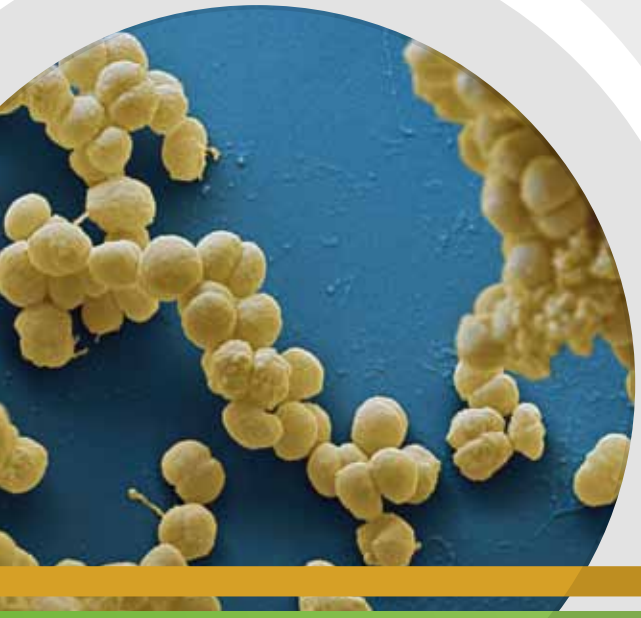

TECHNICAL REPORT

External quality assurance scheme for *Neisseria meningitidis*

2012

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ECDC TECHNICAL REPORT

External quality assurance scheme for *Neisseria meningitidis* – 2012

As part of the IBD-labnet surveillance network

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Adoración Navarro Torné and produced by Steve Gray (Health Protection Agency, Meningococcal Reference Unit, Manchester, UK), on behalf of the IBD-labnet consortium (referring to specific contract ECDC.3335).

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

N. meningitidis is the only agent among the major bacterial agents causing meningitis that may cause epidemic as well as endemic disease. The meningococcus is carried in the human nasopharynx asymptomatically by 5% to 10% of adults in non-epidemic periods but may be greater than 30% for first-year university students. *N. meningitidis* accounts for morbidity and mortality within the cases and may result in sequelae. In addition, it may be responsible for more unusual presentations, such as arthritis, osteomyelitis and cellulitis.

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 genetically related strains (described as clonal complexes) that cause most disease, some of which (e.g. cc ST-11) show particular epidemiological features: relatively low carriage, rapid transmissibility and raised case-fatality ratio.

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

ECDC promotes the performance of external quality assurance (EQA) schemes, in which laboratories are sent simulated clinical specimens or bacterial isolates for testing by routine and/or reference laboratory methods.

EQA schemes or proficiency laboratory testing provides information about the accuracy of different characterisation and typing methods as well as antimicrobial susceptibility testing, and the sensitivity of the methods in place to detect and confirm a specific pathogen or novel resistance patterns. This means that quality assurance enables laboratory performance to be assessed in comparison to reference methods and to other peer laboratories.

In May 2012, a collection of three viable isolates of *N. meningitidis* of the major disease-causing serogroups (A, B and C), together with four simulated blood (non-culture) samples for molecular studies (one of which was negative for *N. meningitidis* DNA), was sent by UK NEQAS to 29 reference laboratories (Annex 1) participating in the IBD-labnet surveillance network for quality assurance testing. The laboratories were asked to perform:

- phenotypic characterisation of viable isolates (serogroup and antimicrobial susceptibility testing: gradient diffusion MIC results), in addition to
- molecular characterisation (*porA* typing, *fetA* typing and MLST). The 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 the ECDC TESSy database.

Overall, the EQA performance has shown that European meningococcus reference laboratories 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.

The 2012 distribution included one *N. meningitidis* isolate that had been included in the 2009 panel (serogroup B) and another (serogroup A) from the 2011 EQA panel. Similarly, two of the non-culture samples (group B and C) were previously included in the 2009 IBDlabnet distribution, and a further group B sample was from a EUIBIS EQA panel in 2007.

The phenotypic characterisation of viable isolates was successful, with reports for serogroup received from 28 (97%) of the participating laboratories for each sample. One laboratory only uses genogrouping and not serogrouping. However, the phenotypic serogrouping reports demonstrated some limited discrepancies or errors, probably due the limited resources or reactivity of the reagents. This was similar to that observed in the 2009 and 2011 EQA exercises.

The comparison of minimum inhibitory concentration (MIC) between laboratories requires a standard methodology such as that recommended by EMGM: gradient diffusion methodology (such as by Etest) and a standardised agar plate medium (Müller-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, namely EUCAST for consistency, which could be achieved within the ECDC TESSy database.

There was a substantial increase in the number of participants reporting *fetA* characterisations and slight increase in *porA* and MLST over the period 2009 to 2012. For culture, nearly 70% of participants recorded *porA* and *fetA*, but approximately 50% for MLST cc. For the non-culture samples, approximately 45% of participants reported *porA* and *fetA*, but only 21% MLST. Given the more exacting demands of non-culture MLST analysis, there was excellent agreement for those reporting.

In conclusion, the results of the IBD-labnet EQA exercise proved that a regular EQA scheme for reference laboratories is required in order to maintain the movement towards improved quality of molecular epidemiological reports. It was also concluded that targeted training and support might be required to assist laboratories that have problems with organism characterisation and in particular the establishment of robust molecular typing techniques according to their particular needs. It should be acknowledged, however, that a more basic requirement may be financial resources in a number of countries not reporting molecular typing and detection.

Overview table. Main findings from 2012 *N. meningitidis* EQA

Main findings	Future direction	Possible actions
Excellent response to EQA distribution (29 responses), but not all laboratories could provide results to all targets.	Need to determine the barriers preventing laboratories completing the range of characterisation data. Why are some laboratories persistent non-responders?	• Targeted questionnaire • Regular EQA distribution • Support partnership working with other participants
Phenotypic serogroup determination was successfully achieved by 93% (27/29) laboratories.	Need to achieve accurate methodology to confirm serogroup A for all laboratories. (2/29 reported incorrect serogroup for the serogroup A sample)	• Targeted training • Regular EQA distribution • Encourage genogroup methodology
Utilising standard methodology for MIC testing greatly improved comparisons. Some laboratories with problems but relatively few (and mainly very minor) differences were observed.	• Maintain EQA. Could reduce MIC data capture and analysis by only reporting specific antibiotic MICs on specific organisms. • Promote standardised methodology.	Only accept EUCAST MIC values
Genogroup is not tested or reported routinely for isolates by many (41%) participants.	Encourage ability to confirm genogroup	Targeted training
62–69% 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.
A minimum of 52% and 21% of laboratories were able to perform sequence-based typing ('fine type') on isolates and non-culture samples, respectively. All those reporting the 'fine type' results were in excellent agreement.	• Support laboratories with training to establish standard assays. Increase the number of laboratories performing MLST for both isolates and non-culture. • Determine if laboratories are routinely determining sequence types on 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 and 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 (EU) agency with a mandate to operate dedicated surveillance networks and to identify, assess, and communicate current and emerging threats to human health from communicable diseases. Within its mission, ECDC shall 'foster the development of sufficient capacity within the Community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes.' (Article 5.3, EC 851/2004[1]).

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

The main purposes of external quality assurance schemes include:

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

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

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

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

The geographical distribution of the serogroups shows that serogroup A strains cause most epidemics in the so-called 'meningitis belt' (the Sahel region of the sub-Saharan Africa) and Asia, but more localised epidemics of serogroup C may also occur. In the Americas, Europe and Australasia, where serogroup C and especially B are the most common, meningococcal disease follows a seasonal pattern and shows lower rates. 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 reported by local and reference laboratories within Europe. The application of PCR-based techniques is such that up to 50% of cases are laboratory-confirmed cases and reported by some countries (e.g. the United Kingdom).

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.

The currently limited but increasing use of whole genome sequencing is compatible with the sequence typing methods previously described and could be used to generate the same characterisations in the future.

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

European surveillance, ECDC programme and IBD-labnet

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

The network was funded by ECDC from October 2006 until October 2007 when the epidemiological and laboratory surveillance was integrated into ECDC. The network has worked in close collaboration with the European Monitoring Group on Meningococci (EMGM) to integrate epidemiological and molecular components of meningococcal disease in Europe.

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

The EMGM consortium concluded in 2009 that the 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 a 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; hence it was recommended as the laboratory variables 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 and 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 results of testing to achieve the same level of characterisation for both culture- and non-culture-(PCR only) confirmed cases of meningococcal disease.

It was accepted that some countries might not be able to provide their own molecular typing data for local and European surveillance due to economic reasons. Outside of the EQA, some countries processing larger numbers of samples, with spare capacity and availability of molecular methods, offered their help to those countries that are not yet able to implement the molecular typing methods: with the aim to provide accurate molecular typing of *N. meningitidis* and thereby more comprehensive European surveillance by ECDC.

This report describes the third ECDC funded EQA following the EQA distributions in 2009 and 2011.

Currently, only the 2009 technical report 'External quality assurance scheme for *Neisseria meningitidis* – 2009' is available [1].

1 Materials and methods

1.1 Objectives

The objectives of the 2012 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) in all EU Member States and candidate countries with suitable reference facilities;
- distribute the panel safely for testing by all participating reference laboratories;
- receive electronic test reports from the participants for analysis;
- compile the consensus report for participant comparison and review;
- compare the EQA results to previous distributions; and thereby improving data quality, assisting in the standardisation of techniques, and facilitating consistent epidemiological data for submission to the ECDC TESSy database; and to
- specifically support the move towards molecular detection, confirmation and accurate characterisation of *N. meningitidis*.

The 2012 *N. meningitidis* EQA was designed to build upon the experience gained in the ECDC IBD-labnet EQAs of 2009 and 2011.

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 undertake 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) in a specified time period. By limiting the result (report) fields to specific criteria and data format acceptance, the participants may be considered to be driven to 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, in part, addressed in a training workshop in Würzburg, Germany, June 2010. Commercially available antisera were demonstrated for use with a standard technique within the confines of a microbiological safety cabinet.

Sensitive to 2009 and 2011 EQA feedback that large panels of isolates (and non-culture samples) may confer a disproportionate workload for some reference laboratories it was decided to reduce the number of isolates from six to three but retain four non-culture (simulated clinical) samples.

The EQA was to be received and tested by the participant laboratories to determine the phenotypic and genotypic results (see Table 1). Results were then reported via the UK NEQAS website using the laboratories' unique identifier.

An anonymised summary was produced by UK NEQAS showing the submitted results of the participant laboratory, the consensus result and, by interpretation, 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. Including the option for reporting of the techniques used for nucleic acid extraction, amplification and detection allowed for a simple (but anonymous) survey of the facilities available within the European laboratories. In addition, methodological information may help to assess how a technique 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 Barcelona, 20–23 November 2012.

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

Table 1. Tests requested of 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

Twenty-nine European meningococcal reference laboratories participated in the 2012 IBD-labnet EQA distribution.

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

The list of the 2012 participating reference laboratories with full contact and address details are given in Annex 1.

All participants were contacted prior to the IBD-labnet EQA distribution in 2012 to confirm the address and contact details for despatch of the potentially hazardous material. In 2009 and 2011 – but not in 2012 – the HPA business and legal department required the agreement of participants to the terms and conditions of the ECDC EQA distribution (Annexes 2 and 3). In essence, it confirmed the recipient's details and their responsibility for safe handling of the material. Also included were clauses relating to the retention and further use of the material, with specific restrictions upon third-party distribution and the necessity for review of any publications relating to the EQA material.

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

1.4 Timelines

Table 2. Timelines for the EQA exercise

Event	Dates
Design and preparation of EQA (isolates and simulated non-culture samples). Includes MIC selection	February 2012
Assessment of prepared simulated septicaemic material.	February–March 2012
Transfer to UK NEQAS from HPA MRU	End March 2012
Preparation of simulated septicaemia samples and freeze drying of panel at UK NEQAS	April 2012
Pre-despatch checks of freeze-dried EQA panel at HPA MRU	April 2012
Distribution of EQA panel by UK NEQAS (8 May 2012)	May 2012
Testing and report (four weeks), return date 22 June 2012	May–June 2012
Analysis of returned reports	June 2012
Distribution of individual reports email (with consensus results) to participants by end of June 2012. Results on UK NEQAS website (https://results.ukneqas.org.uk), distribution 2801 via unique participants' code	June–July 2012
Preliminary EQA summary presentation at IBD-labnet management meeting, Würzburg, Germany, 14 September 2012	Sept 2012
Technical summary report of ECDC IBD-labnet EQA and recommendations (this report)	December 2012

1.5 The EQA panel material

Isolates

The 2012 IBD-labnet EQA panel (UK NEQAS distribution 3212) consisted of three viable isolates of *N. meningitidis*, selected to be representative of major disease-causing serogroups (A, B and C). Ideally, serogroups Y and W135 would have been included too, but it was necessary to limit samples to three isolates only, for reasons of costs. One of the isolates used in the panel was from a case confirmed in England and Wales over the period 2005–10, and two were kindly supplied by Dr M-K Taha, Institut Pasteur, France (Table 3).

Selection was made upon a combination of characterisation factors but primarily upon the MIC values as determined at the HPA MRU, using gradient diffusion methodology (Etest, bioMerieux), and from MICs supplied with isolates from the Institute Pasteur. The aim was to select meningococci that yielded raised MICs to antibiotics other than penicillin. Ideally, organisms were selected that demonstrated unusual MIC levels to more than one antibiotic, which can prove to be difficult. 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 observed it was important to ensure that a variety of *PorA* VR1, *PorA* VR2 and cc and ST results would be determined too. The panel therefore, did not reflect the most commonly characterised meningococci seen in England and Wales or Europe, but provides 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.

Distribution 3212 (2012) was also designed to contain isolates (and non-culture) material that had previously been distributed in EU EQA panels in order to determine if there had been an improvement in participants' abilities to characterise the samples. It was hoped that analysis would be possible at both the total participant and at the anonymised individual laboratory level.

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

Sample 1379: A serogroup A case isolate (08-240233: from blood, 2008); selected as a representative of the rare case isolate 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=0.19 mg/L) and rifampicin (RIF=0.125 mg/L). The CIP MIC would be regarded as resistant by EUCAST guidelines.

Sample 1379 was previously distributed in the 2nd IBD-labnet EQA panel (2011), distribution 2801, as sample 0817.

Sample 1380: A serogroup B organism (08-240850) was selected as a representative RIF resistant isolate (MIC≥32 mg/L). It was supplied by Dr M-K Taha from France (LNP22342). Genotypically, *porA* VR1 and VR2 are 7 and 16 respectively, and MLST ST32 and CC32. *FetA* was F3-3.

The MIC to penicillin was 0.064 mg/L (designated as intermediate by EUCAST), and ciprofloxacin was 0.004 mg/L (designated sensitive by EUCAST).

Sample 1380 was previously distributed in the 1st IBD-labnet EQA panel (2009), distribution 2452, as sample 9201.

Sample 1381: A serogroup C organism (12-240164); supplied by Dr M-K Taha from France (LNP26251). It was characterised as *porA* VR1 and VR2 21-7 and 16, respectively. *FetA* was F5-36, MLST ST 1157 and CC1157. The MIC to penicillin was 0.047 mg/L (designated sensitive by EUCAST guidelines), ciprofloxacin was 0.002 mg/L (designated sensitive by EUCAST) and rifampicin was >32 mg/L (designated resistant by EUCAST). Molecular typing of the organism determined *penA* 22 and *rpoB* 65 for penicillin and rifampicin, respectively.

Sample 1381 had not been used previously in any EU EQA panels.

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 sample). The dilution of meningococci (positive samples) was designed to mimic levels detected by PCR assays of clinical samples (serum or EDTA blood). The three positive samples were to simulate a weak, medium and strong positive sample as indicated by HPA MRU real-time (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 the meningococcal concentration in the samples would be sufficient for detection of *N. meningitidis* and determination of serogroup by commercial latex antigen kits or other serological methods. It should be stated that serological assessment of the samples was not made pre- or post-distribution.

Sample 1382: A heat-killed dilution of a **serogroup B** (02-240691), a case isolate (from blood, England and Wales), was prepared. The organism was phenotypically characterised as B:NT:P1.9 and genotypically as *porA* VR1 and VR2 as 22 and 9, respectively. MLST characterisation was ST1195 and CC 269. The *fetA*VR was F5-1 and *penA* 27. A dilution was made to represent a strong positive when compared to clinical samples.

The sample was selected for non-culture (PCR detection) as it had been observed to be undetected by the initially published *ctrA* assay primers [2]. It was previously distributed in the 1st IBD-labnet EQA, 2009 (distribution 2452, sample 9205), at the same concentration. The 2009 frozen stock dilution was diluted 1/500 for both EQA panels.

Note: To address the deficiency of the published *ctrA* assay [2], the HPA MRU have made a modification to incorporate an additional reverse primer (5'-TTGCCGCGGATTGGCCACCA-3') to enable detection of this variant strain.

