum agreement (74%) was achieved for the CTX MIC of sample 0816 where values were reported by 19 laboratories, which is equivalent to 63% (19/30) of all EQA participants. On the other hand, only five laboratories (27%, 5/19) agreed the CRO MIC for 0816 which was equivalent to 17% (5/30) of all EQA participants The proportion of the submitted MIC values is represented graphically in Figure 1 where the denominator varies by antibiotic. Reports of MICs expressed as <0.016 are excluded from the numerator data.

Figure 1. Proportion (%) of laboratories in agreement with antibiotic mode MIC using the submitted actual values

(Figure 1 utilises data derived from Table 4 and presented as Table 6).



The most successful agreement was for CTX where all samples were sensitive: 47% (9/19), 63% (12/19), 74% (14/19) and 68% (13/19) agreement for 0814, 0815, 0816 and 0817 respectively.

In order to accommodate the small differences in the MIC dilutions on the gradient strips that may be accepted as minor differences with regard to the reported MICs (and clinical interpretation), and to standardise the interpretation, the submitted MIC values were converted to the EUCAST doubling dilution series, as described in Annex 4. The data were reanalysed using + or – one EUCAST MIC dilution (Table7). Figure 2 was derived from an analysis using the varying denominator, reflecting the number of laboratories reporting the sample-antibiotic combination. Figure 2 does not include the <0.016 reports. Laboratories reporting <0.016 were presumed to be using high range strips. Figure 3 shows the proportion of laboratories in agreement with the EUCAST mode +/- one dilution but using the total laboratory denominator –30, thereby accounting for laboratories not testing (reporting) specific antibiotic MICs.

Table 7. Number and proportion (%) of laboratories in agreement with MIC mode (consensus) +/- one dilution, using the converted EUCAST MIC values (% calculated using the total submitted reports denominator)

Antibiotic	No. (%) of labs reporting the consensus +/- one dilution EQA number				Total reports
	0814	0815	0816	0817	
CIP	24 (86)	23 (82)	25 (89)	25 (89)	28
CRO[1]	11 (58)	11 (61)	11 (61)	11 (61)	19
CTX	18 (95)	16 (84)	16 (84)	14 (73)	19
PEN	25 (86)	28 (97)[1]	26 (89)	26 (89)	29
RIF	19 (79)	19 (79)	14 (58)	23 (96)	24

[1] Note PEN bimodal values 0.03–0.06 used for sample 0815 analysis.

Figure 2 Proportion (%) of laboratories in agreement with antibiotic mode MIC (consensus) +/- one EUCAST MIC dilution, variable denominator

Where the proportion of laboratories reporting MICs was calculated using the varying submitted denominators for each antibiotic, 19–29 laboratories (see Table 7).

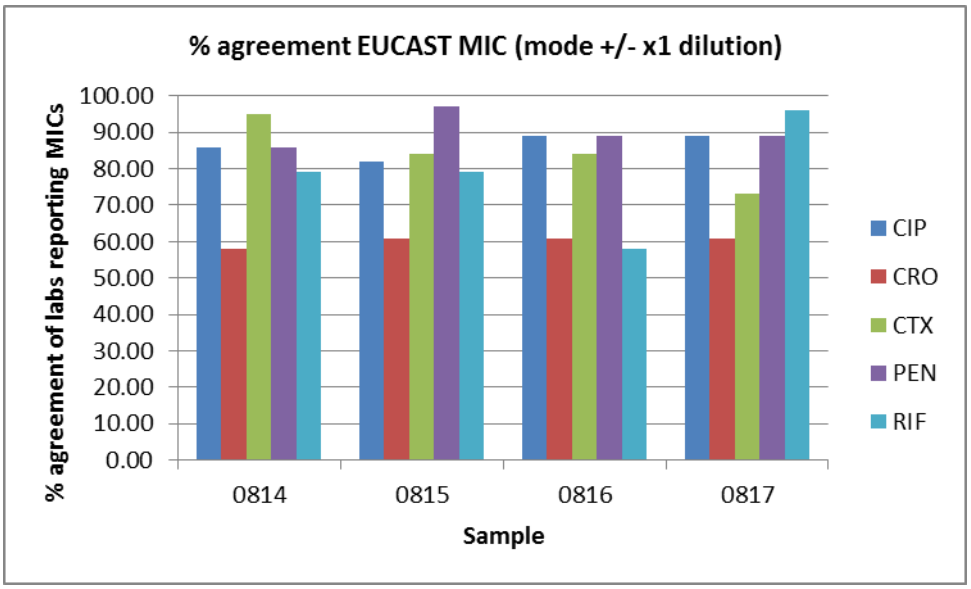
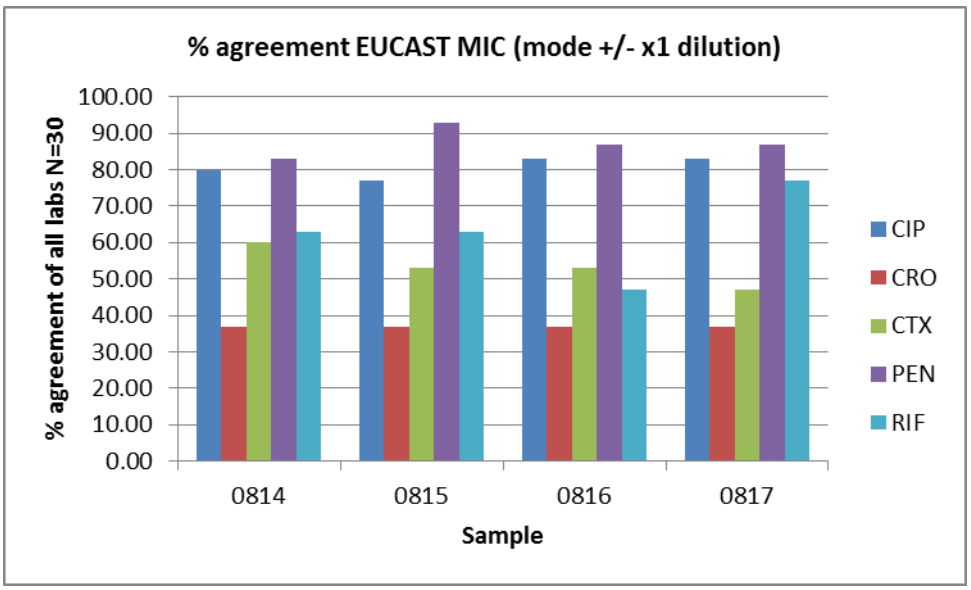


Figure 3. Proportion (%) of laboratories in agreement with antibiotic mode MIC (consensus) +/- one EUCAST MIC dilution, (denominator N=30)

Where the proportion of all laboratories (N= 30) was calculated, a standard denominator is used to account for laboratories not reporting MIC (sample/antibiotic) results.



It was hoped that standardisation would be achieved by using commercially produced gradient diffusion MIC strips and agar plate media. It is possible that the organism suspensions and MIC observations were not so standardised. It is essential that the agar plate medium is supplied or produced to the correct depth (4mm) to allow for the correct MIC gradient to be produced.

In Annex 3 the actual submitted MIC distributions are colour-coded. Orange indicates the mode or mode-range. Yellow indicates values within one dilution of the mode and cream the values within two dilutions of the mode.

The pink fill indicates results that are quite different to the mode and may need to be checked by participants from a technical viewpoint; although the MIC values would not necessarily change the EUCAST interpretation. Annex 5 shows the EUCAST converted MIC reports by participating laboratory for each sample-antibiotic combination.

