TECHNICAL REPORT

External quality assurance scheme for *Haemophilus influenzae*

2011

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2011

As part of the IBD-Labnet surveillance network

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Dr Adoración Navarro Torné and produced by Dr Mary Slack (Health Protection Agency, London, UK) on behalf of the IBD-Labnet consortium participants (referring to specific contract ECD.2273).

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Abbreviations

AMP	Ampicillin
BLNAR	β-lactamase-negative ampicillin-resistant strain
BLPACR	β-lactamase-positive amoxicillin/clavulanate-resistant strain
CAT	Chloramphenicol acetyl transferase
CEC	Cefaclor
CIP	Ciprofloxacin
CHLOR	Chloramphenicol
CLSI	Clinical and Laboratory Standards Institute
COAM	Co-amoxyclav
CRO	Ceftriaxone
CTX	Cefotaxime
CXM	Cefuroxime
EUCAST	European Committee on Antimicrobial Susceptibility Testing
Hinc	Non-capsulated *Haemophilus influenzae*
Hib	*H. influenzae* type b
Hif	*H. influenzae* serotype f
HPA	Health Protection Agency, UK
HRU	Haemophilus Reference Unit
I	Intermediate
MIC	Minimum inhibitory concentration
NE	Not evaluated
ODC	Ornithine decarboxylase
OMP	Outer membrane protein
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
QMS	Quality management systems
R	Resistant
RIF	Rifampicin
S	Susceptible
SDRU	Streptococcus and Diphtheria Reference Unit (UK)
SXT	Trimethoprim-sulphamethoxazole
TET	Tetracycline
TRIM	Trimethoprim

Executive summary

Haemophilus influenzae is a common cause of respiratory tract infections. Most strains of *H. influenzae* are opportunistic pathogens and rarely cause invasive disease unless other factors concur (e.g. viral infections, immunological deficits). Despite the effective prevention of invasive *H. influenzae* serotype b (Hib) infections by the use of conjugated Hib vaccine, infections caused by other capsulated serotypes and non-capsulated strains still occur and are associated with significant morbidity and mortality. Surveillance of *H. influenzae* continues to be of importance, not only to establish the types of *H. influenzae* causing invasive disease but also to monitor the long-term effectiveness of the Hib immunisation programme. An integrated surveillance for this pathogen entails both epidemiological and laboratory surveillance.

ECDC promotes the performance of external quality assessment (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 laboratory proficiency testing provides information about the accuracy of different characterisation and typing methods as well as antimicrobial susceptibility testing, and the sensitivity of the methods in place to detect a certain pathogen or novel resistance patterns.

In February 2011, a collection of six strains of *Haemophilus spp.* [three non-capsulated *H. influenzae*, one *H. influenzae* serotype b (Hib), one *H. influenzae* serotype f (Hif) and one *H. parainfluenzae*] and two simulated samples of cerebrospinal fluid (CSF) (one containing *H. influenzae*, one containing *S. pneumoniae*) was sent to 30 participating reference laboratories in the IBD-Labnet surveillance network for quality assessment testing. The laboratories were asked to perform standard laboratory protocols for the methods usually used by the laboratory for: species identification, biotyping and serotyping by serological methods and/or PCR. Antimicrobial susceptibility testing and β-lactamase testing was also requested for those laboratories that perform antimicrobial susceptibility testing of the isolates on a routine basis.

The results of this EQA distribution have shown that European Haemophilus reference laboratories differ in the level of characterisation of strains, ranging from simple speciation to full identification and typing. All but two laboratories routinely phenotypically serotype isolates. Fifteen laboratories (52%) performed PCR-based capsular genotyping; 23 laboratories (79%) reported antimicrobial susceptibility testing results.

The EQA scheme identified some problems with speciation of strains, slide agglutination for the serotyping of strains and antimicrobial susceptibility testing. The identification of *H. influenzae* was very good, with only one laboratory erroneously identifying one isolate of *H. influenzae* as *H. ducreyi.* The identification of *H. parainfluenzae* was more problematic, with 11 laboratories (38%) misidentifying this organism. The incorrect identifications included *Aggregatibacter segnis* (four laboratories), *H. paraphrophilus* (three laboratories), *H. aphrophilus* (two laboratories), *H. ducreyi* (one laboratory) and *'not H. influenzae'* (one laboratory).

Conventional serotyping is prone to errors of interpretation because of observer error, cross-reactions and auto-agglutination. These problems can be resolved by using a PCR-based capsular genotyping scheme.

The results of the antimicrobial susceptibility testing indicate that almost all reference laboratories routinely test for β-lactamase production in strains of *Haemophilus influenzae* and the results are excellent. Twenty-two laboratories (76%) returned antimicrobial susceptibility testing results. The detection of β-lactamase-negative ampicillin-resistance (BLNAR) proved challenging, with 12 (52%) and five (22%) laboratories reporting strains number 0264 and 0267 as BLNAR, respectively. Low BLNAR strains can have an ampicillin MIC at or around the breakpoint for this agent, and disc diffusions tests or even MIC determinations may fail to identify such strains. The only definitive way of identifying such strains is by partial sequencing of the *ftsI* gene, which is not routinely undertaken by the majority of reference laboratories.