Sample 1383: A heat-killed dilution of a **serogroup C** (08-240857), a case isolate (from blood, England and Wales, 2008), was prepared. The organism was phenotypically characterised as C:NT:NT and genotypically as *porA*VR1 and *porA*VR2 as 7-4 and 14-6, respectively. MLST characterisation was ST1031 and CC334. The *fetA*VR was F3-9 and *penA* was 83. The dilution prepared was aimed to be representative of medium positive clinical sample.

The sample had previously been distributed in the 1st IBD-labnet EQA, 2009 (distribution 2452, sample 9209) at the same concentration. The 2009 frozen stock dilution was diluted 1/5000 for both EQA panels.

Sample 1384: A heat-killed dilution of a **serogroup B** (05-240165), a case isolate (from blood, England and Wales, 2005), was prepared. The organism was phenotypically characterised as B:NT:P1.7 and genotypically as *porA*VR1 and *porA*VR2 as 7-8 and 4-1, respectively. MLST characterisation was ST41 and CC41/44. *Fet*A and *penA* designations were not known. The dilution prepared was aimed to be representative of weaker positive clinical sample.

The sample had previously been distributed in the 3rd EUIBIS EQA, 2007 (distribution 2287, sample 8768) at the same concentration. The 2007 frozen stock dilution was diluted 1/10 000 for both EQA panels.

Sample 1385: Negative; the sample did not include *N. meningitidis* organisms (or meningococcal DNA). It did comprise of a heat-killed dilution of *Streptococcus pneumoniae* (UK HPA NCTC11902, serotype 14), diluted (in horse serum) to be representative of a weaker clinical sample.

The sample had previously been distributed in the 2nd EUIBIS EQA, 2006 (distribution 2146, sample 8331) but at a ten-fold lower dilution to 2012. Used at 1/500 in 2006, but 1/5000 in 2012.

Table 3. Summary of *N. meningitidis* selected for the 2012 EQA panel; based on results available at HPA MRU (prior to distribution)

Sample	HPA MRU LabNo		Clinical Site	serogroup	serotype	*porA*		*fetA*VR	MLST	
						VR1	VR2		ST	cc
1379	M08	0240233	Blood	**A**	4/21	20	9	3-1	4789	5
1380	M08	0240850[1]	NS[3]	**B**	14	7	16	3-3	32	32
1381	M12	0240164[2]	NS[3]	**C**	nt	21-7	16	5-36	1157	1157
1382	M02	0240691	Blood	**B**	nt	22	9	5-1	1195	269
1383	M08	0240587	Blood	**C**	nt	7-4	14-6	3-5	1031	334
1384	M05	0240165	Blood	**B**	4t	7-8	4-1	NK[6]	41	41/44
1385	**NEGATIVE**[4] (*Nm*-ve but *Sp*+ve)									

[1] *08-240850 = LNP 22342 supplied by Dr M-K Taha, Institute Pasteur, France*

[2] *12-240164 = LNP 26251 supplied by Dr M-K Taha, Institute Pasteur, France*

[3] *Clinical site not stated*

[4] *Negative control: contained no* N. meningitidis *but did contain* Streptococcus pneumoniae *organisms*

[6] *fetAVR not known*

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

In order to provide sufficient standardised material and not to incur ethical or blood safety issues, it was decided not to use actual human clinical (blood) samples. Safety considerations necessitated the use of heat-treated

suspensions of meningococci in a protein matrix or diluent; ideally, one that was suitable for freeze-drying and acceptable for import into all States. For that reason 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 1 mL 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) in a heating block.
- HPA MRU *ctrA* and *siaD* real-time 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 were undertaken by UK NEQAS.

Packaging and transport of EQA samples

The 2012 *N. meningitidis* EQA panel (UK NEQAS distribution 3212) 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 (Annex 4).

The EQA distribution was made by UK NEQAS on 8 May 2012.

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 their available methods to confirm the identity and directed characterisation of the samples. Participants were encouraged to use their routinely available methods but were not discouraged from using additional techniques or reagents, for example techniques they may not use on routine samples submitted to their laboratories.

Results were to be returned to UK NEQAS by 22 June 2012 (17:00 GMT) via the UK NEQAS website (https://results.ukneqas.org.uk) or faxed using a copy of the results report included with the EQA samples.

Details of the results required and reporting via the UK NEQAS website are included in Annex 4.

An extensive questionnaire was not included but information was requested on the following topics (similar to the 2011 distribution):

Part 1. Serogroup for isolates (samples 1379–1381)

Comments on serogroup determination; specifically, which reagents were used (tested) if 'Not determined' was recorded.

Part 2. MIC for isolates (samples 1379–1381)

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 (1379–1381) and non-culture (1382–1385) samples.

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

Standards and accreditation

Designation and interpretation of *N. meningitidiis* phenotypic characterisations (apart from MIC interpretations) are not known to be standardised, although the genotypic designations are quite strictly controlled through the use of 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.meningitidiis* EQA distributions highlighted the problem of antibiotic susceptibility interpretation and which guidelines to follow when reporting. 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. ECDC may then interpret using EUCAST guidelines for European surveillance. There was no requirement for participant laboratories to be operating to ISO (international) 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 within the Manchester Medical Microbiology Partnership by Clinical Pathology Accreditation UK Ltd under Centre Reference Number 0635.

Website result submission

Participants were able to enter characterisation results to the website at any time until the closing date (deadline). Should corrections be required, participants could overwrite old data. Results could be updated by re-submission. It was also possible to print the laboratory's results submission at any time.

Assessment of performance

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

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

Participants were not scored on their results or performance but actively encouraged to compare their results to the consensus and determine if they did or did not achieve the 'correct' result. It was then hoped that participants would be able to resolve issues themselves (locally). Opportunities to discuss results at the annual IBD-labnet meeting (either openly or informally with other participants or the co-ordinator) were available. Dr S Gray was also available via email.

The IBD-labnet meeting, including EQA presentations and discussion, was held in Barcelona, 21–23 November 2012.

2 Results and discussion

EQA panels were distributed to 29 countries (see section 1.2 participants) and 29 results reports were returned to UK NEQAS which they considered an excellent response.

UK NEQAS collated the results and produced a draft report that was reviewed by Dr S Gray before individual reports were made available to all the participating reference laboratories via the UK NEQAS website (https://results.ukneqas.org.uk) from 11 July 2012; accessible using the laboratories' unique code.

The summary report (Annex 5) was comprehensive, indicating the individual laboratory's results compared to all other submitted results.

2.1 Characterisation of viable isolates

The phenotypic characterisation of the three viable isolates (samples 1379–1381) was very successful, with serogroup reports returned from 28 (97%) participants. Only one laboratory did not confirm the *N. meningitidis* isolate group by serology but preferred genogrouping (PCR) alone.

The consensus isolate phenotype (serogroup and reported MICs) results are shown in Table 4 as disseminated to the participating laboratories. An example is given as Annex 5.

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

Sample	serogroup	MIC (mg/L)				
		CIP	CRO	CTX	PEN	RIF
1379	A	0.19	<0.002	0.002	0.032	0.125
1380	B	0.004	<0.002	0.008/<0.016[1]	0.032/0.064[2]	>32.0
1381	C	0.002	<0.002	0.003	0.032	>32.0

Actual gradient MIC values as reported to UK NEQAS.

[1] 1380 CTX bimodal distribution was observed
[2] 1380 PEN bimodal distribution was observed

Serogroup/genogroup

Phenotypic and genotypic group

The maximum number of reports for phenotypic (serological) serogroup determination was 28 (97% of the 29 returned reports). See Table 4.

1379: Combining the serogroup and genogroup characterisations, 27 (93%) of the 29 laboratories characterised **group A** for 1379. Twenty-six (93%) of the 28 laboratories reported **serogroup A**; the two anomalous serogroup reports were: one serogroup B and one serogroup Y or Y/W135.

One laboratory reported genogroup results only, correctly as A only. A total of 17 (59% of the 29) laboratories reported genogrouping the isolate. Fourteen (43%) laboratories confirmed **group A**, two laboratories stated correctly that it was 'not B or C', but one erroneously reported group W135.

When the same sample (previously 2801, 0817) was tested in the 2nd IBD-labnet 2011 panel, the consensus serogroup A was confirmed by 29 (100%) of laboratories.

1380: Combining the serogroup and genogroup characterisations, 28 (97%) of the 29 laboratories confirmed **group B** for sample 1380. Twenty-seven (96%) of the 28 laboratories reported **serogroup B**; the sole anomalous serogroup report was 'NG' (not serogroupable).

A total of 17 (59% of the 29) laboratories reported genogrouping the isolate: all confirmed **group B**.

When the same sample (previously 2452, 9201) was tested in the 1st IBD-labnet 2009 panel, the consensus serogroup B was confirmed by 23 (85%) of the 27 serogroup reporting laboratories. One laboratory reported serogroup Y, and three laboratories reported NG (not serogroupable).

1381: Combining the serogroup and genogroup characterisations, 29 (100%) of the 29 laboratories confirmed **group C** for sample 1381. Twenty-eight (100%) laboratories reported **serogroup C.**

All 17 (59% of the 29) laboratories reporting genogrouping confirmed **group C**.

Sample 1381 was only distributed in the current EQA panel (3rd IBD-labnet EQA panel, 2012, distribution 3212).

Antimicrobial susceptibility – MIC results

MIC reports were returned from 28 (97%) of the 29 laboratories. All laboratories reporting MICs tested PEN (penicillin) MICs (97%, 28/29). CIP (ciprofloxacin) was reported by 27 (93%, 27/29) laboratories and RIF (rifampicin) by 23 (79%, 23/29); 72% (21/29) reported CRO (ceftriaxone) and CTX (cefotaxime); see Table 5.

Laboratory NM33 did not report (or test) any antibiotic MICs, and laboratory NM20 reported only CTX and PEN. All other laboratories tested and reported at least three antibiotic MICs, including PEN. RIF was not reported by NM20, NM33, NM35, NM40, NM51 and NM52.

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

Analysis and interpretation of MICs

The analysis of the MIC values reported for each sample and the calculation of frequencies are presented in Annex 6; the actual MICs determined by laboratories were converted (by S Gray) to the EUCAST doubling dilution series. This was done for two reasons, to accommodate the increased and close dilution series of the commercial strips and to allow interpretation to EUCAST guidelines. The conversion table generated by the author is given in Annex 6. The consensus (mode) values and the distribution of the reported MIC values are indicated.

Table 6 shows to sample mode (consensus) converted to EUCAST dilutions.

Table 5. Reporting of gradient diffusion MICs from laboratories

Antibiotic	No. of labs reporting MIC	% labs reporting MIC[1]
CIP	27	93
CRO	21	63
CTX	21	63
PEN	28	97
RIF	23	80

[1] *The denominator was 29 laboratories, even though MIC reports were received from 28 laboratories.*

Table 6. Consensus isolate phenotypic characterisations with EUCAST-mode MIC

Sample	serogroup	MIC (mg/L)				
		CIP	CRO	CTX	PEN	RIF
1379	A	0.25	<0.002	0.002	0.06	0.12/0.25[1]
1380	B	0.004	<0.002	0.008	0.06	>32.0
1381	C	0.004	<0.002	0.004	0.03	>32.0

[1] *1379: RIF bimodal distribution was observed.*
Submitted MICs converted to EUCAST doubling dilutions.

Table 7. Number and proportion of laboratories in agreement with the consensus EUCAST MIC mode

	No. (%) of labs reporting the consensus (mode) MIC[1]			
Antibiotic	**1379**	**1380**	**1381**	**Total reports**
CIP	14 (52)	14 (52)	14 (52)	27
CRO	10 (48)	10 (48)	10 (48)	21
CTX	7 (32)	6 (29)	9 (43)	21
PEN	14 (50)	11 (39)	15 (54)	28
RIF	20 (87)	20 (87)	20 (87)	23

[1] *The proportion (%) of laboratories reporting the specific antibiotic MIC.*
Submitted MICs converted to EUCAST doubling dilutions.

Table 8. Number and proportion of laboratories in agreement with the EUCAST MIC mode +/– x1 dilution

	No. (%) of labs reporting the consensus (mode) MIC +/– x1 dilution[1]			
Antibiotic	**1379**	**1380**	**1381**	**Total reports**
CIP	25 (93)	23 (85)	24 (89)	27
CRO	11 (52)	10 (48)	11 (52)	21
CTX	13 (62)	13 (62)	12 (57)	21
PEN	25 (89)	27 (96)	26 (93)	28
RIF	22 (97)	22 (96)	22 (96)	23

[1] *The proportion (%) of laboratories reporting the specific antibiotic MIC. Submitted MICs converted to EUCAST doubling dilutions.*

Table 9. EUCAST interpretation of all laboratory reported MIC results

	No. (%) of labs reporting the EUCAST designation[1]			
Antibiotic	**1379**	**1380**	**1381**	**Total reports**
CIP	R (100)	S (100)	S (96), I (4)	27
CRO	S (100)	S (100)	S (100)	21
CTX	S (100)	S (100)	S (100)	21
PEN	S (96), I (4)	S (71), I (29)	S (93), I (7)	28
RIF	S (100)	R (100)	R (100)	23

[1] *S (susceptible – sensitive), I (intermediate or reduced susceptibility) and R (resistant) MICs: no reports were anomalous, but there were a number of intermediate designations with regard to PEN for all three samples.*

Table 7 shows that the consensus (mode) result was attributed to a relatively small number (proportion) of laboratories for antibiotics CIP, CRO, CTX and PEN, where the range was 29%–54% agreement. RIF MIC agreement was consistently high at 87%. For example, the maximum agreement, 87% was achieved for all three samples with RIF, but the minimum agreement was 29% for CTX (sample 1380).

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) it was decided to re-analyse the data, increasing the range around the mode by + or – one EUCAST MIC dilution (Table 8), thereby demonstrating great improvement in agreement to the mode. Eight (over half) of the sample and antibiotic combinations were now in agreement at 89% or higher. The minimum agreement was then shown to be 48% for CRO (sample 1380). This was a reflection of the close distribution of the MIC results.

Interestingly, when the results were interpreted to EUCAST breakpoints (Table 9), all the sample and antibiotic designations were in agreement. No results would have been interpreted as sensitive if the mode indicated resistant and vice versa.

The CIP-resistant isolate 1379 was confirmed, as were the two RIF-resistant isolates 1380 and 1381.

The minor discrepancies were the four sample and antibiotic combinations where reports were designated intermediate (or reduced susceptibility). All three samples indicated PEN intermediate susceptibility: only 4% (one laboratory, NM22) and 7% (two laboratories, NM22 and NM51) for 1379 and 1381, respectively, but nearly a third (29%) of the PEN MIC reports for 1380. Specifically, PEN intermediate susceptibility (0.12 mg/L) was reported for 1380 by laboratories: NM22, NM32, NM35, NM41, NM42, NM51, NM52 and NM54. A similar number of laboratories also reported the PEN-sensitive MIC of 0.03 mg/L, demonstrating a normal distribution for the characteristic.

CIP intermediate susceptibility (0.03 mg/L) for 1381 was reported by one laboratory, NM51.

Although not causing an anomalous interpretation, other **'outlier' MICs** of note (greater than x1 EUCAST dilution to the mode) were reported (Annex 6):

Sample 1379: CIP 0.06m/L was reported by NM39 and NM48. RIF 0.03 was reported by NM48.

Sample 1380: CIP 0.03 mg/L and 0.016 mg/L were reported by NM32 and NM37, respectively. PEN 0.016 mg/L was reported by NM45.

Sample 1381: CIP 0.03 mg/L was reported by NM51.

Comparison with previous distribution MICs

Sample 1379: A comparison of laboratories' results of testing the same sample in different EQA panels (Table 10) demonstrated an improved proportion of laboratories which reported the mode for sample 1379, distribution 3212

(tested initially in 2011 as 0817, distribution 2801), with the exception of CTX. The use of high-range strips for CTX and CRO MICs may account for reduced agreement with consensus. Similarly, the closeness of the gradient diffusion MIC dilutions and the correlation to EUCAST doubling dilutions may have accentuated very minor differences.

There was an improvement in the proportions with EUCAST agreed interpretation except for PEN where the number of intermediate (reduced susceptibility) reports increased for 0% to 4%. It is very unlikely that reduced susceptibility interpretation would mean that there would be a clinical effect given the high levels of PEN administered to patients.