2.1.3 Interpretation of MICs

The analysis revealed that there were very few reports that would have resulted in an incorrect MIC interpretation (EUCAST guidelines).

One laboratory (NM28) reported CIP as 0.06 mg/L for 0815 which would be reported as resistant when all other reports were sensitive. Three laboratories (NM39, NM47 and NM48) reported CIP 0.06mg/L for 0817 which, although indicating resistance (and therefore EUCAST correct interpretation), were two dilutions different to the mode.

All samples were CRO sensitive and were reported with values <= 0.12 mg/L. However there were a number of reports for each sample that were more than three dilutions different to the mode. Notably: 0.03 mg/L (NM44), 0.06 mg/L (NM23) and 0.12mg/L (NM45) for 0816; 0.03 mg/L (NM44 and NM45) and 0.06 mg/L (NM23) for 0814.

All the CTX reports would be correctly interpreted using EUCAST but there was one report each for 0.016 mg/L of 0815 (NM40) and 0817 (NM40) that were different to the mode by more than three dilutions.

One laboratory reported PEN for 0814 as 0.03 mg/L (NM48), indicating sensitivity but the consensus was resistant. Three laboratories reported 0814 PEN as 0.12 mg/L (NM26, NM34 and NM45) which may be regarded as intermediate ('less susceptible to penicillin'). Similarly one report for 0815 PEN of 0.12 mg/L (NM32) and four reports for 0816, 0.12 mg/L (NM52) and 0.25 mg/L (NM34, NM43 and NM45) were also intermediate ('less susceptible to penicillin').

Only one report was made indicating RIF resistance for 0817, 0.50 mg/L (NM54) compared to the sensitive consensus at 0.12 mg/L. A dilution of 0.5 mg/L under the EUCAST guidelines would therefore be incorrectly assigned as resistant.

2.1.4 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).

Ninety percent (27/30) of the laboratories reported the use of E-test methodology and 7% (2/30) 'other method' in response to the gradient MIC.

Four laboratories (out of all the MIC testing laboratories) recorded the use of Liofilchem MIC test strips.

In total, 80% of participants (24/30) responded to the question regarding agar plate medium suppliers indicating 10 commercial agar plate medium suppliers: ThermoFisher-Oxoid (8 laboratories), Becton Dickinson (4), bioMerieux (2), Merck (2), AES (1), BioRad, (1), E & O (1), LIP (1) and LabM (1). Three (10%, 3/30) laboratories reported the use of an in-house produced agar plate medium.

Three types of agar plate medium were reported from 23 laboratories: Mueller Hinton with heated sheep blood (70%, 16/23), Mueller Hinton with heated horse blood (26%, 6/23) and one laboratory used IsoSensitest agar with horse blood and NAD.

Reviewing laboratories with more discrepant results in relation to methodology and reagents, the only notable feature was that NM45 used in-house prepared agar plate medium. NM28 and NM34 recorded the use of Oxoid Mueller Hinton plate medium with sheep blood and NM28 Oxoid Mueller Hinton plate medium with horse blood. Both variations of the Oxoid plate Mueller Hinton media were successfully used by other laboratories.

2.1.5 MIC summary

The small panel of isolates 0814–0817 indicated the advantage of the gradient diffusion method for MIC determination; standardised dilutions but also the artefact of very close dilution series. Minor differences in agar plate volume may have affected the depth of medium or the differences in plate manufacture may have been responsible for the range of results around the mode, in some instances resulting in a bimodal distribution.

The use of the high range strips was not foreseen and consequently led to the difficulty in reconciling reports of <0.016 mg/L.

It was quite gratifying to know that if all the reported MICs had been interpreted according to EUCAST guidelines there would have been very few incorrect susceptibility designations. However, it is clear that there is still some way to go to generate accurate data from all laboratories.

Approximately 80% of the laboratories submitting MIC results achieved the mode +/- one dilution. An indication of the ranges for each antibiotic is as follows: CIP (82–89% agreement), CRO (58–61% agreement), CTX (70–95% agreement), PEN (80–97% agreement) and RIF (58–96% agreement) (see Table 7 for details).

Although most laboratories test for PEN and CIP MIC, fewer test for RIF and even fewer (63%) for CTX and CRO (see Tables 5, 6 and 7).

Six laboratories had more problems with the EQA panel and methodology than the others. Specifically, NM23, 26, 28, 34, 37 and 45 reported discrepant MICs for three or more samples, irrespective of the antibiotic. In addition, four of these (laboratories NM23, 28, 34 and 45) reported MICs >four dilutions different to the consensus for more than one isolate-antibiotic combination.

Reviewing the MIC method and agar plate medium reported with the discrepant results did not reveal an obvious trend. This may suggest that a combination of factors including the concentration (density) of organism suspension, medium, strips and operator processing may be involved. It is also not possible to rule out the introduction of contaminants (less susceptible) to the antibiotics during processing.

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

2.2 Simulated non-culture samples

2.2.1 Species detection

Four simulated septicaemia samples (0818, 0819, 0820 and 0821) 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. The samples contained heat treated suspensions of organisms diluted in sterile horse serum.

Three samples contained *N. meningitidis* DNA: 0818 serogroup W135, 0819 serogroup B and 0821 serogroup C. 0820 was negative, containing only horse serum diluent (see Table 8).

Table 8. Consensus species detection and genogroup for the simulated septicaemia samples

EQA number	Species	Genogroup
0818	*N. meningitidis*	W135
0819	*N. meningitidis*	B
0820	Negative	
0821	*N. meningitidis*	C

Sample 0818: All 23 (100%) laboratories reported the sample positive for meningococcal DNA. Assuming a maximum of 30 potential testing laboratories as recorded for the phenotypic results, 77% of the participant laboratories were able to test and detect the non-culture sample.

Fourteen laboratories utilised a *ctrA* PCR assay, three a *crgA* assay, three a *porA* assay and two 16SrDNA-based assays for species detection.

Genogroup W135 was confirmed by 16 laboratories. It was also reported as 'Neither B nor C' by two laboratories, a correct if not specific result. Unfortunately one laboratory reported genogroup C and another 'neither B, C, Y or W135'. The correct specific result, W135, was reported by 73% (16/22) of those submitting a genogroup result which represented 53% (16/30) of the participant laboratories.

SiaD (synonymous with *synF*) based assays were used by 15 of the laboratories with only one incorrect genogroup C result reported. Genogroup W135 was also reported by four laboratories stating a *synG* based assay probably reflecting an error in the designation of reporting options (synonyms).

Sample 0819: All 22 (100%) laboratories reported the sample positive for meningococcal DNA. Assuming a maximum of 30 potential testing laboratories as recorded for the phenotypic results, 73% (22/30) of the participant laboratories were able to test and detect the non-culture sample.

Genogroup B was correctly confirmed by 17 laboratories. The correct specific result, B, was reported by 89% (17/19) of those submitting a genogroup result, which represented 57% (17/30) of the participant laboratories.

Two laboratories reported 'neither A, B, C, Y or W135'. Taken with the fact that 23 laboratories had reported genogroup results for other samples the inability of six laboratories to confirm genogroup B may reflect the design of the 'weak' positive sample.

SiaD based PCR assays were used by all 19 laboratories reporting results for 0819.

Sample 0820: Twenty-three laboratories tested the sample which contained no meningococcal DNA and 22 of them (96%) confirmed the correct negative result. One laboratory (NM41) reported a positive result for meningococcal DNA. Assuming a maximum of 30 potential testing laboratories as recorded for the phenotypic results, 73% (22/30) of the participant laboratories were able to identify the negative sample.

It is not apparent from the responses how one laboratory did not agree with the negative consensus but it could be a genuine technical or contamination issue or possibly a simple clerical error. It is hoped that laboratory NM41 will investigate the initial processing of the sample and consider re-testing it.

Most laboratories did not report genogroup but two laboratories recorded the negative sample as 'neither A, B, C, Y or W135' which is also correct and may reflect their comprehensive testing algorithm.

Sample 0821: Twenty-three (100%) laboratories reported the sample positive for meningococcal DNA. Assuming a maximum of 30 potential testing laboratories as recorded for the phenotypic results, 77% (23/30) of the participant laboratories were able to test and detect the non-culture sample.

Genogroup C was correctly confirmed by 19 laboratories. The correct specific result, C was reported by 82% (19/23) of those submitting a genogroup result, which represented 63% (19/30) of the participant laboratories.

SiaD based PCR assays were used by all 19 laboratories reporting genogroup C for 0821.

2.2.2 Non-culture detection and genogroup summary

Approximately 76% of the 30 participant laboratories were capable of detecting non-culture *N. meningitidis* (see Table 9) which is quite an impressive result. The genogroup confirmation is more exacting as the assays appear to be less sensitive and the more dilute simulated septicaemia samples are challenging. Nevertheless, over 50% of the participant laboratories determined the genogroups.

The use of more dilute (but detectable) positive samples is required to broaden the range of genogroups and challenge the processing of samples at clinical levels.

Table 9. Proportion (%) of participant (30) laboratories agreeing with consensus for detection and genogroup

EQA number	Detection	Genogroup
0818	77%	53%
0819	73%	57%
0820	73%	N/A*
0821	77%	63%

*N/A = Not applicable, negative sample

2.3 Genotyping of isolates and simulated septicaemia (non-culture) samples

Participants were asked to test and report the PorA variable regions VR1 and VR2, the FetA variable region (VR) and to use MLST to determine the sequence type (ST) and clonal complex (cc) for all meningococcal samples.

Where full *N. meningitidis* ST designation required the DNA amplification and sequencing of internal fragments of seven gene loci (*abcZ, adk, aroE, fumC, gdh, pgm* and *pdhC*) and comparison with the PubMLST database, the cc was assigned on the basis of agreement between four or more loci. The full or partial allelic profiles determined by laboratories were not reported.

2.3.1 Genotyping of isolates

The consensus results for PorA, FetA and MLST genotyping of the four isolate samples are presented in Table 10.

Table 10. Isolate sample - consensus genotyping (PorA, FetA and MLST) results

EQA number	Phenotype	PorA		FetA	MLST	
	Group	VR1	VR2		ST	cc
0814	**B**:4: P1.7,9	7	9	F4-1	35	35
0815	**C**:14: P1.19,15	15-11	F1-7		467	269
0816	**Y**:2c: P1.5.2	5-1	2-2	F5-8	23	23
0817	**A**: 4/21: P1.9	20	9	F3-1	4789	5

The total number of respondents to the molecular typing targets varied from sample to sample, ranging from 21–22 for PorA, 20–21 for FetA and 16 for MLST.

0814: All 22 of the 30 participating laboratories (73%) reported the consensus PorA VR1 7. The consensus PorA VR2 9 was determined by 21 of the 30 participating laboratories (70%) with one laboratory (NM37) reporting PorA VR2 9-10.

The explanation for one laboratory reporting PorA VR2 9-10 instead of PorA VR2 9 could be poor quality sequence data or, more likely, a simple technical error when assessing the options on the PorA typing website as the two VR2 variants differ by only three amino acids. VR2 9 = YVDEQSKYHA, compared to VR2 9-10 EQSKYHA. VR 9-10 is missing the YVD motif, equivalent to nine nucleotide bases.

Twenty-one of the 30 participating laboratories (70%) reported FetA VR: all reported F4-1.

Fifteen of the 30 participating laboratories (50%) reported ST results: all reported ST-35.

Sixteen of the 30 participating laboratories (53%) reported cc results: all reported cc35. One laboratory (NM37) may have had difficulty with one or more of the alleles in determining the specific ST-35 but had sufficient data to infer the cc. [Note: It was the cc and not the ST that was requested for the TESSy database].

0815: All 21 of the 30 participating laboratories reported PorA VR1 19-1 and VR2 15-11, representing 70% of the participating laboratories.

Twenty of the 30 participating laboratories (67%) reported FetA VR: all reported F1-7.

Fourteen of the 30 participating laboratories (47%) reported ST results: all reported ST-467.

Sixteen of the 30 participating laboratories (53%) reported cc results, all reported cc269. This indicates that two laboratories (NM37 and NM44) may have had difficulty with one or more of the alleles in determining the specific ST-269 but had sufficient data to infer the cc. [Note: It was the cc and not the ST that was requested for the TESSy database].

0816: All 21 of the 30 participating laboratories reported PorA VR1 5-1 and VR2 2-2, representing 70% of the participating laboratories.

Twenty of the 30 participating laboratories (67%) reported FetA VR: all reported F5-8.

Fifteen of the 30 participating laboratories (50%) reported ST results: all reported ST-23.

Sixteen of the 30 participating laboratories (53%) reported cc results: all reported cc23. This indicates that one laboratory (NM37) may have had difficulty with one or more of the alleles in determining the specific ST-23 but had sufficient data to infer the cc. [Note: It was the cc and not the ST that was requested for the TESSy database].

0817: Twenty-one of the 30 participating laboratories (70%) reported PorA VR1 20 but 22 (73%) reported PorA VR2 9. This indicates that one laboratory (NM22) was unable to determine the PorA VR1. Thus, the full PorA VR consensus was only achieved by 21 laboratories, representing 70% of the participants.

Twenty of the 30 participating laboratories, (67%) reported FetA VR: all reported F3-1.

Fourteen of the 30 participating laboratories (47%) reported ST results: all reported ST-4789.

Sixteen of the 30 participating laboratories (53%) reported cc results, all reported cc5. This indicates that two laboratories (NM34 and NM42) may have had difficulty with one or more of the alleles in determining the specific ST-4789 but had sufficient data to infer the cc. [Note: It was the cc and not the ST that was requested for the TESSy database].

2.3.2 Genotyping of simulated septicaemia samples

The consensus results for PorA, FetA and MLST genotyping of the four simulated septicaemia (non-culture) samples are presented in Table 11.

Table 11. Simulated septicaemia sample – consensus genotyping (PorA, FetA and MLST) results

EQA number		PorA		FetA	MLST	
	Genogroup	VR1	VR2		ST	cc
0818	W135	18-7	9-5	F3-7	184	22
0819	B	17	16-30	F5-8	136	41/44
0820	Negative					
0821	C	18-1	3	F3-9	5133	103

0818: Fourteen of the 30 participating laboratories (47%) reported PorA VR1 18-7 and 15 (50%) reported PorA VR2 9-5. This indicates that one laboratory (NM32) was unable to determine the PorA VR1. Thus, the full PorA VR consensus was only achieved by 14 laboratories, representing 47% of the participants.

Ten of the 30 participating laboratories (33%) reported FetA VR: all reported F3-7.

Six of the 30 participating laboratories (20%) participants reported ST results: all reported ST-184.