Table 10. Sample 1379. MICs reported and interpreted in 2011 and 2012

			% labs reporting the mode MIC				
Year	**Distribution**	**Sample**	**CIP**	**CRO**	**CTX**	**PEN**	**RIF**
2011	**2801**	**0817**	36	50	68	28	46
2012	**3212**	**1379**	52	48	33	50	87
			% labs agreement with EUCAST interpretation				
2011	**2801**	**0817**	R 100	S 94	S 95	S 100	S 96
2012	**3212**	**1379**	R 100	S 100	S 100	S 96	S 100

Sample 1380: The repeat testing of the same sample, 1380 (distribution 3212) and 9201 (distribution 2452 in 2009) demonstrated (Table 11) that a reduced proportion of laboratories matched the mode MICs for all antibiotics except RIF, in 2012. The use of high-range strips for CTX and CRO and the interpretation of the MICs may account for reduced agreement with consensus. Similarly the closeness of the gradient diffusion MIC dilutions and the correlation to EUCAST doubling dilutions may have accentuated very minor differences particularly at the lower values.

The proportion of PEN MIC mode agreement was reduced and reflected an increase in intermediate susceptible MIC reports.

The EUCAST MIC interpretation was in agreement for all reported MICs except PEN where 29% of reports were of reduced susceptibility.

Table 11. Sample 1380. MICs reported and interpreted in 2011 and 2012

			% labs reporting the mode MIC				
Year	**Distribution**	**Sample**	**CIP**	**CRO**	**CTX**	**PEN**	**RIF**
2009	**2452**	**9201**	64	54	64[1]	52	83
2012	**3212**	**1380**	52	48	29	39	87
			% labs agreement with EUCAST interpretation				
2009	**2452**	**9201**	S 100	S 100	S 100	S 92	R 100
2012	**3212**	**1380**	S 100	S 100	S 100	S 71	R 100

Overall, the repeat testing of samples 1379 and 1380 demonstrated that EUCAST interpretative reporting and breakpoints generated slightly improved results. Minor differences in MIC reports may affect the agreement, but epidemiological surveillance based on the laboratories' reports would be satisfactory.

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 use of Etest methodology while 7% (2/30) cited 'other method' in response to the gradient MIC.

A maximum of twenty-eight laboratories reported MIC results, but only 23 laboratories reported the agar plate medium: 65% (15) laboratories used Müller-Hinton base with heated sheep blood or 28% (6) with heated horse blood. Two laboratories (9%) used Iso-Sensitest agar with horse blood and NAD.

Two laboratories reported the use of either 'in-house prepared' (NM45) or 'home-made' (NM40) plate media; all other laboratories used commercially produced agar plates from a number of different suppliers.

Twenty-six responses regarding the commercial gradient strip manufacturer were made. Most frequently mentioned was AB biodisk-bioMerieux (21, 81%), followed by Liofilchem (3, 12%), Oxoid (1, 4%) and Lucron (1, 4%).

There was no apparent association of 'outlier' MIC reports with medium composition, supplier, or commercial strip supplier, which suggests that although EUCAST standardisation would require Müller-Hinton base with heated

sheep blood, in practice interpretable *N. meningitidis* MICs may be adequately obtained by using different methodologies. It would be highly desirable for all participating laboratories to adopt the recommended EUCAST methodology.

MIC summary

The small panel of three isolates 1379–13814 indicated the advantage of the gradient diffusion method for MIC determination, with regard to standardised dilution and the artefact caused by very close dilution series. Minor differences in agar plate volume may have affected the depth of medium, or the differences in plate manufacture were responsible for the range of results around the mode, in some instances resulting in a bimodal distribution. Most likely, the small difference (one dilution) to the mode was due to the reading of the intersection of the growth with the strips: influenced by the initial organism suspension (concentration) and operator. The use of the high-range MIC strips proved difficult to reconcile reports of ≤0.016 mg/L for CRO and CTX.

This demonstrated that if all the reported MICs were interpreted to EUCAST guidelines, there would be very few incorrect susceptibility designations, in fact, only a few intermediate MICs that would be unlikely to be clinically significant. The reporting of actual MICs to ECDC's TESSy database for EUCAST interpretation has been discussed previously. The practicality of laboratories converting their own MICs before submission should be discussed for subsequent EQA panels.

Greater than 80% of the laboratories submitting CIP, PEN and RIF MIC results achieved the mode +/– one dilution.

Although most laboratories test for PEN and CIP MIC, few test for RIF, and even fewer (72%) for CTX and CRO.

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

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

2.2 Simulated non-culture samples

Species detection and *N. meningitidis* genogroup confirmation

Four simulated septicaemia samples (1382, 1383, 1384 and 1385) 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: 1382 serogroup B, 1383 serogroup C, and 1384 serogroup B; 1385 was negative *for N. meningitidis* but contained heat-killed *Streptococcus pneumoniae* in horse serum diluent (see Table 12).

A maximum of 21 (72% of the 29) laboratories returned reports of species detection and serogroup confirmation for at least one of the four non-culture samples. Conversely, eight (28%, over a quarter of the laboratories) did not report non-culture detection or serogroup and may be assumed to be unable to carry out non-culture PCR investigations.

Table 12. Consensus species detection and genogroup of the simulated septicaemia samples

Sample	Species	Genogroup	Concentration[1] (orgs/mL)
1382	*N. meningitidis*	B	6.0×10^4
1383	*N. meningitidis*	C	2.6×10^4
1384	*N. meningitidis*	B	6.8×10^3
1385	NEGATIVE *N. meningitidis*	(*S. pneumoniae* POSITIVE)	5.2×10^3

[1]*Approximate concentrations of viable organisms were determined by Miles and Misera and calculated for dilutions used in the simulated samples.*

Sample 1382: Twenty-one laboratories reported results for strong positive sample 1382. Eighteen (62% of the total 29) laboratories returned the consensus (*N. meningitidis*-positive result), with three laboratories reporting the sample incorrectly as '*N. meningitidis* negative'.

Eighteen (62%) laboratories confirmed the consensus serogroup B (equating to 86% of laboratories reporting PCR detection); one laboratory incorrectly reported 'not A, B, C or W135'.

Laboratory NM48 reported species DNA detection but not genogroup, and NM32 reported genogroup and not species.

In 2009, 12 of the 27 (44%) laboratories determined the consensus *N. meningitidis* detection, demonstrating a considerable improvement in proportion of correct reports, following an increase in the number of laboratories performing non-culture PCR in 2012.

Sample 1382 would not have been detected using the *ctrA* primers described by Corless et al. [2], thereby requiring additional or modified reverse primers or another target gene for detection. The three laboratories reporting a negative result were all using *ctrA* assays: NM22, NM35 and NM37.

Sample 1383: Nineteen laboratories reported results for strong positive sample 1383. Eighteen laboratories (62% of the total 29) returned the consensus result (*N. meningitidis* positive), with one laboratory reporting the sample incorrectly as *N. meningitidis* negative.

Sixteen (55%) laboratories confirmed the consensus genogroup C (equating to 76% of laboratories reporting PCR detection), two laboratories (NM21 and NM32) incorrectly reported genogroup B, and one laboratory reported 'ND' (group not determined).

In 2009, 20 (74% of 27) laboratories determined the consensus *N. meningitidis* detection for 1383; in 2012 it was only 62%. In 2009, 100% of the laboratories detecting *N. meningitidis* confirmed genogroup C; in 2012, correct confirmation of the genogroup was down to 76%.

Sample 1384: Twenty laboratories reported results for weaker positive sample 1383. Twenty laboratories (69% of the total 29) returned the consensus result (*N. meningitidis* positive), with no laboratories reporting the sample incorrectly as *N. meningitidis* negative.

Fifteen (52%) laboratories confirmed the consensus serogroup B (equating to 75% of laboratories reporting PCR detection), one laboratory incorrectly reported genogroup C (NM35), three laboratories incorrectly reported 'not A, B, C or W135' (NM23, NM28 and NM37), and one laboratory reported 'ND' (genogroup not determined).

In both 2007 and 2012, 100% of the laboratories reporting non-culture results confirmed *N. meningitidis* positive detection. Seventeen laboratories reported in 2007, and 20 laboratories in 2012 provided evidence of increased capacity (number of laboratories) and consistent sensitivity. In 2007, only 13 laboratories reported genogroup results: 10 laboratories reported genogroup B, one laboratory 'not B or C', one laboratory 'not A, B, C, Y or W135', and one laboratory 'ND'. This would suggest improved capacity to genogroup in 2012, but there could be sensitivity issues with weaker samples, particularly with the genogroup assays that appear less sensitive.

Sample 1385: Twenty-one laboratories reported results for the *N. meningitidis* negative sample 1385. Thirteen laboratories (45% of the total 29) returned the consensus result (*N. meningitidis* negative), but three laboratories reported the sample incorrectly as *N. meningitidis* positive (NM20, NM28 and NM45). Five laboratories correctly reported that sample 1385 contained another species: *S. pneumoniae* (serotype 14). The total number of correct reports was 15 (52% of laboratories).

There should have been no genogroup confirmations for 1385, but incorrect reports included one laboratory reporting genogroup B (NM32) and one laboratory reporting genogroup C (NM45). There was also one report of *Haemophilus influenzae* detection (NM37).

A report from one laboratory (NM41) of genogroup 'not A, B, C, Y or W135' was in fact correct but unnecessary; this may reflect confusion regarding the use of 'not determined' (four laboratories) and 'not tested' (two laboratories) comments in the genogroup results.

In 2006, sample 1385 was prepared at a 10-fold stronger concentration (slightly more positive) and two (11%) of the 19 laboratories reported the negative sample as *N. meningitidis* positive. Seventeen (89%) confirmed the sample correctly as *N. meningitidis* negative. Five laboratories commented that 'another species', *S. pneumoniae*, was present. There were no incorrect genogroup or species reports in 2006, but, similar to 2012, some laboratories reported negative results for genogroup assays.

Non-culture detection and genogroup summary

A minimum of 62% of the 29 participant laboratories were capable of non-culture *N. meningitidis* detection in 2012, compared with 76% in 2011 (see Table 13).

The genogroup confirmation is more exacting as the assays appear to be less sensitive, and the more diluted simulated septicaemia samples are more challenging. Even so, more than 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 genuine clinical levels.

Table 13. Proportion of participant (29) laboratories agreeing with consensus for detection and genogroup

Sample	Detection	Genogroup
1382	62%	62%
1383	62%	55%
1384	69%	52%
1385	45%	N/A*

** N/A = Not applicable, negative sample*

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

Participants were requested to test and report the *porA* variable regions VR1 and VR2, *fetA* variable region (VR), and use MLST to determine the sequence type (ST) and clonal complex (cc) of 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.

Genotyping of isolates

The consensus results for *porA*, *fetA* and MLST genotyping of the four isolate samples are presented as Table 14.

Table 14. Isolate consensus genotyping (*porA*, *fetA* and MLST) results

Sample	*porA*	*fetA*	MLST		
	Group	VR1	VR2	ST	cc
1379	A	20	9	F3-1	4789	5
1380	B	7	16	F3-3	32	32
1381	C	21-7	16	F5-36	1157	1157

The total number of respondents to the molecular typing targets varied from sample to sample, ranging from 20 for *porA*VRs, 20 for *fetA* and 15–16 for MLST.

1379: Twenty (69% of 29) laboratories reported *porA* genotyping results and all reported the consensus *porA* VR1 20 and VR2 9. Twenty participants (69%) reported *fetA* VR; all reported F3-1.

Ten laboratories (34% of the 29) reported ST results for 1379, and all reported MLST ST 4789. Fifteen laboratories (52%) reported cc results; all reported cc 5.

Five laboratories reported cc but not ST, possibly indicating difficulties with particular alleles. Three laboratories commented that *pdhC* had proved exacting. Two of those laboratories did not determine *pdhC* (NM24 and NM42), but laboratory NM30 described the successful determination after using sequencing primers for initial amplification.

[Note: It is the cc and not the ST that has been requested for the ECDC TESSy database; this was part of the 'fine type' assignment].

In 2011, sample 0817 was characterised similarly to 1379 in 2012. Seventy percent of participants reported *porA* VR1 and 73% *porA* VR2; 67% for *fetA*. MLST ST 47% and cc 53% were also comparable. The issue with *pdhC* and the use of sequencing primers to determine the allele had also been noted in the 2011 EQA summary report. Interestingly, NM24 did report ST-4789 but NM42 did not report MLST ST for 0817 in 2011.

1380: Twenty of 29 (69%) laboratories reported *porA* genotyping results, and all reported the consensus *porA* VR1 7 and VR2 16. Twenty (69%) laboratories reported *fetA* VR, and all reported F3-3.

Seventeen of the 29 (59%) laboratories reported ST results for 1380, 16 (55%) reported MLST ST 32. Similarly, 17 of the 29 (59%) laboratories reported cc, and 16 (55%) reported MLST cc 32.

Laboratory NM37 incorrectly reported the ST 2145. A detailed description of the erroneous report of ST 2145 instead of ST 32 is given in Annex 7. It appears that analysis of the *aroE* sequence may have been the issue.

Laboratory NM39 reported the ST 32 but incorrectly reported cc5. This may indicate a simple reporting confusion with the designation ST-32 complex/ET-5 complex: ET-5 indicates the compatibility to the previously used multilocus enzyme electrophoresis (MEE) typing scheme.

In 2009, sample 9201 (1380, 2012) was correctly characterised *porA* VR1 and VR2 by 18 (67%) of all participants, a similar proportion to 2012. A considerable improvement in the number and proportion of laboratories reporting

fetA was made between 2009 (33%) and 2012 (69%). In the same period, consensus MLST ST and cc reports increased from 44% (12 laboratories) to 55% (16 laboratories). Interestingly, participant NM41 had difficulty assigning the MLST ST (unable to confirm *pgm* 8 allele) in 2009, but not in 2012.

1381: Twenty (69% of 29) laboratories reported *porA* genotyping results, and all laboratories reported the consensus *porA* VR1 21-7 and VR2 16. Twenty participants (69%) reported *fetA* VR; all reported F5-36.

Sixteen laboratories (55% of the 29) reported ST results for 1381, and all reported MLST ST 1157. Similarly, 16 (55% of the 29) laboratories reported cc, and all reported MLST cc 1157.

Genotyping of simulated septicaemia (non-culture) samples

The consensus results for *porA*, *fetA* and MLST genotyping of the three positive simulated septicaemia (non-culture) samples are presented as Table 15.

Table 15. Simulated septicaemia sample – consensus genotyping (*porA*, *fetA* and MLST) results

Sample	Genogroup	*porA*		*fetA*	MLST	cc
		VR1	VR2		ST	
1382	B	22	9	F5-1	1195	269
1383	C	7-4	14-6	F3-9	1031	334
1384	B	7-8	4-1	F1-5	41	41/44

1382: Fourteen laboratories (48%, 14/29) reported *porA* VR1 and VR2, but only 13 (45%) the consensus VR1 22 and VR2 9. One laboratory (NM24) reported *porA* VR1 as 2-2, which could be a clerical error during online results submission. Another laboratory (NM32) incorrectly reported VR2 4.

Thirteen of the 29 laboratories (45%) reported *fetA* VR, where 12 (41%) reported the consensus F5-1. One laboratory (NM26) incorrectly reported *fetA* VR F1-5. While *fetA* F1-5 may be a simple clerical error upon submission, it should be noted that the sample or result may have been confused with 1384.

Six of the 29 laboratories (21%) reported ST results, all reported ST-1195. Six of the 29 laboratories (21%) reported cc – all reported cc ST-269.

[Note: It is the cc and not the ST that was requested for the TESSy database].

In 2009, 9205 (1382, 2012) the consensus *porA*VR1 was agreed by eight (30%) laboratories and VR2 by nine (33%) laboratories. *Fet*A was reported and agreed as F5-1 by three (11%) laboratories. Similarly, three (11%) laboratories agreed the consensus MLST ST-1195, but four (15%) agreed cc 269. Comparing the characterisations from 2009 and 2012, it has to be noted that the number of participants which undertake *porA* and *fetA* typing has substantially increased. Similarly, there had been a doubling in MLST capacity from three to six laboratories over the three-year period: a definite improvement in European molecular typing capacity.

1383: Thirteen of the 29 laboratories (45%) reported *porA* VR1 and 15 (52%) reported VR2. All 13 *porA* VR1 agreed 7-4. The consensus VR2 14-6 was confirmed by 14 laboratories, with one laboratory (NM32) incorrectly reporting 4.

Thirteen of the 29 laboratories (45%) reported *fetA* VR F3-9.

Six of the 29 laboratories (21%) reported ST results, and all reported ST-1031. Seven of the 29 laboratories (24%) reported cc – all reported cc ST-334.

[Note: It is the cc and not the ST that was requested for the TESSy database].

In 2009, 9209 (1383, 2012) the consensus *porA*VR1 and VR2 were achieved by all 11 (41%) laboratories reporting as VR1 7-4 and VR2 14-6. *FetA* was agreed as F3-9 by five laboratories (19%) . Only five (19%) participants reported MLST ST. The consensus MLST ST-1031 was only based on two (7%) laboratory reports, where the other three laboratories reporting ST had reported 'UA' (unassigned ST). MLST cc was reported by six laboratories (22%): the consensus was agreed as cc 334 by five laboratories (19%), with one (4%) reporting 'UA' (Unassigned).