Seven of the 30 participating laboratories (23%) reported cc: all reported cc ST-22. This indicates that one laboratory (NM37) may have had difficulty with one or more of the alleles in determining the specific ST-184 but had sufficient data to infer the cc. [Note: It was the cc and not the ST that was requested for the TESSy database].

0819: Fifteen of the 30 participating laboratories (50%) reported PorA VR1: 13 as PorA VR1 17 (the consensus), one as PorA VR1 18-1 and one as PorA VR1 7-2. Two laboratories (NM27 and NM32) therefore reported incorrect porA VR1 values. Similarly, thirteen of the 30 participating laboratories reported PorA VR2: 13 (43%) as PorA VR2 16-30 (the consensus), one as PorA VR2 3 and one as PorA VR1 4-21

The full PorA VR consensus was therefore only achieved by 13 laboratories, representing 43% of the participants.

The incorrect PorA VR1 and VR2 combinations were: 18-1, 3 and 7-2, 4-21. One laboratory may have had a sample switch (or 'mix-up') with 0821 but it is unclear how 7-2, 4-21 was obtained. It could also be an internal laboratory sample switch ('mix-up') error.

Nine of the 30 participating laboratories (30%) reported FetA VR: all reported F5-8.

Six of the 30 participating laboratories (20%) reported ST results: all reported ST-136.

Seven of the 30 participating laboratories (23%) reported cc: all reported cc ST-41/44. This indicates that one laboratory (NM37) may have had difficulty with one or more of the alleles in determining the specific ST-136 but had sufficient data to infer the cc. [Note: It was the cc and not the ST that was requested for the TESSy database].

0820: The sample did not contain meningococcal DNA (= negative). Molecular typing assays were not applicable.

0821: All sixteen of the 30 participating laboratories reported PorA VR1 18-1 and PorA VR2 3, representing 53% of the participants.

Ten laboratories (33%) of participants reported FetA VR: all reported F3-9.

Seven laboratories reported ST: with six laboratories reporting ST-5133 and one laboratory reporting ST-513. The consensus was reported by six of the 30 participants (20%). ST-513 was incorrectly reported (by NM21) due to a clerical or transcription error (corroborated by the correct reporting of the consensus cc).

Seven of the 30 participating laboratories, (23%) reported cc results, all reported cc103; including the laboratory that incorrectly reported ST-513 (which is not part of cc103) due to a transcription error.

2.4 Summary of genotyping consensus reporting

The proportion of laboratories reporting genotyping results from the 30 participants is summarised in Table 12. The proportion of participants achieving full PorA characterisation is the lower of the two VR1 and VR2 values.

The complete PorA sequence typing (VR1 and VR2) of isolates 0814–0817 was achieved by a minimum of 21 (70%, 21/30) of the participating laboratories but dropped to 43–53% for the non-culture samples 0818–0821.

Similarly, the range of FetA consensus agreement for the isolates (0814–0817) was 67–70%, but only 20% for the non-culture samples (0818–0821).

The ST consensus ranged from 47–50% for the isolates (0814–0817) but was only 20% for the non-culture samples.

The cc consensus reports were 53% for the isolates (0814–0817) and 23% for the non-culture samples.

Table 12. Proportion (%) of all participant (30) laboratories in agreement with consensus for genotyping

Sample	PorA		FetA	MLST	
	VR1	VR2	VR	ST	cc
0814	73%	70%	70%	50%	53%
0815	70%	70%	67%	47%	53%
0816	70%	70%	69%	50%	53%
0817	70%	73%	67%	47%	53%
0818	47%	50%	33%	20%	23%
0819	50%	43%	30%	20%	23%
0820	N/A*		N/A*	N/A*	
0821	53%	53%	33%	20%	23%

*N/A = Not applicable, negative sample

An analysis of the data that considers the proportion of consensus results for those laboratories (countries) that actually submitted results gives a slightly different, but perhaps more impressive result (see Table 13).

Table 13. The proportion (%) of laboratories achieving consensus of those submitting genotyping results

EQA number	PorA		FetA	MLST	
	VR1	VR2	VR	ST	cc
0814	100%	**95%**	100%	100%	100%
0815	100%	100%	100%	100%	100%
0816	100%	100%	100%	100%	100%
0817	**95%**	100%	100%	100%	100%
0818	**93%**	100%	100%	100%	100%
0819	**87%**	**87%**	100%	100%	100%
0821	100%	100%	100%	**86%**	100%

The denominator varied by molecular target.

All laboratories reporting FetA, ST and cc for isolates or simulated septicaemia (non-culture) samples achieved the consensus and are therefore represented as 100% in Table 13. The one transcription error for the 0821 ST result reduced the proportion to 86%.

There were only three other deviations from 100% agreement for all the submitted molecular typing results. All three related to PorA: two for VR1 and one for VR2. Nevertheless, PorA agreement was over 93% for all samples and laboratories (countries).

It was observed that laboratories NM22 (one error), NM27 (one), NM32 (two) and NM37 (one) had some problems with PorA.

MLST problems were encountered by NM34 (one error), NM37 (five), NM42 (one) and NM44 (one).

MLST problems were inferred from the unreported ST where a cc was reported for the same sample. Laboratories may have encountered problems with the determination of the allelic variants at one to three loci as cc definition requires the confirmation of identical alleles at four out of seven loci. It is possible that the four laboratories indicated only had problems with one locus or that one or two loci were problematic.

Whilst determining the ST for sample 0817 (the serogroup A isolate) the laboratory of the author encountered some difficulty sequencing the pdhC allele using two standard (alternate) primer sets. To obtain a PCR product the recommended sequencing primers were used for both initial PCR amplification and the cycle sequencing reactions. This is not best technical practice but produced the desired result. Problems could have been due to variation in the pdhC primer region although this has not been investigated. Interestingly, there were only four isolates in the PubMLST database which had pdhC allele 334. These belonged to serogroup A and had PorA VR1=20 VR2=9 which matched the results for isolate 0817. The sample was not selected as part of the EQA panel with regard to pdhC primer variation.

2.4.1 'Fine type' – agreement with full data requested by ECDC for TESSy.

The proportion of laboratories reporting the consensus 'fine type' (serogroup: PorA: FetA: cc) as requested by ECDC for the TESSy dataset is dependent on the sample: 16/30 (53%) for isolates but only 6/30 (20%) for the simulated septicaemia (non-culture) samples. The main reason for the proportion being lower is the smaller number of laboratories currently carrying out MLST, and particularly MLST of non-culture samples.

There are more difficulties associated with genotyping non-culture material, in particular with regard to the amount of genomic material available. This necessitates nested (or two rounds of) PCR amplification before the cycle sequencing. It becomes even more difficult with the multiple targets required for MLST. Often alternate (or additional) sets of primers are required which may involve the use of additional, optimised thermal cycling parameters.

2.5 Molecular methodology reports

It was accepted that most participants would use conventional slide agglutination or serological techniques to establish the serogroup and that the MIC investigation was specifically targeted to gradient diffusion (by E-test or similar). The MIC gradient strip of the manufacturer/supplier was requested and a review of the responses associated with MIC results is discussed in Section 2.1.2. Possible problems due to in-house media production were mentioned for one laboratory.

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

The methods used for the isolates are presented in Table 14. Simple heated (boiled) suspensions of meningococci are confirmed as a suitable genotyping technique, with a number of laboratories using conventional PCR and gel detection to determine results. More than 43% (13/30) laboratories achieved PorA, FetA and MLST sequencing, while DNA sequencing was only reported by four laboratories.