Similar to sample 1382, an increase in the number of laboratories reporting molecular characterisations was observed in 2012 compared with 2009. There was an improvement in MLST characterisation as no UA reports were received for either ST or cc in 2012.

1384: Twelve (41%, 12/29) laboratories reported *porA* VR1 and 15 (52%) reported VR2. Eleven laboratories confirmed *porA* VR1 7-8, but one laboratory (NM27) incorrectly reported VR1 7. This may have been due to a technical mix-up rather than incorrect editing of poor sequence data (see Annex 8). *PorA* VR2 4-1 was reported by 14 laboratories (48%), with one laboratory (NM32) incorrectly reporting VR2 4.

Thirteen laboratories (45%, 13/29) reported *fetA* VR results; 12 (41%) laboratories correctly identified F1-5 and one laboratory (NM23) incorrectly detected F3-9. F3-9 was the correct result for 1383, which suggests an online reporting error or technical mix-up during testing.

Six laboratories (21%, 6/29) reported ST results, and all reported ST-41. Seven laboratories (24%, 7/29) reported cc – all reported cc ST-41/44.

[Note: It is the cc and not the ST that was requested for the TESSy database].

In 2007, 11 (40%) of 29 participants reported *porA* VR characterisations for sample 8768 (same as 1384 in 2012): Seven (24%) reported the VR1 consensus 7-8 and 10 (34%) reported VR2 4-1. Four VR1 reports of 7-15 and one 'NT' (not typed) were recorded. The VR1 7-15 variant is three amino acids shorter than 7-8, and it is most likely that laboratories not reporting the longer consensus 7-8 were not fully conversant with the typing website. Improvements with the PubMLST *Neisseria* typing website since 2007 show the *porA* designation options more clearly and guide users to the most applicable designation by presenting the matches and length.

There was an increase in the number of laboratories performing molecular *porA* typing and an improvement in quality in 2012 compared to 2007.

In 2007, only three participants (10%) reported MLST ST, and five (17%) reported cc. While five laboratories reported cc, only two reported ST-41 (the consensus) and one ST-2188. In 2012, there was an increase in the number and proportion of laboratories elucidating non-culture MLST and achieving a genuine consensus. It should be noted that MLST investigation of 1384 (8768) was the most exacting task within both panels as the samples were designed for a low level of positivity.

FetA was not requested in 2007 and again demonstrates the increased molecular typing capacity of participants for *fetA* by 2012.

2.4 Summary of genotyping consensus reporting

The proportion of laboratories reporting consensus genotyping results from the 29 participants is summarised in Table 16. The complete *porA* sequence typing (VR1 and VR2) of isolates 1379–1381 was achieved by 20 (69%, 20/29) of participating laboratories but dropped to 45–52% for the non-culture samples (1381–1385). Similarly, the range of *fetA* consensus agreement for the isolates (1379–1381) was 69% but only 38–45% for the non-culture samples (1382–1385). The ST consensus ranged from 52–55% for the isolates (1379–1381) but was only 21–24% for the non-culture samples (1382–1385). The cc consensus reports were 35–69% for the isolates (1379–1381) and 21–24% for the non-culture samples.

Table 16. Summary of molecular typing results for isolates and non-culture samples

					MLST	
	Detection	Group	*porA*	*fetA*	ST	MLST
Isolates	N/A	59%*	69%	69%	35–69%**	52–55%
Non-culture	62–69%	52–62%	45–52%	38–45%	21%	21–24%

** A number of labs do not routinely determine molecular group for isolates.*
*** Range for the x3 samples, either isolates or non-culture.*

If data are analysed considering the proportion of consensus results of those laboratories (countries) that actually submitted results, the table appears slightly different but perhaps more impressive and an indication of the characterisation quality (Table 17). The isolate genotypic characterisations submitted were in 100% agreement for all samples, except 1380 ST and CC, which only dropped slightly to 94%.

The non-culture samples were more exacting but consensus was achieved by greater than 92% for all sample and molecular target combinations.

Table 17. Number and proportion of laboratories achieving the consensus of those submitting genotyping results

Sample	*porA*	*fetA*	MLST		
	VR1	VR2	VR	ST	cc
1379	20 (100%)	20 (100%)	20 (100%)	10 (100%)	16 (100%)
1380	20 (100%)	20 (100%)	20 (100%)	16 (94%)	16 (94%)
1381	20 (100%)	20 (100%)	16 (100%)	16 (100%)	16 (100%)
1382	13 (93%)	13 (93%)	12 (92%)	6 (100%)	6 (100%)
1383	13 (100%)	14 (93%)	13 (100%)	6 (100%)	7 (100%)
1384	11 (92%)	14 (93%)	12 (92%)	6 (100%)	7 (100%)

'Fine type', agreement with full data requested by ECDC for TESSy

The proportion of laboratories reporting the consensus 'fine type' (serogroup: PorA*:* FetA*:* cc) for isolates as requested by ECDC for the TESSy dataset was dependent on the sample: for all three isolates (1379, 1380 and 1381) it was 52% (15/29 laboratories), for greater or equal to two samples it was 55% (16/29). See Table 18.

The proportion of laboratories reporting the consensus 'fine type' (serogroup: PorA*:* FetA*:* cc) for non-culture samples as requested by ECDC for the TESSy dataset was also dependent on the sample: for all three *N. meningitidis*-positive simulated septicaemia samples (1382, 1383 and 1384) it was 10% (3/29 laboratories), for greater or equal to two samples it was 21% (6/29), and for greater or equal to one sample it was 24% (7/29).

It is important to note that in Table 18, 'No report' indicates that 45% of the 29 participants were unable to designate fine types for isolates and 76% for non-culture samples.

The proportions are reduced mainly due to 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, not least of which is the amount of genomic material available. This necessitates nested (or two rounds of) PCR amplification before the cycle sequencing. This becomes even more difficult with the multiple targets required for MLST. Often, alternate (or additional) sets of primers are required, which may require the use of additional, optimised thermal cycling parameters.

The number of laboratories capable of reporting the fine-type designation for each specific sample is shown as Table 19.

Table 18. Number and proportion of participant laboratories reporting 'fine type' designation

Isolates	Non-culture	
All 3 samples[1]	15 (52%)	3 (10%)
≥2 samples	16 (55%)	6 (21%)
≥1 sample	16 (55%)	7 (24%)
No report	13 (45%)	22 (76%)

[1] *Results matched consensus for all five components of the 'fine type' for each of the* N. meningitidis *isolates or positive non-culture samples.*

Table 19. No. of laboratories (%) capable of designating the consensus fine type by sample

Sample	No.	%
1379	15	52%
1380	16	55%
1381	16	55%
1382	6	21%
1383	5	17%
1384	5	17%

Laboratories achieving complete consensus typing reports

Rather than concentrate upon the negative aspects of the EQA (no reports or non-consensus analysis) it should be recorded that 10 (34%) of all the participants returned reports in complete agreement for the isolate consensus typing results: namely, NM20, NM23, NM27, NM28, NM29, NM30, NM31, NM34, NM35 and NM41.

There were three (10%) laboratories that achieved consensus typing for all the isolate and non-culture samples: NM30, NM31 and NM34.

If MIC reports were included in the overall analysis, no participants would have achieved the complete consensus of all results. This observation probably reflects the normal (statistical) distribution of MIC results: laboratories were only one dilution different to the mode in most instances as shown previously.

It should be understood that the EQA panel required 18 typing results for isolates and 22 typing results for the non-culture samples (allowing one result for the negative non-culture sample). In fact, to achieve the complete consensus, the amount of DNA sequencing required to determine the MLST ST actually meant that 11 results would be required for each isolate and 12 for each non-culture sample (except the negative).

Should MIC (mode) results be included too, then to achieve total isolate consensus, a participant laboratory would need to report 16 results per isolate, a total of 48.

It was possible but unlikely that any laboratory would report 84 consensus results (48 for isolates and 36 for non-culture).

This reinforces the achievement of participants: NM30, NM31 and NM34.

2.5 Methodology review

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 Etest or similar). The MIC gradient strip manufacturer/supplier were requested and a review of the responses in association with MIC results is discussed in Section 2.1.5.

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 as Table 20. Simple heated (boiled) suspensions of meningococci are confirmed as suitable genotyping, with a number of laboratories using conventional PCR and gel detection to determine results. DNA sequencing was only reported by four laboratories when *porA*, *fetA* and MLST all required sequencing, which was achieved by more than 43% (13/30) laboratories.

Table 20. Methods used for genotyping of isolates, samples 1379–1381

	Extraction	Amplification	Detection
	Capture column (5) Boil (10) Magnetic beads (3) Salt precipitation (1) Other (1)	Conventional PCR (17) Real-time PCR (4)	Gel electrophoresis (11) Real-time PCR (4) Sequencing (6)
Total	**21**	**21**	**21**

Similar responses were reported for the non-culture samples 1382–1385 (Table 21). 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 by two laboratories of sequencing could refer to the DNA sequencing of PCR products to confirm species or genogroup or the other molecular typing assays. It is not clear why increased reports of extraction and amplification methods were made for the non-culture samples when it is apparent that fewer sample reports were made.

It should be stated that in hindsight the questions regarding molecular processes and techniques were not specific enough (as previously noted in the 2011 panel also). That most (nearly all) laboratories applying the molecular methods achieved the consensus results is sufficient to record their utility. If the non-culture samples had been designed to be more exacting (weaker positives), the methods may have been better challenged.

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

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

Table 21. Methods used for genotyping of non-culture, samples 1382–1385

	Extraction	Amplification	Detection
	Capture column (16) Magnetic beads (5) Other (2)	Conventional PCR (11) Real-time PCR (11)	Gel electrophoresis (9) Real-time PCR (10) Sequencing (2)
Total	**23**	**22**	**21**

2.6 Review of non-responding participants to typing characteristics

It is too easy to dwell upon the submitted positive results and compare them to the consensus, analysing minor discrepancies, when it is of most importance to increase the number of laboratory submissions to all characterisation targets to fulfil the maximum 'fine-typing' capacity.

The non-submission of MIC results was described in section 2.1.2 previously.

Table 22 describes the typing characteristics that laboratories did not report for the isolates 1379–1281, and Table 23 is the same information presented by non-responding participants to suggest molecular typing capacity issues: finance, space, equipment, staff, or training. It should be noted that all (29) participants returned results for

serogroup or genogroup. Twelve (41%) participants did not provide at least one of the characteristics required for fine typing of isolates, Table 22.

Similarly, Tables 24 and 25 describe the laboratories not reporting non-culture sample characterisations. There were 22 (76%) participants (excepting NM37) that did not report at least one of the molecular characteristics for the non-culture samples.

Not surprisingly, the participants not reporting *fetA*, *porA* or MLST for the isolates did not report for the non-culture samples either.

Table 22. Isolate typing characteristics not returned by participants

	No. (%)	Lab ID
*fetA*VR	9 (31%)	NM36, NM38, NM40, NM43, NM45, NM48, NM51, NM52, NM54
*porA*VR1 & VR2	9 (31%)	NM36, NM38, NM40, NM43, NM45, NM48, NM51, NM52, NM54
MLST CC & ST	12 (41%)	NM22, NM26, NM36, NM38, NM40, NM43, NM45, NM47, NM48, NM51, NM52, NM54

Table 23. Laboratories not returning isolate characterisations

Lab ID	*fetA*VR	*porA* VR1 and VR2	MLST CC	ST
NM22			✓	✓
NM26			✓	✓
NM36	✓	✓	✓	✓
NM38	✓	✓	✓	✓
NM40	✓	✓	✓	✓
NM43	✓	✓	✓	✓
NM45	✓	✓	✓	✓
NM47			✓	✓
NM48	✓	✓	✓	✓
NM51	✓	✓	✓	✓
NM52	✓	✓	✓	✓
NM54	✓	✓	✓	✓

Table 24. Non-culture typing characteristics not returned by participants

	No. (%)	Lab ID
Detection	8 (28%)	NM32, NM33, NM36, NM38, NM40, NM51, NM52, NM54
Genogroup	8 (28%)	NM33, NM36, NM38, NM40, NM48, NM51, NM52, NM54
*fetA*VR	15 (52%)	NM20, NM28, NM32, NM33, NM35, NM36, NM38, NM40, NM42, NM43, NM45, NM48, NM51, NM52, NM54
*porA*VR1	15 (52%)	NM20, NM28, NM33, NM35, NM36, NM37, NM38, NM40, NM42, NM43, NM45, NM48, NM51, NM52, NM54
*porA*VR2	14 (48%)	NM20, NM28, NM33, NM35, NM36, NM38, NM40, NM42, NM43, NM45, NM48, NM51, NM52, NM54
MLST CC	22 (76%)	NM20, NM22, NM23, NM26, NM27, NM28, NM29, NM32, NM33, NM35, NM36, NM38, NM39, NM40, NM42, NM43, NM45, NM47, NM48, NM51, NM52, NM54
MLST ST	22 (76%)	NM20, NM22, NM23, NM26, NM27, NM28, NM29, NM32, NM33, NM35, NM36, NM38, NM39, NM40, NM42, NM43, NM45, NM47, NM48, NM51, NM52, NM54

Table 25. Laboratories not returning non-culture characterisations

			porA		MLST	
LabID	detection	Group	*fetA*VR	VR1&VR2	CC	ST
NM20			✓	✓	✓	✓
NM22					✓	✓
NM23					✓	✓
NM26					✓	✓
NM27					✓	✓
NM28			✓	✓	✓	✓
NM29					✓	✓
NM32	✓		✓		✓	✓
NM33	✓	✓	✓	✓	✓	✓
NM35			✓	✓	✓	✓
NM36	✓	✓		✓	✓	✓
NM37[1]				✓[1]		
NM38	✓	✓	✓	✓	✓	✓
NM39					✓	✓
NM40	✓	✓	✓	✓	✓	✓
NM42			✓	✓	✓	✓
NM43			✓	✓	✓	✓
NM45			✓	✓	✓	✓
NM47					✓	✓
NM48		✓	✓	✓	✓	✓
NM51	✓	✓	✓	✓	✓	✓
NM52	✓	✓	✓	✓	✓	✓
NM54	✓	✓	✓	✓	✓	✓

[1] *NM37 only porAVR2s reported, no porAVR1; suggestive of technical not capacity issues*

2.7 Review of participants' non-consensus trends

Although it was expected that participants would review their own results following the designation and distribution of the consensus results, it is possible to review submissions over the three IBD-labnet EQAs 2009, 2011 and 2012. Clearly, it is important to increase the submissions to all characterisations for as many laboratories as possible (Section 2.6), but the quality of the reports is fundamentally important and the reason for regular EQA panel distributions. Consideration should be given to the fact that non-consensus results are relatively few, as detailed in the sample specific results.

Table 26 indicates that a small number of laboratories did not match the consensus in two or more of the distributions. The detailed non-consensus reports by characteristic for isolates and non-culture are respectively shown as Tables 27 and 28. It may be seen that some laboratories are observed more than once in a distribution and occasionally in more than one distribution.

Laboratory NM37 is notable for non-consensus isolate typing reports in 2011 and also in 2009, but not in 2012. NM45 would appear to have problems achieving consensus serogroup or genogroup results in all three EQAs (2009 to 2012). There were fewer non-consensus reports for isolates in the current 2012 EQA panel.

The non-culture non-consensus summary (Table 28) also identifies NM37 in 2012, but other participants (NM32 and NM35) are also listed with more than one erroneous result. The relatively larger number of laboratories having problems with non-culture (species) detection and genogroup in 2012 could mirror those of 2009 as sample 1379 and 1380 were included in both distributions: NM22, NM35 and NM37 featuring in both years.

Table 26. Non consensus in two or more EQA panels

Characteristic	No. of EQA panels (LabID)
Isolates	
Serogroup	3 (NM45); 2 (NM38, NM40)
porA	2 (NM37)
MIC Cip	2 (MN48)
MIC Pen	2 (NM48)
Non-culture	
Detection	2 (NM22, NM35, NM37)
Genogroup	2 (NM23, NM37)
PorA	2 (NM24, NM32)
MLST	2 (NM37)

Table 27. Detailed non-consensus reports for participants with respect to isolate molecular typing characteristics in EQAs 2009, 2001 and 2012

	2009	2011	2012
Serogroup	NM36(2)[1], NM38, NM40, NM45, NM48	NM22, NM38, NM40, NM45	NM45
Genogroup	NM32	NM23	NM45
porA	NM22(2), NM23, NM26, NM27, NM28, NM32, NM34, NM37, NM41	NM37	
MLST		NM37	
CC		NM37	

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

Table 28. Detailed non-consensus reports for participants with respect to non-culture molecular typing characteristics in EQAs 2009, 2001 and 2012

	2009	2011	2012
Detection	NM22(5)[1], NM25, NM31,NM35, NM37(2), NM38(3), NM39, NM43,	- NM35, NM37, NM45	NM20(2), NM22, NM28 NM44
Genogroup	NM31, NM49	NM23, NM37	NM21, NM23, NM28, NM32(2), NM35, NM37, NM45
porA	NM26, NM32	NM24, NM37	NM24, NM27, NM32(5)
fetA	-	-	NM23, NM26
MLST	NM20(2), NM34, NM37(3), NM41(2)	NM21	NM37
CC	NM25(2)	-	NM39

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

Using the non-consensus Tables 26, 27 and 28, a summary table indicating participant laboratories that may require support was compiled as Table 29. There were three laboratories that had problems with serogroup: NM38, NM40 and NM45. Six laboratories attempting non-culture PCR detection had some problems and six also when determining the genogroup. To put that in perspective, there were eight participants (Section 2.6, Table 24) that did not carry out PCR detection or genogroup at all.

Without contacting the indicated laboratories individually it is not possible to ascertain from the submitted results if there are serious problems or simple data entry (website reporting) issues. Access to appropriate resources could be an issue for the non-consensus reports.

Table 29. Participant laboratories that may require support (based on EQA)

	Serogroup	Molecular techniques		
		Detection	Genogroup	Typing[1]
NM20		✓		
NM22		✓		
NM23			✓	✓
NM28		✓	✓	
NM32		✓	✓	
NM35		✓	✓	
NM37		✓	✓	✓
NM38	✓			
NM40	✓			
NM45	✓		✓	✓

[1] *Molecular typing includes porA, fetA or MLST*

2.8 Summary comparison of IBD-labnet *N. meningitidis* EQA panels 2009, 2011 and 2012

The 3rd IBD-labnet EQA panel 2012 (this report) was distributed to 29 laboratories but was previously distributed to 31 countries in 2011 and 30 in 2009. In 2012, there were 29 returned reports, but in 2011 there were 30 returned reports compared with 29 in 2009. With regard to the typing of the isolates, there were relatively few problems with any of the panels although it should be noted that there were six isolates in 2009, four in 2011 and only three in 2012.

To re-assess the capabilities of the European meningococcal reference laboratories, two of the isolates distributed in 2012 were selected from previously distributed EQAs. Sample 1379 demonstrated that serogroup A can still be a problem for one or two laboratories and in fact was better characterised when tested in 2009. The problems encountered with serogroup Y isolates in 2011 and 2009 were not re-assessed in 2012.

The evaluation of MICs results in 2012 was facilitated by conversion of the submitted MIC values (dilutions) to the EUCAST doubling dilution series. Differences were observed, but they appeared to be minor, with very few 'outlier' reports in 2012. EUCAST interpretation demonstrated that the 2012 participant MIC reports were consistent with the few 'reduced susceptibility' reports, and that it was unlikely that there would have been clinical consequences.

There could still be an issue with the website reporting and feedback (conversations) with participants since the 2012 distribution has suggested better explanations to inform participants how to enter and save results on the UKNEQAS website, and that it is possible to amend, update and print laboratory reports until the closing date (deadline).

The reduction in the number of genotyping fields (results) for reporting and specifying options appeared to cause less confusion and clerical errors upon website reporting in 2011 and 2012.

With regard to the molecular typing of isolates, there was good agreement, with few laboratories indicating problems.

Although there has been a general increase in laboratories reporting isolate molecular typing – and particularly the adoption of *fetA* in 2011 and 2012 – it is apparent that a number of laboratories do not have the capability for molecular typing.

The non-culture samples again proved more difficult than the isolates as they 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 1384 as a simulated 'weak positive' septicaemia sample.

There was an increase in the maximum number of laboratories reporting molecular detection results from 2009 to 2011: 20 (69%, 20/29) in 2009 to 23 (77%, 23/30) in 2011. However, in 2012 this number decreased to 20 (69% 20/29). That the proportion is dependent on the sample was illustrated by the inclusion in 2012 of 'potential *ctrA* negative' isolate, previously distributed in the 2009 panel. With regard to the maximum genogroup reports for the non-culture samples, 19 (66%, 19/29) laboratories reported in 2009; in 2011, this number was up to 22 (73%, 22/30) laboratories, but declined to 18 (62%, 18/29) in 2012.

The investigation of the genotyping of the non-culture samples in 2012 revealed very few reports that were different to the consensus, and where identified it appeared that simple laboratory or transcription errors could be implicated.

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

30). In 2011, 16 (53%, 16/30) laboratories confirmed the maximum isolate 'fine type', constrained by the number assigning the cc. The non-culture 'fine types' achieved in 2011 were six (20%, 6/30), again constrained by the non-culture MLST. In 2012, the maximum number of participants capable of isolate 'fine type' designation was 16 (55%) and six (21%) for non-culture. In 2012, like 2011, the constraint was in assigning cc. There was no increase in the number of laboratories in 2012 but slight improvement in proportion due to the withdrawal of one laboratory not reporting molecular results.

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 30. Maximum number (proportion) of laboratories capable or reporting 'fine type' by EQA distribution

Serogroup: porA, *fet*A, cc

Year	'Fine type'	
	Isolates	**Non-culture**
2009	9 (31%)	2 (7%)
2011	16 (53%)	6 (20%)
2012	16 (55%)	6 (21%)

Note: 29 reports received in 2009, 30 in 2011, and 29 in 2012.

2.9 IBD-labnet EQA workshop report, Barcelona, Spain, 21 November 2012

A brief summary presentation of the 2012 EQA distribution (UK NEQAS 3212) was given by Dr S Gray based upon preliminary analysis of the laboratory reports returned to UK NEQAS. The results and general conclusions within the presentation are consistent with this summary report, but the subsequent careful review of data for the summary report has allowed for a more detailed analysis than at the time of presentation.

The details of the presentation were not challenged. The conclusion was that characterisation and accurate typing were improving although there a still a number of laboratories not reporting molecular results. There was a limited discussion regarding an analysis of non-responders. A request for a certificate of enrolment or participation in the EQA scheme was made by a number of countries.

A discussion was held regarding whether possible local (intra-country) distribution of the EQA material was allowed. The potential safety issues were raised, but the question was not resolved.

3 Conclusions

Overall, the 2012 3rd *N. meningitidis* IBD-labnet EQA was successful. Some improvements were noted in the number and quality of responses to the requested detection and characterisation targets. The reduction in requested information and restricted options for website reporting greatly facilitated the review of results. Even after reducing the number of isolates from four to three in 2012, the EQA panel was a considerable amount of work for participants. The seven sample panel generated a large amount of data for comparative analysis.

Serogrouping was again identified as an issue for two laboratories, notably with regard to the serogroup A isolate. Whether it was training or availability of specific and pooled agglutination reagents was not determined. The utility of genogrouping for the laboratories using it has been highlighted by the non-culture samples, and it is possible that widespread adoption of PCR-based genogrouping could become important for more accurate typing (knowing if a capsule is being expressed, and whether a polysaccharide vaccine would actually be an effective intervention).

Participation in the three ECDC IBD-labnet EQAs was on the understanding of participant anonymity, and as such laboratories (countries) have only been indicated by their codes NM 'xy'. It was agreed that much of the EQA evaluation and review of procedures would be carried out by the laboratories themselves on receipt of their individual reports, comparing their results to the consensus and repeating or re-evaluating their results as required. It is too easy to dwell on the reported results and relatively minor errors observed and thus overlook the fact that nearly all the genotyping data submitted were in agreement. Laboratories testing and submitting results for genotyping of the isolates, and particularly the non-culture samples, are to be encouraged.

This report has drawn attention to (anonymous) laboratory performance as requested by ECDC, notably the laboratories that have not submitted specific characterisation reports, those reporting non-consensus (over the three EQAs), and those capable of 'fine-type' determination. The achievement of participants that reported complete isolate 'fine types' and those which also reported the non-culture 'fine types' should not be understated.

It may be 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 the position to characterise all their routine samples and submit the data to TESSy. To determine the overall capacity and barriers to routine testing and reporting to ECDC, it would be more appropriate to use a questionnaire than this EQA process.

Opportunities were given to participants to feedback their comments on the EQA panel or specific results in person at the annual IBD-labnet meeting in Barcelona, Spain 2012, or by email, but to date there have been very few comments other than appreciation of the EQA and ECDC's support.

Interestingly, one laboratory (NM32) requested a repeat set of samples from UK NEQAS on receipt of their individual report. They thought they had a contamination problem affecting their non-culture typing (as noted in previous sections). They were able to demonstrate the issue related to the sole microbiological safety cabinet that they had access to. Using their experience of the 2012 EQA, they were able to convince senior management to provide another cabinet, thereby separating culture and non-culture activity and reducing opportunities for cross-contamination of (particularly non-culture) samples.

Another laboratory discussed the issue regarding the non-detection of *N. meningitidis* in sample 1382 using *ctrA* PCR and requested information regarding the level of positivity (concentration of *N. meningitidis*) in the non-culture samples.

Similar to 2011, the consensus levels were generally excellent (for those reporting results), but it should be noted that there are a number of laboratories (countries) unable to detect *N. meningitidis* in non-culture samples and then apply the more exacting molecular typing methods. It is not possible to determine from the EQA if it is lack of resources or expert knowledge, but one may speculate that it is more likely to be the former. Therefore, one may assume that countries not reporting non-culture detection in the EQA may be unable to confirm non-culture cases and as such may be underreporting meningococcal cases. It may be argued that it is a requirement of local hospitals to confirm the non-culture cases, and not the national reference facility.

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 one would need to generate the DNA sequences. Similarly, to set up routine non-culture detection service, a laboratory would require considerably more training with the equipment a laboratory would have local access to. To address these issues, it would be appropriate to send out a short but directed questionnaire to ascertain which laboratories were/are unable to complete all the

requested typing targets and then to set up specific training to meet their needs. Some effort has been made in this direction, with laboratory placements in early 2011; the reports of which are awaited.

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. It is noted that a UK NIBSC-produced standard available through 2010–2012 has just been withdrawn due to lack of orders (interest).

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

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

4 References

1 European Centre for Disease Prevention and Control. External quality assurance scheme for *Neisseria meningitidis* – 2009. As part of the IBD-Labnet surveillance network. Stockholm: ECDC; 2011. Available from: http://ecdc.europa.eu/en/publications/Publications/1103_EQA_NMening_2009.pdf

2 Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicaemia using real-time PCR. J Clin Microbiol. 2001 Apr;39(4):1553-8.

Annex 1. Participating *Neisseria* reference laboratories, 2012

Austria
Dr Sigrid Heuberger
National Reference Centre for Meningococci, Pneumococci and Haemophilus influenzae
Austrian Agency for Food and Health Safety
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Belgium
Sophie Bertrand
WIV-ISP
DO Maladies transmissibles et infectieuses – Service scientifique des maladies bactériennes
Rue Juliette Wytman 14
1050 Bruxelles, 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
Lotte Lambertsen
Neisseria and Streptococcus Reference Laboratory
Department of Bacteriology, Mycology and Parasitology
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5 Artillerivej, building 211/117B
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Estonia
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Finland
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Unit Invasive Bacterial Infections
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Institute of Laboratory Medicine
Landspitali University Hospital
Baronsstigur
101 Reykjavik, Iceland

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Hôpitaux Universitaires de Géneva
Laboratoire Central de Bactériologie
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1211 Genève 14, Switzerland

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National Public Health Surveillance Laboratory
Zolyno str. 36
10210 Vilnius, Lithuania

Luxembourg
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United Kingdom
Dr Edward Kaczmarski
HPA Meningococcal Reference Unit
Manchester Royal Infirmary
Oxford Road, Manchester M13 9WL, UK

Annex 2. Cover letter

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

Dear [name of participant],

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

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

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

Thank you for your cooperation.

Yours sincerely,

[Signed]

Annex 3. Terms and conditions of participation distributed in 2009 but not 2012

Health Protection Agency EQA scheme and/or *H. influenzae* EQA scheme

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

We hereby acknowledge receipt and accept the conditions outlined above.

Signed .....

Name …….

For and on behalf of

Name of Recipient Organisation ……..

Address ……..

……..

……

Date .....

Annex 4. The UK NEQAS sample handling and reporting documentation, *N. meningitidis*, EQA distribution 3212, 8 May 2012

11/05/12

UK NEQAS Microbiology Quality Assessment
Special survey for *Neisseria meningitidis* identification and typing
Date: 08.05.2012

Distribution No. 3212
Lab ID No:

NM30

This survey has been organised jointly between UK NEQAS for Microbiology and the Meningococcal Reference Unit, Manchester, UK on behalf of ECDC IBD LabNet.

To address results required for ECDC TESSy database

See page 5 for reconstitution methods of the specimens – this distribution closes 22 June 2012.

Part 1. Specimens 1379 to 1381. Results for *Neisseria meningitidis* **isolates (viable cultures)** strain characterisation

Please fill in all columns, if not tested enter ND

Report **serogroup** (as A, B, C etc. {NG = Not groupable, ND = Not determined})

Report **phenotypic or genotypic results in this section**

Serogroup may be determined by either serology or PCR techniques.

If a serogroup or genogroup is recorded as ND (Not determined) state in "General Comments" which serogroups were tested.

Specimen number	Serogroup (phenotypic e.g. serology)	Serogroup (genotypic e.g. PCR)
1379		
1380		
1381		

General comments:

Return results as soon as possible via the Website: www.ukneqasmicro.org.uk **no later than: 22 June 2012** (17.00 GMT).

UK NEQAS Microbiology Quality Assessment
Special survey for *Neisseria meningitidis* identification and typing
Date: 08.05.2012

Distribution No. 3212
Lab ID No:

NM30

Part 2. Specimens 1379 to 1381. Antimicrobial Susceptibility Testing
Instructions: After growth following incubation and identification determine the **Minimum Inhibitory Concentration (MIC) using commercial gradient diffusion methodology (e.g. E-test or similar).**

Please enter details of methods used:

Your method(s) of testing:

Gradient MIC:
Specify manufacturer _____

Plate agar medium used:
Specify manufacturer _____

Notes on completing reply forms:
Test only those agents you would routinely test. If you do not test for any antimicrobial please leave the result box blank.

Spec. number	Antimicrobial agent:	MIC (mg/L)
1379	Ciprofloxacin	
	Ceftriaxone	
	Cefotaxime	
	Penicillin	
	Rifampicin	
1380	Ciprofloxacin	
	Ceftriaxone	
	Cefotaxime	
	Penicillin	
	Rifampicin	
1381	Ciprofloxacin	
	Ceftriaxone	
	Cefotaxime	
	Penicillin	
	Rifampicin	

MAY 11 12:59

UK NEQAS Microbiology Quality Assessment
Special survey for *Neisseria meningitidis* identification and typing
Date: 08.05.2012

Distribution No. 3212
Lab ID No:

NM30

Part 3. Results for *Neisseria meningitidis* strain genotyping. Specimens 1382 to 1385 contain killed organisms and have been included as non-culture samples for molecular testing only.

Please see instructions for handling on page 5.

Samples 1379 to 1381 are viable cultures and 1382 to 1385 are non-culture samples for molecular confirmation

Report ***porA*** sequence type (geno-subtype) (report as 7-2, 4 {where VR1= 7-2 and VR2= 4})

Report ***fetA*** sequence type (as F5-1 or F4 etc.)

Specimen number	Result (pos/neg)	Target gene(s) (ID)	Genogroup	Target gene(s) (genogroup)	*por*AVR1	*por*AVR2	*fetA*
1379	N/A*	N/A*					
1380	N/A*	N/A*					
1381	N/A*	N/A*					
1382							
1383							
1384							
1385							

***N/A = Not applicable to viable *N. meningitidis* isolates**

UK NEQAS Microbiology Quality Assessment
Special survey for *Neisseria meningitidis* identification and typing
Date: 08.05.2012

Distribution No. 3212
Lab ID No:

NM30

Report **MLST sequence type ST** (as 32, 41, 11, 1, 2269, 275, 8881 etc.)

Report **MLST Clonal Complex** (as, 11, 5, 269, 41/44, 213 etc)

Part 3. Continued

Specimen number	MLST	MLST CC
1379		
1380		
1381		
1382		
1383		
1384		
1385		

Specimens	Extraction method used	Amplification method	Detection method
1379-1381			
1382-1385			

General comments:

Return results as soon as possible via the Website: www.ukneqasmicro.org.uk **no later than: 22 June 2012** (17.00 GMT).