Table 14. Methods used for genotyping of isolates, samples 0814–0817

Method		
Extraction	**Amplification**	**Detection**
Salt precipitation (1)	Not stated (1)	Other (1)
Boil (13)	Conventional PCR (17)	Gel (11)
Spin column (5)	RealTime PCR (6)	Sequencing (4)
Magnetic beads (3)		Taqman probes (3)
Other (2)		Fluorescence (3)
		Not stated/Not examined (2)
Total 24	Total 24	Total 24

Similar responses were reported for the non-culture samples 0818–0821 (Table 15). 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. The report of sequencing by two laboratories could refer to the DNA sequencing of PCR products to confirm species or genogroup or to the other molecular typing assays.

Table 15. Methods used for genotyping of isolates, samples 0818–0821

Extraction	Amplification	Detection
Spin column (15)	RealTime PCR (11)	Taqman probes (6)
Magnetic beads (3)	Conventional (nested) PCR (8)	Fluorescence (5)
Capture column (1)		Gel (6)
		Sequencing (2)
Total 19	Total 19	Total 19

2.6 Summary comparison of IBD-labnet *N. meningitidis* EQA panels 2009 and 2011

The second IBD-labnet EQA panel was distributed to 31 countries in 2011 (sent to 30 in 2009). In 2011, 30 reports were returned, compared to 29 in 2009.

With regard to the phenotyping of isolates, there were relatively few problems for either panel although it should be noted that there were six isolates in 2009 and only four in 2011. None of the isolates distributed in 2011 were the same (repeats) as the 2009 panel but there were examples of the same serogroups.

The determination of serogroup A did not cause problems in 2011, compared to 2009. This could be related to the isolate or assistance provided by the training workshop in 2010. Unfortunately, there were still some problems with serogroup Y. Two out of 27 (7%) laboratories incorrectly identified the serogroup Y isolate in 2009 and four out of 30 (13%) in 2011. This is an area of interest as in recent years a small but increasing number of serogroup Y cases have been being reported by a number of European laboratories. There is also some anecdotal evidence of an increase in serogroup Y carriage in some areas.

In 2011, the evaluation of MIC results was considerably easier due to the requirement for similar method and reporting dilutions. Differences were observed but only a small number of laboratories appeared to have problems.

Reducing the number of phenotyping and genotyping fields (results) for reporting and specifying options appeared to cause less confusion and clerical errors with website reporting.

With regard to the molecular assays there was broad agreement, with few laboratories indicating problems. The non-culture samples proved more difficult than the isolates as they are more exacting. However, those testing the non-culture material were generally very successful. This could reflect the strong positivity of the material even though efforts were made to produce 0819 as a simulated, 'weak positive' septicaemia sample.

There was an increase in the maximum number of laboratories reporting molecular detection results: 20 out of 29 (69%) in 2009 and 23 out of 30 (77%) in 2011, although this was dependent on the sample. With regard to the genogroup for the non-culture samples, in 2009 19 out of 29 (66%) laboratories submitted results whereas in 2011 this increased to 22 out of 30 (73%) laboratories.

In 2011, the genotyping revealed very few reports that were different to the consensus and, where identified, it appeared that simple laboratory or transcription errors could be implicated.

Only nine out of 29 (31%) laboratories could successfully report a complete 'fine type' in 2009 for the six isolates and two (7%) for the exacting non-culture samples: the constraint being the number of laboratories testing FetA (Table 16). In 2011, 16 out of 30 (53%) laboratories confirmed the maximum 'fine type', constrained by the number assigning the cc. With regard to non-culture 'fine types' achieved in 2011, there were six out of 30 (20%), again constrained by the non-culture MLST.

Undoubtedly there has been a marked improvement in 'fine type' ascertainment as more laboratories have demonstrated the 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 the lack of a designated non-culture protocol with defined nested and sequencing primer sets.

Table 16. Number (proportion) of laboratories reporting 'fine type' (serogroup: PorA: FetA: cc)

Year	'Fine type'	
	Isolates	Non-culture
2009	9/29 (33%)	3/29 (11%)
2011	16/30 (53%)	6/30 (20%)

Note: 29 participants (reports) received in 2009 and 30 in 2011.

Conclusions

Overall the 2011 *N. meningitidis* IBD-labnet EQA was successful. Improvements were noted in the number and quality of responses to the requested detection and characterisation targets. The reduction in the amount of information requested and the restricted options for website reporting greatly facilitated the review of results. Nevertheless, the 2011 panel still requested a total of 66 technical results (for the eight samples) and responses to several questions regarding MIC and molecular reagents and methods. The ST and cc designations required 14 sequencing reactions per sample, making a total of 98 (7x14) reactions and analyses, which involved a considerable amount of work for participants, particularly when coming as an addition to their routine workload. The comprehensive distribution also generated a large amount of data for comparative analysis.

Participants' ability to characterise the material, both as culture and non-culture, using molecular techniques demonstrated improvement since the last EQA distribution. The MLST characterisation of cultures, and particularly the non-culture samples, requires increased usage to provide comprehensive, accurate strain characterisation coverage for Europe. Similarly, the application of non-culture detection, although increasing among participants, is not available to all laboratories.

MIC reports (when converted to EUCAST dilutions) allowed for valid comparisons, revealing limited interpretation differences. This would suggest that current gradient diffusion MIC reports would be representative of antibiotic susceptibility in the EU region.

Serogrouping is still an important issue, even following the IBD-labnet training workshop in 2010. Availability of specific and pooled agglutination reagents could still be an issue. International availability of a standard serogroup Y monoclonal antibody similar to the serogroup A, B, C and W135 produced by NIBSC (UK) could be helpful.

The usefulness of genogrouping has been highlighted by the non-culture samples and it is possible that widespread adoption of PC- based genogrouping could become important for more accurate typing. However, that is different to knowing if a capsule has been expressed and whether a polysaccharide vaccine would actually be an effective intervention.

Similarly, the method responses could have been targeted to a specific sample or samples. The best way to determine which reagents and method a laboratory uses may be to employ a specific questionnaire, separate to an EQA panel distribution.

It is easy to dwell on the reported results and relatively minor errors observed rather than the fact that nearly all the genotyping data submitted was in agreement. Laboratories testing and submitting results for the genotyping of the isolates, and particularly the non-culture samples, are to be encouraged. It is important to focus attention on the laboratories that only partially responded to the available characterisations. The fact that a number of laboratories were unable to report certain characterisations at all is a significant finding. To build Europe-wide capacity it could be necessary to resource laboratories directly or to create partnerships between those that are less well equipped and expert, fully-resourced laboratories.

It should be stated that in hindsight the questions regarding molecular processes and techniques were not specific enough. The fact that most (nearly all) laboratories applying the molecular methods achieved the consensus results is sufficient to record how useful they are. If the non-culture samples had been designed to be more exacting (weaker positives) they would have offered a better test of laboratory methods.

It was assumed that if results were not submitted then laboratories (countries) were not in a position to test the material. The resources and technical procedures required to molecularly characterise material by all the requested assays should not be underestimated and it was 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 infer that a laboratory (country), although capable of accurate characterisation (e.g. 'fine type'), is in a position to characterise all their routine samples and submit the data to TESSy.

Opportunities were given to participants to give feedback on the EQA panel or specific results in person at the annual IBD-labnet meeting in Slovenia 2011, or by email to steve.gray@hpa.org.uk. To date there have been very few comments other than appreciation of the EQA and ECDC's support. Interestingly, one laboratory mentioned their difficulties with 0817 and the *pdhC* allele as previously described (Section 2.3).