11/05/12

UK National External Quality Assessment Service for Microbiology

These simulated specimens may contain virulent pathogenic organisms of any category other than hazard group 4

Safety Notes

- All EQA samples may contain fully virulent organisms other than those of hazard group 4
- These samples must be handled with the same degree of care as equivalent clinical samples and by the same appropriately qualified and supervised staff
- Safeguards should be included to protect at-risk members of staff
- Local and national safety guidelines and regulations must be followed
- Containment facilities used must be those appropriate to similar clinical samples. As with clinical samples it may be necessary to transfer organisms from containment level 2 to 3 during processing once preliminary tests suggest the presence of derogated category 3 organisms
- Follow the instructions for opening (below) carefully
- Inspect packages for evidence of breakage and leakage and discard by autoclaving if this is evident
- In the event of an accident involving exposure of staff contact UK NEQAS (++ 44 (0) 20 8905 9890) in normal working hours or the Colindale Duty Safety Officer (++ 44 (0) 870 084 2000) out of hours and the identity of the pathogens will be revealed

Special survey for *Neisseria meningitidis* identification and typing

Instructions for specimens 1379-1381 for culture, phenotypic/genotypic identification and susceptibility testing (MIC) (Parts 1 and 2)

1. The vials containing freeze-dried material should be opened in an exhaust protective cabinet. Gloves should be worn during reconstitution and subsequent handling of the vials. For safe removal of the plastic tear-off seals, please proceed as follows:-
2. With the arrow on the plastic flip top pointing away from you, carefully but deliberately pull the flip top up and away from you. When it reaches the far edge, pull downwards and to the right or to the left (depending on whether you are right or left-handed) until the seal separates; then still holding onto the plastic top, gently remove altogether and dispose into a sharps container.
3. Slowly remove the bung and add 1mL of nutrient broth to the vial and allow 1 minute to reconstitute. Treat the resulting suspension as the simulated specimen **using a drop from a Pasteur pipette or dipped swab as the inoculum** before spreading.
4. This distribution contains strains of *Neisseria meningitidis* for identification and typing.
5. Record only findings that you would normally include in your final report.

Instructions for specimens for Genotyping (Part 3)

Specimens **1379-1381** should be processed according to instructions above. After growth following incubation and identification please extract DNA and gentotype using your routine methods for isolates.

Specimens **1382-1385** contain horse serum spiked with heat killed meningococci.

1. Open the vial as instructed in 1 and 2 above.
2. Slowly remove the bung and add 1mL of molecular grade water to the vial and allow 15 minutes to reconstitute (may require agitation). Treat the resulting suspension as the simulated specimen and extract DNA and genotype using your routine methods for blood samples.
3. It is recommended that laboratories extract the sample immediately after reconstitution. However the reconstituted samples should be retained at +4°C until all testing is complete as samples may degrade if repeatedly freeze-thawed.
4. Record only findings that you would normally include in your final report.

11/05/12

UK NEQAS FOR MICROBIOLOGY
UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICES

Your laboratory is already registered for web-based access to UK NEQAS results and reports. Guidance notes are enclosed, and your attention is directed particularly to the section on Certification. The password is renotified below in anticipation that you will use the web based results service launched on our website on April 2nd 2007, via http://www.ukneqasmicro.org.uk. The web capture results service is rapid, accurate and overcomes the issues of failed fax transmissions, lost post and unidentified e-mail responses.
You are reminded that you are responsible for administering password disclosure, access and changing within your laboratory and if you have already changed your password, then the password displayed below will now be invalid.

Username: NM30 Password: que8056

Please note:

- The service can be accessed by more than one person or computer, using the same password.
- You may change the password if you wish (see notes below on validation rules and good practice); we recommend that you change it from the password above to something memorable.
- You **must** change the password if you believe it has been disclosed inappropriately or otherwise compromised.
- If you have registered several laboratory codes for this service and wish to use the same password for each, you will need to change their passwords accordingly.
- This renotification presumes that you have not changed your password; if you have done so and have forgotten it, we require a written email request from the Head of Department to reset the password to that shown above. Please contact the web service provider with the request at:

web@ukneqas.org.uk

Password change validation rules:

- Passwords are case-sensitive; any password character resembling an upper case 'I' is actually a lower case 'L'.
- Passwords must have at least 7 characters, of which at least 3 must be letters and at least 3 must be numbers.
- Passwords must not include any spaces or underscores.
- The numeric part of your laboratory code must not be included.
- No 3 adjacent characters (irrespective of case) can be the same or be in simple progression.
- the sequences "eqa", "neqas", "neq", "nqs" and "qc" are forbidden (irrespective of case).

Password change good practice:
The password gives access to your laboratory's UK NEQAS records. The information available on the web site only remains confidential if managed properly. Therefore, consider changing passwords periodically, and especially if compromise is suspected.

11/05/12

UK NEQAS for Microbiology
An International Quality Assessment Service for Microbiology

UK NEQAS for Microbiology PO Box 63003, NW9 1GH
Tel: + 44 (0)20 8905 9890 Fax: + 44 (0)20 8205 1488 E-mail: organiser@ukneqasmicro.org.uk web:http://www.ukneqasmicro.org.uk

Using the Site

Report & Results Selection page

After your lab code and password have been accepted, the first page displayed (see Figure 1) allows you to select various options for the schemes and distributions in which your laboratory participates and for which this service is available. It also allows download of additional information relating to the UK NEQAS organisation, individual laboratory, UK NEQAS centres, schemes and/or distributions. The buttons do the following:

- **Lab** allows you to access information that is directly targeted to your laboratory, for example, certificate of participation and record sheets.
- **Centre** allows you to access information specific to a particular UK NEQAS centre. schemes operated from that centre.
- **Scheme** will allow participants to view instruction sheets and other information about that scheme.
- **Report** will allow you to view your laboratory specific report.
- **Dist** will allow you to view the intended results and images of results obtained in UK NEQAS where available
- **Result** will take you to the page where you can enter your results for that scheme.
- **'Latest' Drop Down menu** allows you to choose which distribution number you require. Latest is the default setting and may not necessarily be the distribution you require.

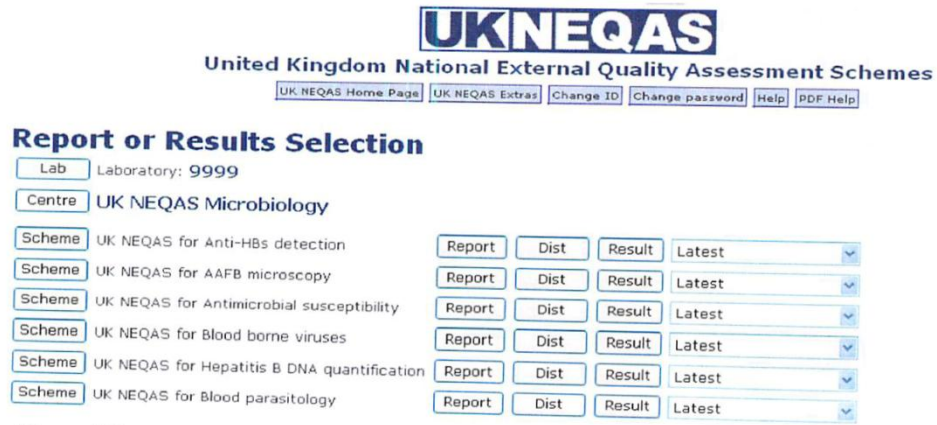

Figure. 1 Reports and results

If new information has been uploaded which you have not yet viewed the relevant button will be bordered red.

UK NEQAS for Microbiology
An International Quality Assessment Service for Microbiology

UK NEQAS for Microbiology PO Box 63003, NW9 1GH
Tel: + 44 (0)20 8905 9890 Fax: + 44 (0)20 8205 1488 E-mail: organiser@ukneqasmicro.org.uk web:http://www.ukneqasmicro.org.uk

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Participation The reports and results screen does not necessarily reflect the schemes to which you currently subscribe if your laboratory has recently registered for a new scheme. It will list every scheme and distribution that you have taken, but may not show any newly added schemes until the first dispatch date.

Results Entry

If the distribution is open you may enter results by pressing the RESULT button

Follow the on-screen instructions in order fill in your results

Intended Results

We aim to publish intended results the day after a scheme has closed

Reports

We aim to publish participant reports within 10 days of the closing date; for some scheme types this may be longer, especially where additional analysis is necessary.

Participants are sent an email when a report is available

After viewing a report do NOT use the BACK button to return to the reports and results screen. Instead, click on the small inverted triangle to the right of the BACK button and select Results and Reports Selection from the drop down menu.

Troubleshooting

Please report all problems with access or function (giving as much detail as possible) by email.

Send the email to organiser@ukneqasmicro.org.uk with **'lab <your lab number> web problem'** in the subject line.

Please do **NOT** use the results page message box for this purpose as it is only for problems related to methods and specimens.

Disclaimer

Because of the wide variety of hardware and software employed for internet connection and web browsing, UK NEQAS (Birmingham), the service provider, gives no warranty that the service will be accessible to all registered participants or that the advice given in this document will be suitable for all users of the service. We have tested the service on a number of different platforms and believe that the information provided will be helpful in most situations. If you are in any doubt, please seek help from your local IT personnel before accessing the service.

11/05/12

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Guidance for Microbiology Participants

General Overview

Description of the service

The UK NEQAS web-based results entry and individual reports access area provides an interface with a protected area of the web site and is accessed by entering your unique laboratory identifier code as the user name and entering the password sent to you. The site is used for:

- Laboratory specific result entry pages.
- Intended results and associated images (if applicable) following the close of the distribution.
- Individual report(s).
- Annual record sheets for each scheme detailing the results reported by you (available from July 2010).
- A certificate of your participation for the current year.
- Additional information relating to the UK NEQAS organisation, individual laboratory, UK NEQAS centres, schemes and/or distributions.

The information can be viewed, printed or downloaded as required.

New participants are only able to access information on distributions that have been sent after the date they registered.

How to find the service on the web

A link to the service is provided from the UK NEQAS for Microbiology Home page at:
http://www.ukneqasmicro.org.uk

Technical Requirements

Browsers

The site has been written for Microsoft Internet Explorer (IE) and some functionality, primarily some drop down and predictive menus, will not work in other browsers.

If you use FIREFOX or CHROME please do the following:

1. Download the latest version of the browser
2. Go to www.ietab.net and download the 'IE tab' software for the browser you wish to use
3. Go to our site www.ukneqasmicro.org.uk
4. For FIREFOX right click on the page and select 'view page in IE Tab' and carry on as normal
5. For CHROME click on the IE symbol to the right of the address bar and carry on as normal
6. Note: if you open links up in another tab it may revert to the browsers default rendering. If the IE symbol doesn't appear next to the address bar then you are not in IE mode. If this is the case repeat step 4 or 5 on the new tab.

UKNEQAS for Microbiology

CPA W.03201
Accredited EQA scheme
Reference No: 001

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Security Certificate

Currently, we are having some problems with our security certificate. This may lead to various warnings appearing on the screen when you try to login. We wish to assure participants that the website is safe and that it is a technical issue we hope to be resolving in the future.

For now please press what ever button – yes, accept, allow, ignore - allows you to enter you login credentials. If your local IT system does not allow you to do this please contact us.

Adobe

Participant reports are published in Adobe's Portable Document Format (PDF). In order to view, print or save a report you will require Adobe's READER or ACROBAT software installed on your computer.

READER is available as a free download from www.adobe.com. Follow the link on-screen or under the Downloads menu and follow the on-screen instructions.

Alternatively, click on Get Acrobat Reader which you will find at the bottom of the page when you log in.

If you already have READER installed please ensure that you have version 4.0 or higher.

You may need permission from your IT administrator to install this software.

User ID and Passwords

Your user ID will be your Lab ID number. Your initial password will have been sent to you separately.

You may change your password at any time by clicking on the Change Password button in the Report & Results Selection Page header and following the instructions on the page displayed.

The validation rules for passwords are included in the attached notification. Please read these notes and guidance carefully and **take note of your responsibility to administer the password**.

Multiple Lab IDs If you have more than one lab code, e.g. for different laboratory sections or instruments then you will have to log in separately for each. If you have already logged in under one set of credentials you can use the 'Change ID' option found in the top menu (see Figure 1 below)

Annex 5. An example of an individual laboratory results report, distribution 3212, 2012

UK National External Quality Assessment Service for Microbiology

Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 1 of 19
Dispatch Date : 08-May-2012	

Intended Result			Your Report	Your Score
Specimen 1379				
	Serogroup - phenotypic	A	A	1
	Serogroup - genotypic	A	Not tested	Not scored
	Genogroup	A	Not tested	Not scored
Specimen 1380				
	Serogroup - phenotypic	B	B	1
	Serogroup - genotypic	B	Not tested	Not scored
	Genogroup	B	Not tested	Not scored
Specimen 1381				
	Serogroup - phenotypic	C	C	1
	Serogroup - genotypic	C	Not tested	Not scored
	Genogroup	C	Not tested	Not scored
Specimen 1382				
	Result for genotyping	positive for N. meningitidis DNA	positive for N. meningitidis DNA	1
	Genogroup	B	B	1
Specimen 1383				
	Result for genotyping	positive for N. meningitidis DNA	positive for N. meningitidis DNA	1
	Genogroup	C	C	1
Specimen 1384				
	Result for genotyping	positive for N. meningitidis DNA	positive for N. meningitidis DNA	1
	Genogroup	B	B	1
Specimen 1385				
	Result for genotyping	negative for N. meningitidis DNA	negative for N. meningitidis DNA	1
	Genogroup	Not determined	Not determined	Not scored

Comments

Specimens were sent to 29 laboratories and results were reported by all participants. Phenotypic typing was undertaken by 28 laboratories with 17 laboratories genotyping the viable organisms.
Antimicrobial susceptibility testing was performed by 28 participants with 27 reporting for ciprofloxacin, 21 for ceftriaxone and cefotaxime, 28 for penicillin and 23 for rifampicin.
The antimicrobial susceptibility test results are presented in a revised format. In the table displaying the number, range and mode of results (page 3) for each organism/agent combination where there was no single mode for the reported results both values are displayed. On the subsequent pages MICs reported are presented by gradient manufacturer and agar medium used. If no manufacturer or agar type was entered on the web form no results are displayed.

Genotypic identification of the non culture specimens was undertaken and reported by 21 laboratories with 19 reporting genogroup results. False negative results were reported by three laboratories for specimen 1382. Six participants identified specimen 1385, which was negative for *N. meningitidis* DNA, as positive for *Streptococcus pneumoniae* with one laboratory characterising it as serotype 19A. One participant stated it was negative for both *Streptococcus pneumoniae* and *Haemophilus influenzae* . False positive results were reported by three laboratories for specimen 1385. All three laboratories used conventional PCR with gel electrophoresis, 2 extracted the samples using capture column and one used IntaGene extraction.

Please note In the histograms in this report your results are indicated by an arrow. If method data was not provided results are not displayed in the histograms. If your results are not indicated in the report and you wish to have the report updated please provide us with your method data.

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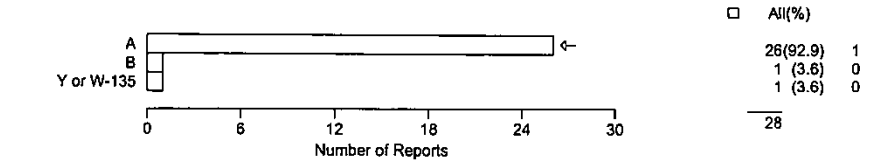
Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 2 of 19
Dispatch Date : 08-May-2012	

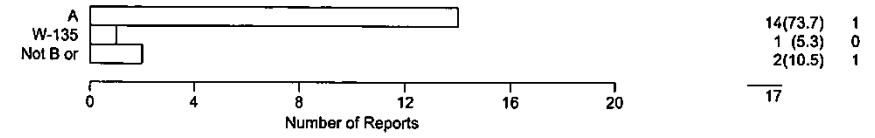
PART 1

Specimen : 1379 (Serogroup - phenotypic)



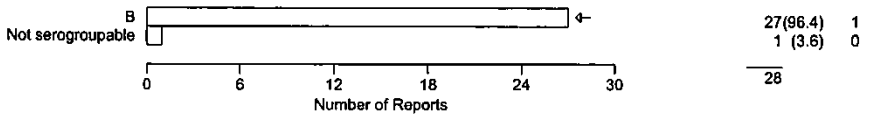
	All(%)	
A	26(92.9)	1
B	1 (3.6)	0
Y or W-135	1 (3.6)	0
	28	

Specimen : 1379 (Serogroup - genotypic)



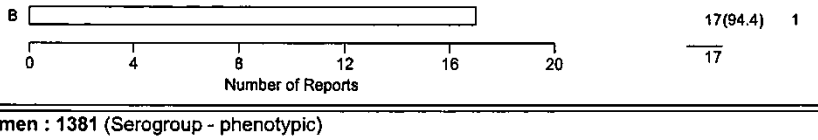
	All(%)	
A	14(73.7)	1
W-135	1 (5.3)	0
Not B or	2(10.5)	1
	17	

Specimen : 1380 (Serogroup - phenotypic)



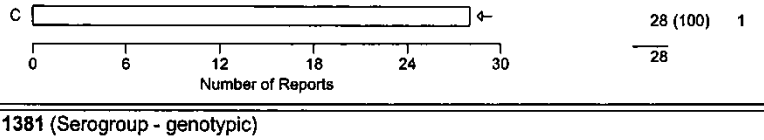
	All(%)	
B	27(96.4)	1
Not serogroupable	1 (3.6)	0
	28	

Specimen : 1380 (Serogroup - genotypic)