Participation in the 2009 and 2011 ECDC IBD-labnet EQAs was on the understanding of participant anonymity and as such laboratories (countries) have only been 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 the individual reports by comparing their results to the consensus or repeating or re-evaluating their results as required. To identify laboratories with specific issues relating to serogroup determination, MIC-related problems, non-culture detection or genotypic characterisation, it would probably be best to consider a questionnaire designed around the EQA panels. This could possibly be done by asking for responses associated with particular samples or techniques. A questionnaire would also be the best way to determine whether laboratories are capable of characterising all

their routine samples to the required TESSy typing targets. Unfortunately the EQA can only estimate the number of laboratories capable of carrying out the detection and typing methods and not what is likely to be routinely available or reported to ECDC (TESSy).

Although there are many encouraging signs within the EQA, such as the generally excellent consensus levels, it should be noted that there are a number of laboratories (countries) unable to confirm non-culture samples and apply the more exacting molecular typing methods. It is not possible to determine from the EQA if this is due to lack of resources or expert knowledge but one may speculate that it is more likely to be the former. The IBD-labnet training workshop (Würzburg, 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 databases website was demonstrated but not the intensive 'hands-on' training which would be necessary to generate the DNA sequences. Similarly, to set up a routine non-culture detection service a laboratory would require considerably more training with the equipment it would have access to. To address the issues it would be appropriate to send out a short, but targeted questionnaire to ascertain which laboratories were/are unable to complete all the requested typing targets and then to set up specific training to meet their needs. Some effort has been made in this direction with laboratory placements in early 2011. Reports from these placements will be available soon.

To assess the sensitivity of molecular assay methods or processes in participants' laboratories it may be useful to consider the distribution of a DNA standard within a subsequent EQA panel.

There are also problems comparing EQA distributions when the samples are not identical. This is compounded with meningococci as there are innumerable strains that could be used, although only relatively few clonal complexes, which are responsible for disease in Europe. Selecting only four isolates immediately 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 is not the intention to distribute non-culture samples from which it is too difficult to detect and determine molecular types. In reality there are a wide variety of meningococci that do not cause disease but that laboratories may be required to assess as part of a potential case investigation. Moreover, there are certainly many confirmed cases with very low positivity in terms of PCR detection (that may not allow serogroup determination or molecular typing).

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

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 Maryse Fauville Dufaux	National Meningococcal Reference Laboratory Scientific Institute of Public Health Rue Juliette Wytsman 14-16 1050 Brussels, Belgium
Bulgaria	Dr Dimitar Nashev	National Centre for Infectious and Parasitic Diseases 26 Y Sakazov Blvd 1504 Sofia, Bulgaria
Cyprus	Dr Despo Pieridou Bagatzouni	Microbiology department, Nicosia general hospital, 1450 Nicosia, Cyprus
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 Jens Jorgen Christensen	Neisseria and Streptococcus Reference Laboratory Department of Bacteriology, Mycology and Parasitology Statens Serum Institut Artillerivej 5, Building 211/117B 2300 Copenhagen, Denmark
Estonia	Dr Rita Peetso	Terviseament Health Board, Paldiski Road 81, 10617 Tallinn, Estonia
Finland	Dr Maija Toropainen	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
Hungary	Dr Ákos Tóth	Department of Bacteriology Johan Bela National Centre for Epidemiology Gyali ut 2-6 1097 Budapest, Hungary
Iceland	Dr Hjordis Hardardóttoir	Department of Clinical Microbiology Institute of Laboratory Medicine Landspitali University Hospital Baronsstigur, 101 Reykjavik, Iceland

Country	Contact person	Institution
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Italy	Dr. Paola Mastrantonio	Department of Infectious, Parasitic and Immunomediated Diseases. Instituto Superiore di Sanitá Viale Regina Elena 299 00161 Rome, Italy
Latvia	Dr Jelena Galajeva	Laboratory of the State Agency Infectology Center of Latvia Bacteriology Department 3 Linezera street Riga, LV 1006, Latvia
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 Migle Janulaitiene	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 Paul Caruana	Matai Dei Hospital Tal-Qroqq Mside MSD 2090, 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
Norway	Prof Dominique A. Caugant	Division of Infectious Disease Control Norwegian Institute of Public Health Lovisenberggata 8 0403 Oslo, Norway
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 Lisbon, 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

Country	Contact person	Institution
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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
UK	Dr Eduard Kaczmarski	HPA Meningococcal Reference Unit Manchester Royal Infirmary Oxford Road, Manchester M13 9WZ, UK

Annex 2. Comparison of the corrected MIC modes *N.meningitidis* EQA distribution, 2801 compared to those initially reported

Erroneous MIC modes reported April 2011

EQA number	Serogroup	MIC (mg/L)				
		CIP	CRO	CTX	PEN	RIF
0814	B	0.006	0.016	0.016	0.5	0.032
0815	C	0.006	0.016	0.016	0.032	0.047
0816	Y	0.006	0.016	0.016	1.5	0.032
0817	A	0.38	0.016	0.016	0.032	0.5

Corrected MIC modes July 2011 (as used in this report)

EQA number	Serogroup	MIC (mg/L)				
		CIP	CRO	CTX	PEN	RIF
0814	B	0.004	0.003	0.016	0.5	0.008
0815	C	0.003	<0.002	0.002/0.003[1]	0.032	0.016
0816	Y	0.003/0.004[2]	0.003	0.016	0.5	0.004-0.008[3]
0817	A	0.19	<0.002	0.002/0.004[4]	0.047	0.125

[1]0815 CTX bimodal distribution was observed

[2]0816 CIP bimodal distribution was observed

[3]0816 RIF bimodal distribution was observed

[4]0817 CTX bimodal distribution was observed

Annex 3. Analysis of the submitted MIC values for the viable isolates to determine % agreement

Ciprofloxacin (CIP) MICs reported

CIP mg/L	EQA number 0814	0815	0816	0817
<0.002	2	2	2	
0.002	1	4	5	
0.003	8	**8**	**6**	
0.004	**10**	4	**6**	
0.006	3	5	5	
0.008	2	2	3	
0.012	2	1		
<0.016				
0.016		1		
0.023			1	
0.032				
0.047				
0.064		1		3
0.094				3
0.125				5
0.19				**10**
0.25				6
0.38				1
0.5				
TOTAL	**28**	**28**	**28**	**28**
No. +/- x1 diln. of mode	**21**	**16**	**22**	**21**
% +/- x1 diln. of mode	**75%**	**57%**	**76%**	**75%**

KEY:

Mode (consensus) indicated in bold and orange ◇
+/- x1 dilution is indicated in yellow ◇
+/- x2 dilutions is indicated in cream ◇
Blue fill indicates the <0.016 reports ◇
Pink fill indicates outliers and results that require checking by participants ◇

Ceftriaxone (CRO) MICs reported

CRO mg/L	EQA number 0814	0815	0816	0817
<0.002		**9**		**9**
0.002		2	1	2
0.003	**7**		**5**	1
0.004	4		2	
0.006		1	2	1
0.008			1	
0.012	1		1	1
<0.016	**4**	**3**	**3**	**3**
0.016		3		1
0.023	2		1	
0.032				
0.047	1			
0.064			1	
0.094				
0.125			1	
0.19				
0.25				
TOTAL	**19**	**18**	**18**	**18**
No. +/- x1 diln. of mode	**11**	**11**	**8**	**11**
% +/- x1 diln. of mode	**58%**	**61%**	**44%**	**61%**

KEY:

Mode (consensus) indicated in bold and orange ◇

+/- x1 dilution is indicated in yellow ◇

+/- x2 dilutions is indicated in cream ◇

Blue fill indicates the <0.016 reports ◇

Pink fill indicates outliers and results that require checking by participants ◇

Cefotaxime (CTX) MICs reported

CTX mg/L	EQA numbers 0814	0815	0816	0817
<0.002				2
0.002		**6**		**5**
0.003		**6**		3
0.004		3		**5**
0.006				
0.008	1	1	1	1
0.012				
<0.016	**1**	**2**	**2**	**2**
0.016	**9**	1	**5**	1
0.023	4		**4**	
0.032	4		**5**	
0.047			1	
0.064			1	
0.094				
0.125				
TOTAL	**19**	**19**	**19**	**19**
No. +/- x1 diln. of mode	**13**	**15**	**15**	**15**
% +/- x1 diln. of mode	**68%**	**79%**	**79%**	**79%**

KEY:

Mode (consensus) indicated in bold and orange ◇
+/- x1 dilution is indicated in yellow ◇
+/- x2 dilutions is indicated in cream ◇
Blue fill indicates the <0.016 reports ◇
Pink fill indicates outliers and results that require checking by participants ◇

Penicillin (PEN) MICs reported

PEN mg/L	EQA number 0814	0815	0816	0817
<0.002				
0.002				
0.003				
0.004				
0.006				
0.008				
0.012				
<0.016		**1**		**1**
0.016		3		2
0.023		1		6
0.032	1	**11**		5
0.047		6		**8**
0.064		6		7
0.094		1		
0.125	3		1	
0.19	2		1	
0.25	8		2	
0.38	5		5	
0.5	**9**		**13**	
0.75			1	
1	1		4	
1.5			2	
TOTAL	**29**	**29**	**29**	**29**
No. +/- x1 diln. of mode	**14**	**18**	**19**	**20**
% +/- x1 diln. of mode	**82%**	**62%**	**66%**	**69%**

KEY:

Mode (consensus) indicated in bold and orange ◇

+/- x1 dilution is indicated in yellow ◇

+/- x2 dilutions is indicated in cream ◇

Blue fill indicates the <0.016 reports ◇

Pink fill indicates outliers and results that require checking by participants ◇

Rifampicin (RIF) MICs reported

RIF mg/L	EQA number 0814	0815	0816	0817
<0.002				
0.002				
0.003				
0.004	6		**5**	
0.006	4		**3**	
0.008	**8**	3	**5**	
0.012	3	6	2	
<0.016	**1**	**1**	**1**	
0.016		**9**	**2**	
0.023		1	1	
0.032	1	2	3	
0.047		1		
0.064	1	1		2
0.094				4
0.125			2	**11**
0.19				4
0.25				2
0.38				
0.5				1
TOTAL	**24**	**24**	**24**	**24**
No. +/- x1 diln. of mode	**15**	**16**	**15**	**19**
% +/- x1 diln. of mode	**63%**	**67%**	**63%**	**79%**

KEY:

Mode (consensus) indicated in bold and orange ◇

+/- x1 dilution is indicated in yellow ◇

+/- x2 dilutions is indicated in cream ◇

Blue fill indicates the <0.016 reports ◇

Pink fill indicates outliers and results that require checking by participants ◇

Annex 4. Analysis of the submitted MIC values converted to EUCAST doubling dilution series for the viable isolates to determine % agreement

Table used for the conversion of participant-submitted MIC results to the EUCAST doubling dilution series.

Etest Gradient MIC	EUCAST equivalent
<0.002	<0.002
0.002	**0.002**
0.003	0.004
0.004	**0.004**
0.006	0.008
0.008	**0.008**
0.012	0.016
0.016	**0.016**
0.023	0.03
0.032	**0.03**
0.047	0.06
0.064	**0.06**
0.094	0.12
0.125	**0.12**
0.19	0.25
0.25	**0.25**
0.38	0.5
0.5	**0.5**
0.75	1
1	**1**
1.5	2
2	**2**
3	4
4	**4**
6	8
8	**8**
12	16
16	**16**
24	32
32	**32**
>32	>32

Conversion of actual reported MIC was made to the closest EUCAST dilution where possible but to the next highest if the submitted value (MIC) was greater than the EUCAST MIC.

For example, if 0.016 was the submitted actual MIC it was correlated to EUCAST 0.013 mg/L. If 0.094 were submitted it was equated to 0.12. Similarly 0.003 was equated to EUCAST 0.004.

Ciprofloxacin (CIP) MICs converted to EUCAST values

CIP mg/L	EQA number 0814	0815	0816	0817
<0.002	2	2	2	
0.002	1	4	5	
0.004	**18**	**12**	**12**	
0.008	5	7	8	
0.016	2	2		
<0.016				
0.03			1	
0.06		1		3
0.12				8
0.25				**16**
0.5				1
TOTAL	**28**	**28**	**28**	**28**
No. +/- x1 diln. of mode[1]	**24**	**23**	**25**	**25**
% +/- x1 diln. of mode[2]	**86%**	**82%**	**89%**	**89%**

KEY:

[1] Mode (consensus) indicated in bold

[2] % of those reporting MIC values

Ceftriaxone (CRO) MICs converted to EUCAST values

CRO mg/L	EQA number 0814	0815	0816	0817
<0.002		**9**		**9**
0.002		2	1	2
0.004	**11**		**7**	1
0.008		1	3	1
0.016	1	3	1	2
<0.016	4	3	3	3
0.03	2		1	
0.06	1		1	
0.12			1	
0.25				
TOTAL	**19**	**18**	**18**	**18**
No. +/- x1 diln. of mode[1]	**11**	**11**	**11**	**11**
% +/- x1 diln. of mode[2]	**58%**	**61%**	**61%**	**61%**

KEY:

[1]Mode (consensus) indicated in bold

[2] % of those reporting MIC values

Cefotaxime (CTX) MICs converted to EUCAST values

CTX mg/L	EQA numbers 0814	0815	0816	0817
<0.002				2
0.002		6		5
0.004		**9**		**8**
0.008	1	1	1	1
<0.016	1	2	2	2
0.016	**9**	1	5	1
0.03	8		**9**	
0.06			2	
0.12				
TOTAL	**19**	**19**	**19**	**19**
No. +/- x1 diln. of mode[1]	**18**	**16**	**16**	**14**
% +/- x1 diln. of mode[2]	**95%**	**84%**	**84%**	**73%**

KEY:

[1] Mode (consensus) indicated in bold
[2] % of those reporting MIC values.

Penicillin (PEN) MICs converted to EUCAST values

PEN mg/L	EQA number 0814	0815	0816	0817
<0.002				
0.002				
0.004				
0.008				
<0.016		1		1
0.016		3		2
0.03	1	**12**		11
0.06		**12**		**15**
0.12	3	1	1	
0.25	10		3	
0.5	**14**		**18**	
1	1		5	
2			2	
TOTAL	**29**	**29**	**29**	**29**
No. +/- x1 diln. of mode[1]	**25**	**28**	**26**	**26**
% +/- x1 diln. of mode[2]	**86%**	**97%**	**89%**	**87%**

KEY:

[1]Mode (consensus) indicated in bold. Note bimodal values 0.03-0.06 for sample 0815**.**

[2] % of those reporting MIC values

Rifampicin (RIF) MICs converted to EUCAST values

RIF mg/L	EQA number 0814	0815	0816	0817
<0.002				
0.002				
0.004	6		5	
0.008	**12**	3	**8**	
<0.016	1	1	1	
0.016	3	15	4	
0.03	1	3	4	
0.06	1	2		2
0.12			2	**15**
0.25				6
0.5				1
TOTAL	**24**	**24**	**24**	**24**
No. +/- x1 diln. of mode[1]	**19**	**19**	**14**	**23**
% +/- x1 diln. of mode[2]	**79%**	**79%**	**58%**	**96%**

KEY:

[1] Mode (consensus) indicated in bold.