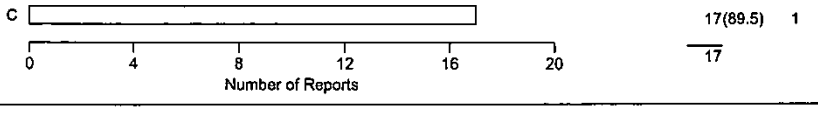
	All(%)	
B	17(94.4)	1
	17	

Specimen : 1381 (Serogroup - phenotypic)



	All(%)	
C	28 (100)	1
	28	

Specimen : 1381 (Serogroup - genotypic)



	All(%)	
C	17(89.5)	1
	17	

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Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 3 of 19
Dispatch Date : 08-May-2012	

PART 2

		MIC for ciprofloxacin	MIC for ceftriaxone	MIC for cefotaxime	MIC for penicillin	MIC for rifampicin
1379	n	27	21	21	28	23
	range	0.064 - 0.38	<0.002 - 0.016	<0.002 - 0.016	0.012 - 0.125	0.032 - 0.25
	mode	0.19	<0.002	0.002	0.032	0.125
1380	n	27	21	21	28	23
	range	<0.002 - 0.06	<0.002 - 0.016	0.002 - 0.03	0.016 - 0.125	16 - >32
	mode	0.004	<0.002	0.008,<0.016	0.032,0.064	>32
1381	n	27	21	21	28	23
	range	<0.002 - 0.03	<0.002 - 0.016	<0.002 - 0.016	0.016 - 0.64	16 - >32
	mode	0.002	<0.002	0.003	0.032	>32

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Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 4 of 19
Dispatch Date : 08-May-2012	

1379 - MIC for ciprofloxacin
Mode 0.19
Your reported MIC : 0.19

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.125	1
		0.19	1
	Mueller Hinton with heated horse blood	0.19	1
		0.25	1
		0.38	1
	Mueller Hinton with heated sheep blood	0.064	1
		0.094	1
		0.12	1
		0.125	3
		0.19	5
		0.25	1
		0.38	1
Liofilchem	Mueller Hinton with heated horse blood	0.064	1
		0.25	1
	Mueller Hinton with heated sheep blood	0.19	1
		0.25	1

1379 - MIC for ceftriaxone
Mode <0.002
Your reported MIC : <0.002

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.016	1
		<0.002	1
	Mueller Hinton with heated horse blood	<0.002	2
		<0.016	1
	Mueller Hinton with heated sheep blood	0.016	1
		<0.002	4
		<0.016	5
Liofilchem	Mueller Hinton with heated horse blood	<0.016	2

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Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 5 of 19
Dispatch Date : 08-May-2012	

1379 - MIC for cefotaxime
Mode 0.002
Your reported MIC : <0.002

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.016	1
		<0.002	1
	Mueller Hinton with heated horse blood	0.002	1
		<0.016	1
	Mueller Hinton with heated sheep blood	0.002	2
		0.003	1
		0.004	1
		0.006	1
		0.012	1
		0.016	1
		<0.016	2
Liofilchem	Mueller Hinton with heated horse blood	0.002	1
		<0.016	1
	Mueller Hinton with heated sheep blood	0.002	1
		0.003	1

1379 - MIC for penicillin
Mode 0.032
Your reported MIC : 0.032

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.032	1
		0.047	1
	Mueller Hinton with heated horse blood	0.032	1
		0.047	2
	Mueller Hinton with heated sheep blood	0.012	1
		0.016	2
		0.032	4
		0.047	3
		0.06	1
		0.064	2
Liofilchem	Mueller Hinton with heated horse blood	0.023	1
		0.032	1
	Mueller Hinton with heated sheep blood	0.032	1
		0.064	1

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Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 6 of 19
Dispatch Date : 08-May-2012	

1379 - MIC for rifampicin
Mode 0.125
Your reported MIC : 0.064

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.064	1
		0.094	1
	Mueller Hinton with heated horse blood	0.19	3
	Mueller Hinton with heated sheep blood	0.032	1
		0.094	1
		0.125	3
		0.19	2
		0.190	1
		0.25	1
Liofilchem	Mueller Hinton with heated horse blood	0.064	1
		0.125	1
	Mueller Hinton with heated sheep blood	0.19	1
		0.25	1

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Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 7 of 19
Dispatch Date : 08-May-2012	

1380 - MIC for ciprofloxacin
Mode 0.004
Your reported MIC : 0.004

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.003	1
		0.004	1
	Mueller Hinton with heated horse blood	0.003	1
		0.004	1
		0.006	1
	Mueller Hinton with heated sheep blood	0.002	1
		0.003	2
		0.004	4
		0.006	1
		0.008	1
		0.012	1
		0.03	1
		0.06	1
		<0.002	1
Liofilchem	Mueller Hinton with heated horse blood	0.002	1
		0.008	1
	Mueller Hinton with heated sheep blood	0.004	1
		0.008	1

1380 - MIC for ceftriaxone
Mode <0.002
Your reported MIC : <0.002

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.016	1
		<0.002	1
	Mueller Hinton with heated horse blood	<0.002	2
		<0.016	1
	Mueller Hinton with heated sheep blood	0.002	1
		0.016	1
		<0.002	3
		<0.016	5
Liofilchem	Mueller Hinton with heated horse blood	<0.016	2

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Neisseria meningitidis	Laboratory : **NM30**
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Dispatch Date : 08-May-2012	

1380 - MIC for cefotaxime
Mode 0.008, <0.016
Your reported MIC : 0.002

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.002	1
		0.016	1
	Mueller Hinton with heated horse blood	0.006	1
		<0.016	1
	Mueller Hinton with heated sheep blood	0.002	1
		0.003	1
		0.004	1
		0.006	1
		0.016	2
		0.03	1
		<0.016	2
Liofilchem	Mueller Hinton with heated horse blood	0.002	1
		<0.016	1
	Mueller Hinton with heated sheep blood	0.008	2

1380 - MIC for penicillin
Mode 0.032, 0.064
Your reported MIC : 0.064

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.032	1
		0.064	1
	Mueller Hinton with heated horse blood	0.064	2
		0.125	1
	Mueller Hinton with heated sheep blood	0.016	1
		0.023	2
		0.032	3
		0.047	2
		0.06	2
		0.094	1
		0.12	1
		0.125	1
Liofilchem	Mueller Hinton with heated horse blood	0.032	1
		0.064	1
	Mueller Hinton with heated sheep blood	0.032	1
		0.094	1

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Neisseria meningitidis	Laboratory : **NM30**
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Dispatch Date : 08-May-2012	

1380 - MIC for rifampicin
Mode >32
Your reported MIC : >32

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	32	1
		>32	1
	Mueller Hinton with heated horse blood	>32	3
	Mueller Hinton with heated sheep blood	16	1
		>32	8
Liofilchem	Mueller Hinton with heated horse blood	>32	2
	Mueller Hinton with heated sheep blood	32	1
		>=32	1

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Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 10 of 19
Dispatch Date : 08-May-2012	

1381 - MIC for ciprofloxacin
Mode 0.002
Your reported MIC : 0.004

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.002	1
		0.004	1
	Mueller Hinton with heated horse blood	0.002	1
		0.003	1
		0.004	1
	Mueller Hinton with heated sheep blood	0.002	3
		0.003	4
		0.004	2
		0.006	1
		0.03	1
		<0.002	2
Liofilchem	Mueller Hinton with heated horse blood	0.002	1
		0.003	1
	Mueller Hinton with heated sheep blood	0.002	1
		0.008	1

1381 - MIC for ceftriaxone
Mode <0.002
Your reported MIC : <0.002

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.016	1
		<0.002	1
	Mueller Hinton with heated horse blood	<0.002	2
		<0.016	1
	Mueller Hinton with heated sheep blood	0.016	1
		<0.002	4
		<0.016	5
Liofilchem	Mueller Hinton with heated horse blood	<0.016	2

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Neisseria meningitidis	Laboratory : **NM30**
Distribution : **3212**	Page 11 of 19
Dispatch Date : 08-May-2012	

1381 - MIC for cefotaxime
Mode 0.003
Your reported MIC : <0.002

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.016	1
		<0.002	1
	Mueller Hinton with heated horse blood	0.003	1
		<0.016	1
	Mueller Hinton with heated sheep blood	0.002	1
		0.003	3
		0.008	1
		0.016	2
		<0.016	2
Liofilchem	Mueller Hinton with heated horse blood	<0.002	1
		<0.016	1
	Mueller Hinton with heated sheep blood	0.004	2

1381 - MIC for penicillin
Mode 0.032
Your reported MIC : 0.032

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	0.032	2
	Mueller Hinton with heated horse blood	0.032	1
		0.064	2
	Mueller Hinton with heated sheep blood	0.016	3
		0.023	2
		0.03	1
		0.032	3
		0.047	1
		0.064	1
		0.12	1
		0.64	1
Liofilchem	Mueller Hinton with heated horse blood	0.023	1
		0.032	1
	Mueller Hinton with heated sheep blood	0.032	1
		0.047	1

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1381 - MIC for rifampicin
Mode >32

Your reported MIC : >32

Gradient MIC manufacturer	Plate agar medium	MIC	Count
AB Biodisk	ISO sensitest with horse blood and NAD	32	1
		>32	1
	Mueller Hinton with heated horse blood	>32	3
	Mueller Hinton with heated sheep blood	16	1
		>32	8
Liofilchem	Mueller Hinton with heated horse blood	>32	2
	Mueller Hinton with heated sheep blood	32	1
		>=32	1

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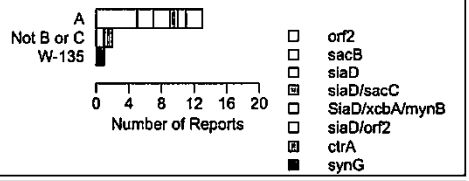
Specimen : 1379

Target gene(s) (ID)

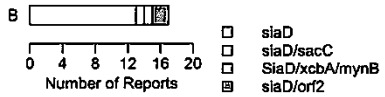
Target gene(s) (genogrp)



Specimen : 1380

Target gene(s) (ID)

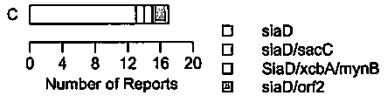
Target gene(s) (genogrp)



Specimen : 1381

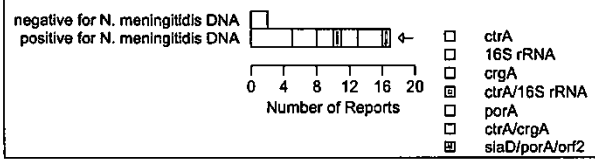
Target gene(s) (ID)

Target gene(s) (genogrp)

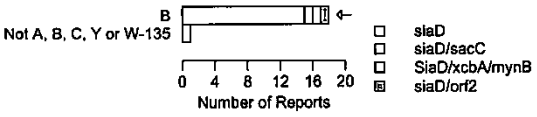


Specimen : 1382

Target gene(s) (ID)



Target gene(s) (genogrp)



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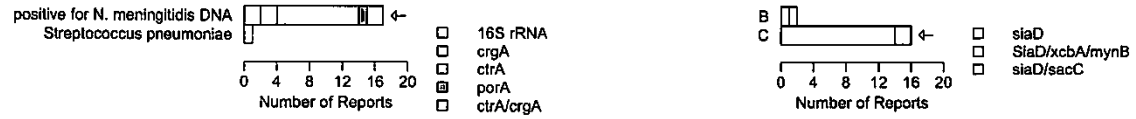
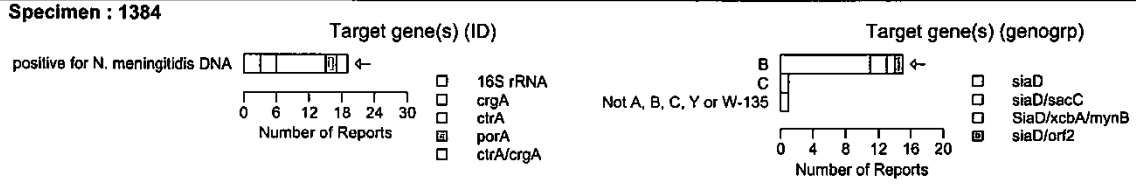
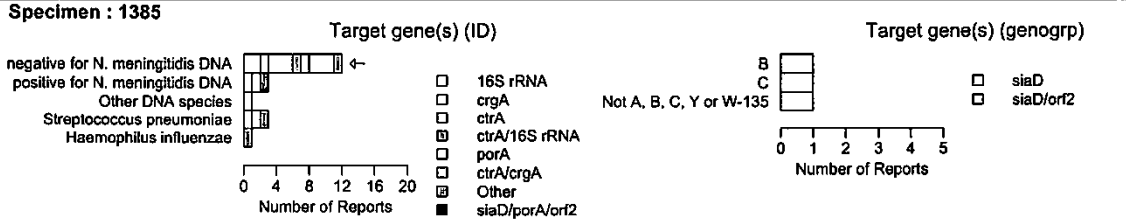
Specimen : 1383



Specimen : 1384



Specimen : 1385



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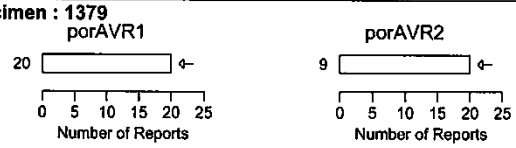
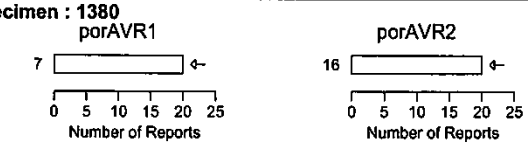
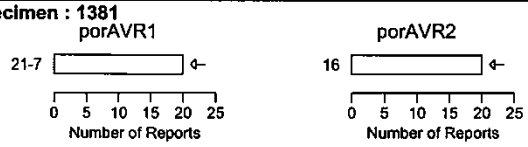
Specimen : 1379



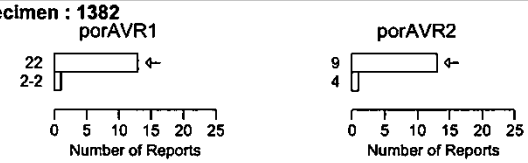
Specimen : 1380



Specimen : 1381



Specimen : 1382



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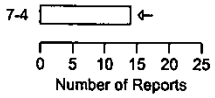
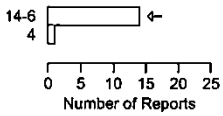
Specimen : 1383

porAVR1

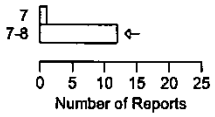


porAVR2

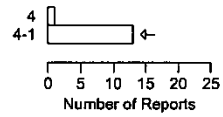


Specimen : 1384

porAVR1

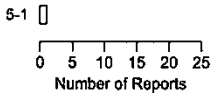


porAVR2

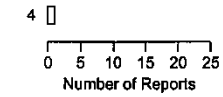


Specimen : 1385

porAVR1



porAVR2



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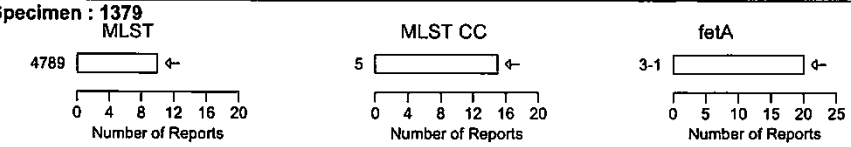
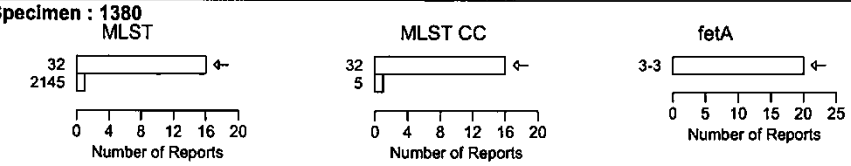
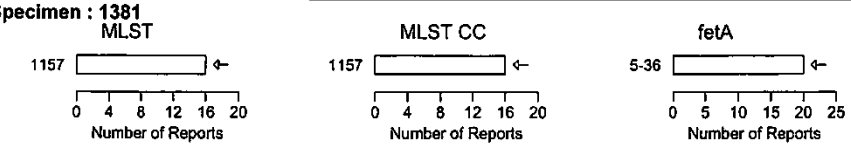
Specimen : 1379



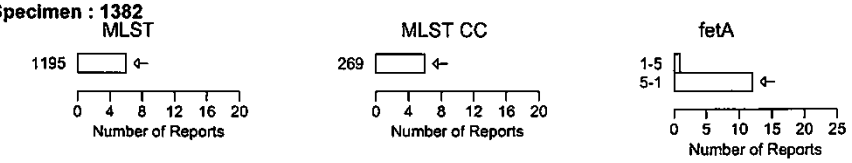
Specimen : 1380



Specimen : 1381



Specimen : 1382



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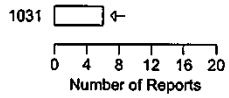
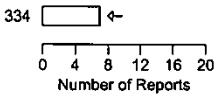
Specimen : 1383

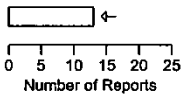
MLST



MLST CC

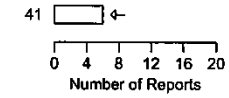


fetA

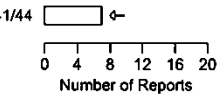


Specimen : 1384

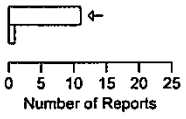
MLST



MLST CC



fetA



Specimen : 1385

MLST MLST CC fetA

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Specimen type		Extraction method used	Amplification method	Detection method	Combination
Organisms	ID	Salt precipitation	PCR - conventional	Sequencing	1
Organisms	ID	Boil	PCR - conventional	Gel electrophoresis	5
Organisms	ID	Boil	PCR - conventional	Sequencing	5
Organisms	ID	Magnetic bead	Real-time PCR	Real-time Taqman probes	2
Organisms	ID	Magnetic bead	Real-time PCR	Real-time fluorescence	1
Organisms	ID	Capture column (with centrifugation)	PCR - conventional	Gel electrophoresis	4
Organisms	ID	Capture column (with centrifugation)	Real-time PCR	Real-time Taqman probes	1
Organisms	ID	Other	PCR - conventional	Gel electrophoresis	2
Serum	ID	Magnetic bead	PCR - conventional	Gel electrophoresis	1
Serum	ID	Magnetic bead	Real-time PCR	Real-time Taqman probes	3
Serum	ID	Magnetic bead	Real-time PCR	Real-time fluorescence	1
Serum	ID	Capture column (with centrifugation)	PCR - conventional	Gel electrophoresis	7
Serum	ID	Capture column (with centrifugation)	PCR - conventional	Sequencing	1
Serum	ID	Capture column (with centrifugation)	Real-time PCR	Real-time Taqman probes	5
Serum	ID	Capture column (with centrifugation)	Real-time PCR	Real-time fluorescence	1
Serum	ID	Other	PCR - conventional	Gel electrophoresis	1
Serum	ID	Other	Real-time PCR	Real-time Taqman probes	1
Serum	ID	Capture column (no centrifugation)	Real-time PCR	Real-time Taqman probes	1

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Annex 6. MIC results analysis

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.