[2] % of those reporting MIC values

Annex 5. Participant laboratory submitted MIC values converted to EUCAST by sample

Sample 0814					
LAB ID	CIP	CRO	CTX	PEN	RIF
NM20	0.004	0.004	0.016	0.25	0.004
NM21	0.004		0.016	0.25	0.004
NM22	0.004			0.5	
NM23	0.016	0.06	0.03	0.5	0.008
NM24	0.004	0.004	0.03	0.25	0.008
NM25	0.004	0.004	0.016	0.5	0.008
NM26	0.004			0.12	0.016
NM27	0.008		0.03	0.5	0.016
NM28	0.008		0.03	0.5	0.5
NM29	0.004		0.016	0.5	0.008
NM30	0.004	0.004	0.03	0.5	0.004
NM31	0.004	0.004	0.03	0.25	0.008
NM32	0.004	0.004		0.5	0.016
NM34	0.004	0.004		0.12	0.008
NM35		<0.016		0.25	
NM36	0.004	0.004		0.5	0.004
NM37	0.008	0.016	0.03	0.25	0.008
NM38	0.002		0.016	0.5	0.004
NM39	<0.002	0.004		1	0.008
NM40	0.004		0.016	0.25	
NM41	0.004		0.016	0.5	0.008
NM42	<0.002		0.03	0.25	0.004
NM43	0.004	0.004	0.016	0.25	0.008
NM44	0.008	0.03		0.5	0.008
NM45	0.016	0.03		0.12	0.008
NM47	0.004	<0.016		0.5	<0.016
NM48	0.004	<0.016	<0.016	0.03	
NM52	0.004	0.004	0.016	0.25	
NM54	0.008	<0.016	0.016	0.5	0.03
MODE	**0.004**	**0.004**	**0.016**	**0.5**	**0.008**

Sample 0815					
LAB ID	**CIP**	**CRO**	**CTX**	**PEN**	**RIF**
NM20	0.008		0.002	0.03	0.008
NM21	0.004		0.004	0.06	0.016
NM22	0.008	0.002		0.06	
NM23	0.016	0.016	0.004	0.06	0.016
NM24	0.004	<0.002	0.002	0.03	0.008
NM25	0.004		0.004	0.06	0.016
NM26	0.004	<0.002		0.06	0.03
NM27	0.008		0.004	0.06	0.03
NM28	0.06		0.002	0.06	0.06
NM29	0.008		0.002	0.03	0.016
NM30	0.004	<0.002	0.004	0.06	0.016
NM31	0.002	<0.002	0.004	0.03	0.016
NM32	0.004	<0.002		0.12	0.016
NM34	0.004	<0.002		0.016	0.016
NM35				0.03	
NM36	0.004	0.002		0.03	0.008
NM37	0.008	0.002	0.002	0.06	0.016
NM38	0.002		0.004	0.03	0.016
NM39	<0.002	<0.002		0.06	0.016
NM40	0.002		0.016	0.03	
NM41	0.004		0.004	0.03	0.016
NM42	<0.002		0.002	0.06	0.016
NM43	0.004	<0.002	0.004	0.016	0.016
NM44	0.008	0.008		0.06	0.016
NM45	0.016	0.016		0.016	0.03
NM47	0.004	<0.016		0.03	<0.016
NM48	0.004	<0.016	<0.016	<0.016	
NM52	0.002	<0.002	0.002	0.03	
NM54	0.008	<0.016	<0.016	0.03	0.06
MODE	**0.004**	**<0.002**	**0.004**	**0.03-0.06**	**0.016**

Sample 0816					
LAB ID	**CIP**	**CRO**	**CTX**	**PEN**	**RIF**
NM20	0.008		0.03	0.5	0.016
NM21	0.004		0.016	0.5	0.004
NM22	0.008	0.008		1	
NM23	0.008	0.06	0.06	0.5	0.004
NM24	0.004	0.004	0.03	0.5	0.004
NM25	0.004		0.03	0.5	0.016
NM26	0.002	0.008		0.5	0.03
NM27	0.008		0.03	0.5	0.008
NM28	0.004		0.03	0.5	0.12
NM29	0.008		0.03	0.5	0.004
NM30	0.004	0.004	0.03	0.5	0.004
NM31	0.004	0.004	0.016	0.5	0.008
NM32	0.004	0.004		2	0.016
NM34	0.004	0.008		0.25	0.03
NM35		<0.016		0.5	
NM36	0.004	0.004		0.5	0.008
NM37	0.008	0.016	0.06	1	0.008
NM38	0.002		0.016	0.5	0.12
NM39	<0.002	0.004		1	0.008
NM40	0.004		0.016	0.5	
NM41	0.004		0.03	1	0.008
NM42	<0.002		0.016	0.5	0.016
NM43	0.002	0.004	0.03	0.25	0.008
NM44	0.008	0.03		1	0.008
NM45	0.03	0.12		0.25	0.03
NM47	0.002	<0.016		0.5	<0.016
NM48	0.004	<0.016	<0.016	0.5	
NM52	0.002	0.002	0.008	0.12	
NM54	0.008	<0.016	<0.016	2	0.03
MODE	**0.004**	**0.004**	**0.03**	**0.5**	**0.008**

Sample 0817					
LAB ID	**CIP**	**CRO**	**CTX**	**PEN**	**RIF**
NM20	0.25		0.004	0.03	0.06
NM21	0.25		0.002	0.03	0.12
NM22	0.25	0.002		0.06	
NM23	0.25	0.016	0.004	0.06	0.12
NM24	0.12	<0.002	0.002	0.03	0.12
NM25	0.12		0.004	0.06	0.25
NM26	0.12	<0.002		0.06	0.25
NM27	0.25		0.004	0.06	0.25
NM28	0.25		0.004	0.06	0.12
NM29	0.12		0.004	0.06	0.12
NM30	0.25	<0.002	<0.002	0.06	0.12
NM31	0.25	<0.002	0.002	0.03	0.12
NM32	0.25	<0.002		0.06	0.25
NM34	0.25	<0.002		0.016	0.12
NM35				0.03	
NM36	0.25	0.002		0.06	0.06
NM37	0.25	0.004	0.008	0.06	0.25
NM38	0.12		0.004	0.06	0.12
NM39	0.06	<0.002		0.03	0.12
NM40	0.25		0.016	0.03	
NM41	0.12		0.002	0.06	0.12
NM42	0.12		0.002	0.06	0.12
NM43	0.12	<0.002	0.004	0.03	0.12
NM44	0.25	0.008		0.06	0.25
NM45	0.25	0.016		0.016	0.12
NM47	0.06	<0.016		0.03	0.12
NM48	0.06	<0.016	<0.016	<0.016	
NM52	0.016	<0.002	<0.002	0.03	
NM54	0.5	<0.016	<0.016	0.03	0.5
MODE	**0.25**	**<0.002**	**0.004**	**0.06**	**0.12**