Example: If 0.016 was the submitted actual MIC, it was correlated to EUCAST 0.013 mg/L.

If 0.094 was submitted, it was equated to 0.12. Similarly, 0.003 was equated to EUCAST 0.004.

Sample 1379: reported MICs converted to EUCAST doubling dilutions

LabID	CIP	CRO	CTX	PEN	RIF
NM20A	0.25	NT	0.002	0.03	NT
NM21	0.12	NT	0.002	0.016	0.25
NM22	0.25	0.002	NT	0.12	0.25
NM23	0.25	<0.016	0.002	0.03	0.06
NM24	0.12	<0.002	0.004	0.06	0.12
NM26	0.25	<0.002	NT	0.03	0.25
NM27	0.25	NT	0.004	0.06	0.25
NM28	0.25	NT	0.002	0.03	0.25
NM29	0.25	NT	0.002	0.06	0.12
NM30	0.25	<0.002	<0.002	0.03	0.06
NM31	0.12	<0.002	0.002	0.03	0.12
NM32	0.5	<0.002	NT	0.06	0.25
NM33	NT	NT	NT	NT	NT
NM34A	0.25	<0.002	NT	0.03	0.12
NM35A	NT	NT	NT	0.06	NT
NM36	0.5	<0.002	0.002	0.06	0.25
NM37A	0.12	<0.016	0.016	0.03	0.12
NM38	0.12	0.016	0.016	0.006	0.12
NM39	0.06	<0.016	<0.016	0.03	0.12
NM40	0.25	<0.016	<0.016	0.06	NT
NM41	0.12	<0.002	0.004	0.06	0.12
NM42	0.25	NT	0.004	0.06	0.25
NM43	0.25	<0.002	0.004	0.03	0.25
NM45	0.12	0.016	0.016	0.016	0.12
NM47	0.25	<0.016	NT	0.06	0.12
NM48	0.06	<0.016	<0.016	0.016	0.03
NM51	0.12	<0.016	NT	0.06	NT
NM52	0.12	<0.002	0.008	0.06	NT
NM54	0.25	<0.016	<0.016	0.06	0.25
Total	**27**	**21**	**21**	**28**	**23**
Mode	**0.25**	**<0.002**	**0.002**	**0.06**	**0.12/0.25**

Sample 1379: frequency distribution of MICs to determine mode and range

EUCAST MIC	CIP	CRO	CTX	PEN	RIF
<0.016		8	4		
<0.002		10	1		
0.002		1	7		
0.004			5		
0.008			1		
0.016		2	3	3	
0.03				10	1
0.06	2			14	2
0.12	9			1	10
0.25	14				10
0.5	2				
1					
2					
4					
8					
16					
32					
>32					
Total	**27**	**21**	**21**	**28**	**23**
Mode	**0.25**	**<0.002**	**0.002**	**0.06**	**0.12/0.25**

Sample 1379: summary of MIC reports 2012 and comparison to 2011 mode

1379		Cipro	Ceftr[1]	Cefo[2]	Pen[3]	Rif[4]
	n	27	21	21	28	23
	range	0.06–0.5	<0.002-0.016	<0.002–0.016	0.016-0.12	0.03–0.25
	mode	**0.25**	**<0.002**	**0.002**	**0.06**	**0.12/0.25**
EUCAST interpretation						
		R	**S**	**S**	**S**	**S**
Mode exact match		**14/27**	**10/21**	**7/21**	**14/28**	**20/23**
		52%	**48%**	**33%**	**50%**	**87%**
mode+/-x1EUCAST diln.						
		25/27	**11/21**	**13/21**	**25/28**	**22/23**
		93%	**52%**	**62%**	**89%**	**97%**
Interpretation of all reports						
		R 100%	**S 100%**	**S 100%**	**S 96%, I 4%**	**S 100%**
2011 2nd EQA mode		**0.25**	**<0.002**	**0.002/0.004**	**0.06**	**0.12**
	n	**28**	**18**	**19**	**29**	**24**

[1] Ten labs used high-range strips, where ≤0.016 is lowest value, therefore unable to specify <0.002

[2] Seven labs used high-range strips, where ≤0.016 is lowest value, therefore unable to specify 0.002, and one lab reported 0.008 (just outside mode+/-x1diln.)

[3] 0.016 (three labs) and 0.12 (one lab)

[4] 0.03 reported by one lab. Bimodal 0.12–0.25

Sample 1380: reported MICs converted to EUCAST doubling dilutions

LabID	CIP	CRO	CTX	PEN	RIF
NM20A	0.004	NT	0.002	0.03	NT
NM21	0.004	NT	0.004	0.03	>32
NM22	0.008	0.004	NT	0.12	>32
NM23	0.008	<0.016	0.002	0.03	>32
NM24	0.008	<0.002	0.008	0.06	>32
NM26	0.004	<0.002	NT	0.06	>32
NM27	0.008	NT	0.008	0.06	>32
NM28	0.004	NT	0.008	0.03	32
NM29	0.004	NT	0.004	0.06	>32
NM30	0.004	<0.002	0.002	0.06	>32
NM31	0.004	<0.002	0.004	0.06	>32
NM32	0.03	<0.002	NT	0.12	>32
NM33	NT	NT	NT	NT	NT
NM34A	0.004	<0.002	NT	0.03	>32
NM35A	NT	NT	NT	0.12	NT
NM36	0.008	<0.002	0.008	0.06	>32
NM37A	0.016	<0.016	0.03	0.06	>32
NM38	0.004	0.016	0.016	0.03	32
NM39	0.002	<0.016	<0.016	0.06	>32
NM40	0.004	<0.016	<0.016	0.03	NT
NM41	0.004	<0.002	0.008	0.12	>32
NM42	0.008	NT	0.008	0.12	>32
NM43	0.004	<0.002	0.004	0.03	>32
NM45	0.008	0.016	0.016	0.016	16
NM47	0.004	<0.016	NT	0.06	>32
NM48	0.002	<0.016	<0.016	0.03	>32
NM51	0.06	<0.016	NT	0.12	NT
NM52	<0.002	0.002	0.016	0.12	NT
NM54	0.004	<0.016	<0.016	0.12	>32
Total	**27**	**21**	**21**	**28**	**23**
Mode	**0.004**	**<0.002**	**0.008**	**0.06**	**>32**

Sample 1380: frequency distribution of MICs to determine mode and range

EUCAST MIC	CIP	CRO	CTX	PEN	RIF
<0.016		8	4		
<0.002	1	10			
0.002	2		3		
0.004	14	1	4		
0.008	7		6		
0.016	1	2	3	1	
0.03	1		1	8	
0.06	1			11	
0.12				8	
0.25					
0.5					
1					
2					
4					
8					
16					1
32					2
>32					20
Total	**27**	**21**	**21**	**28**	**23**
Mode	**0.004**	**<0.002**	**0.008**	**0.06**	**>32**

Sample 1380: summary of MIC reports 2012 and comparison to 2009 mode

1380		**CIP**	**CRO**	**CTX**	**PEN**	**RIF**
	n	27	21	21	28	23
	range	<0.002-0.06	<0.002–0.016	0.002–0.03	0.016–0.12	32->32
	mode	**0.004**	**<0.002**	**0.008**	**0.06**	**>32**
EUCAST interpretation		**S**	**S**	**S**	**S**	**S**
Mode exact match		**14/27**	**10/21**	**6/21**	**11/28**	**20/23**
		52%	**48%**	**29%**	**39%**	**87%**
mode+/-x1EUCAST diln.		**23/27**[1]	**10/21**[2]	**13/21**[3]	**27/28**[4]	**22/23**
		85%[1]	**48%**[2]	**62%**[3]	**96%**[4]	**96%**
Interpretation of all reports		**S 100%**	**S 100%**	**S 100%**	**S 71%, I 29%**	**R 100%**
2009 1st EQA mode		**0.004**	**0.002**	**0.008**	**0.016**	**32**
	n	**28**	**18**	**19**	**29**	**24**

[1] *One lab <0.002 just outside mode+/-x1 diln; also, one lab 0.012, one lab 0.06, and one lab 0.03.*

[2] *Eight labs used high-range strips where ≤0.016 is lowest value, therefore unable to specify <0.002, 2 labs reporting 0.016. One lab reported 0.004.*

[3] *Seven labs used high-range strips where ≤0.016 is lowest value hence bimodal, therefore unable to specify 0.008. One lab reported 0.03.*

[4] *One lab reported 0.016. Eight labs reported 0.12, designated EUCAST intermediate susceptibility (I).*

Sample 1381: reported MICs converted to EUCAST doubling dilutions

LabID	CIP	CRO	CTX	PEN	RIF
NM20A	0.004	NT	0.002	0.016	NT
NM21	0.002	NT	0.004	0.016	>32
NM22	0.004	0.002	NT	0.12	>32
NM23	0.004	<0.016	<0.002	0.03	>32
NM24	0.004	<0.002	0.004	0.03	>32
NM26	0.004	<0.002	NT	0.03	>32
NM27	0.004	NT	0.004	0.06	>32
NM28	0.002	NT	0.004	0.03	32

LabID	CIP	CRO	CTX	PEN	RIF
NM29	0.004	NT	0.002	0.06	>32
NM30	0.004	<0.002	<0.002	0.03	>32
NM31	0.004	<0.002	0.004	0.03	>32
NM32	0.004	<0.002	NT	0.06	>32
NM33	NT	NT	NT	NT	NT
NM34A	0.002	<0.002	NT	0.03	>32
NM35A	NT	NT	NT	0.06	NT
NM36	0.004	<0.002	0.004	0.06	>32
NM37A	0.008	<0.016	0.016	0.03	>32
NM38	0.002	0.016	0.016	0.03	32
NM39	0.002	<0.016	<0.016	0.03	>32
NM40	0.004	<0.016	<0.016	0.03	NT
NM41	0.002	<0.002	0.004	0.03	>32
NM42	0.008	NT	0.004	0.06	>32
NM43	0.004	<0.002	0.004	0.03	>32
NM45	0.004	0.016	0.016	0.016	16
NM47	0.002	<0.016	NT	0.03	>32
NM48	<0.002	<0.016	<0.016	0.03	>32
NM51	0.03	<0.016	NT	0.12	NT
NM52	<0.002	<0.002	0.008	0.06	NT
NM54	0.002	<0.016	<0.016	0.06	>32
Total	**27**	**21**	**21**	**28**	**23**
Mode	**0.004**	**<0.002**	**0.004**	**0.03**	**>32**

Sample 1381: frequency distribution of MICs to determine mode and range

EUCAST MIC	CIP	CRO	CTX	PEN	RIF
<0.016		8	4		
<0.002	2	10	2		
0.002	8	1	2		
0.004	14		9		
0.008	2		1		
0.016		2	3	3	
0.03	1			15	
0.06				8	
0.12				2	
0.25					
0.5					
1					
2					
4					
8					
16					1
32					2
>32					20
Total	**27**	**21**	**21**	**28**	**23**
Mode	**0.004**	**<0.002**	**0.004**	**0.03**	**>32**

Sample 1381: summary of MIC reports 2012

1381		CIP	CRO	CTX	PEN	RIF
	n	27	21	21	28	23
	range	<0.002-0.03	<0.002-0.016	<0.002–0.016	0.016–0.12	16->32
	mode	**0.004**	**<0.002**	**0.004**	**0.03**	**>32**
EUCAST interpretation						
		S	**S**	**S**	**S**	**R**
Mode exact match		**14/27**	**10/21**	**9/21**	**15/28**	**20/23**
		52%	**48%**	**43%**	**54%**	**87%**
mode+/-x1EUCASTdiln.						
		24/27[1]	**11/21**[2]	**12/21**[3]	**26/28**[4]	**22/23**
		89%[1]	**52%**[2]	**57%**[3]	**93%**[4]	**96%**
Interpretation of all reports						
		S 96%, I 4%	**S 100%**	**S 100%**	**S 93%, I 7%**	**R 100%**

[1] *One lab reported 0.03, intermediate susceptibility*

[2] *Ten labs used high-range strips where ≤0.016 is lowest value, therefore unable to specify 0.002: two labs reported 0.016*

[3] *Seven (or six) labs used high-range strips. Three labs reported 0.016*

[4] *Two labs reported 0.12*

Annex 7. Analysis of *aroE* sequence interpretation for sample 1380

ST	abcZ	adk	aroE	fumC	gdh	pdhC	pgm	clonal complex
32	4	10	5	4	6	3	8	ST-32 complex/ET-5 complex
2145	4	10	4	4	6	3	8	ST-32 complex/ET-5 complex

Annex 8. Sample 1384 *porA* VR1 review

Fourteen laboratories reported *porA* VR1 7-8 but one laboratory reported *porA* VR1 7. Aligning the amino acid sequences as shown below suggests that either the laboratory edited poor sequence data or there was a technical mix-up. The latter is more likely as to delete 2 amino acids in one place and 3 in another (a total of 15 nucleotides) is most unlikely.

*porA*VR1 amino acid sequence

7–8	AQAANGGAGASGQVKVTKVTKVTKA
7	AQAANGGASGQVKVTKVTKA
7	AQAANGGA___SGQVKVTKVTK___A

The introduction of spaces to accommodate the additional amino acids in variant 7-8 to align with variant 7 suggestive of more than editing.

aroE 4 and aroE 5 contig

TATCGGTTTGACCAACGACATCACGCAGGTCAAAAATATTGCCATCGAGGGCAAAACCATTTTGCTTTTGGGCGCAGGCGGCG
CGGTGCGCGGCGTGATTCCKGTTTTGAAAGAACACCGYCCTGCCCGTATCGTCATTGCCAACCGTACCCGCGCCAAAGCCGAG
GAATTGGCGCAGCTTTTCGGCATTGAAGCCGTCCCGATGGCGGAYGTGAACGGCGGTTTTGATATCATCATCAACGGCACGT
CSGGCGGTCTAAACGGTCAGATTCCCGATATTCCGCCCGATATTTTTCAAAACTGCGCGCTTGCCTACGATATGGTGTACGGC
TGCGCGGCAAAACCGTTTTTAGATTTTGCACGACAATCGGGTGCGAAAAAAACTGCCGACGGACTGGGTATGCTAGTCGGTC
AAGCGGCGGCTTCCTACGCCCTCTGGCGCGGATTTACGCCCGATATCCGCCCCGTTATCGAATACATGAAAGCCMTR

	anomalous base positions					
	103	121	211	250	488	490
aroE 5	T	T	C	G	C	A
aroE 4	G	C	T	C	A	G

Conclusion: suggests that laboratory NM37 reporting ST 2145 may have edited poor sequence.