Eight laboratories used the EUCAST criteria for antimicrobial susceptibility testing while 13 are still using CLSI guidelines. This makes the comparison of results difficult. It is recommended that all European reference laboratories move to using EUCAST guidelines as soon as possible.

Two simulated CSF samples were included in the quality assurance panel to assess methods used for the non-culture detection of *Haemophilus influenzae*. Eighteen laboratories (62%) submitted results for this exercise and all were correct. One of the samples contained *S. pneumoniae* DNA and any of the following results were regarded as correct – '*S. pneumoniae*', 'not *H. influenzae*', 'negative' – since not all of the European Haemophilus reference laboratories also act as pneumococcal reference laboratories. With such a small number of samples it was not possible to evaluate whether participants were reporting results appropriate to the gene targets that they were using for their PCRs. Some gene targets are species-specific whereas others are designed for typing of strains of a particular species.

Introduction

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

External quality assessment (EQA) is part of quality management systems (QMS) and evaluates performance of laboratories, by an outside agency, on material that is supplied specifically for the purpose. ECDC's disease-specific networks organise a series of EQAs for EU/EEA countries. In some specific networks, non-EU/EEA countries are also involved in the EQA activities organised by ECDC, although at their own costs. The aim of the EQA is to identify needs for 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 the:

- 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.

Haemophilus influenzae is a common cause of serious disease in children worldwide. Pneumonia and meningitis are the most frequent manifestations. However, it can also be responsible for epiglottitis and infections of bones, joints, skin, soft-tissues and other body sites. Invasive bacterial diseases are an important cause of morbidity and mortality in neonates and children worldwide. Highly safe and effective protein-polysaccharide conjugate Hib vaccines have been available for almost 20 years and have completely changed the epidemiology of invasive *H. influenzae* infections. Nevertheless, the availability of vaccines requires a more accurate surveillance system. Completeness and accuracy become key objectives of surveillance when vaccines are introduced and the incidence of the infection approaches low levels, as it is in invasive diseases due to *H. influenzae*. Not only epidemiological surveillance but also laboratory data, especially serotyping are needed to ensure optimal European surveillance for *H. influenzae*.

The European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) was a successful dedicated surveillance network for the surveillance of invasive diseases caused by *Neisseria meningitidis* and *Haemophilus influenzae*. The network had epidemiological and laboratory components. The epidemiological activities focused on the collection and analysis of data on *N. meningitidis* and *H. influenzae* cases, and the evaluation of the impact that vaccination programmes using conjugate vaccines have on the epidemiology of meningococcal disease. The laboratory activities focused on EQA and were aimed at strengthening the laboratory capacity in Member States for accurately characterising the isolates of *N. meningitidis* and *H. influenzae*. EU-IBIS was coordinated by the Health Protection Agency (HPA) in London, United Kingdom from 1999 to 2006. Since October 2007, the coordination of the activities of EU-IBIS has been integrated into the activities of ECDC and the epidemiological and the laboratory data collected by the EU-IBIS network have been transferred to ECDC.

The implementation of laboratory surveillance activities, namely the External Quality Assurance (EQA) activities and training, have been outsourced by the framework contract No ECDC/08/008 to a consortium of European experts (the European Monitoring Group on Meningococci – EMGM – and some other experts in *H. influenzae* and *N. meningitidis*), coordinated by Prof Dr Matthias Frosch, University of Würzburg, Germany.

The specific objectives of this EQA exercise are:

- further harmonisation of molecular typing of *H. influenzae*;
- further harmonisation of methods for antimicrobial susceptibility testing of *H. influenzae;*
- training and dissemination of methods for the laboratory surveillance of invasive bacterial infections;
- assisting the countries in capacity building, when required;
- supporting ECDC in linking laboratory surveillance data and epidemiological data.

[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

1 Material and methods

The objectives of this exercise were:

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

1.1 Study design

The design of the project allowed individual reference laboratories to test the material using their routinely available techniques in order to complete some or all of the requested criteria (Table 1) in the allocated time period.

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

The EQA distribution used the availability of the large collection of *H. influenzae* isolates and expert knowledge of the Health Protection Agency's (HPA) Haemophilus Reference Unit (HRU, Microbiology Services Division, HPA Colindale, London) together with the expert knowledge of Dr Vivienne James (UK NEQAS for Microbiology) and facilities in the External Quality Assurance Department (eQAD), HPA Colindale, London.

UK NEQAS for Microbiology undertake several International EQA schemes for other organisms that also require freeze-drying, distribution, results analysis and web-based reporting. The samples for the EQA scheme were selected by the HPA by agreement of the University of Würzburg, as coordinator of the IBD-Labnet project.

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

Table 1. Tests requested from the participating laboratories

Procedure	Tests requested	
	Bacterial isolates	Non-culture samples (simulated CSF)
Phenotypic identification	Species	
	Serotype	
	Biotype	
	Antimicrobial susceptibility testing	
	β-lactamase production	
Genotypic identification	Species	Detection of *H. influenzae*
	Capsule type	

Participants were strongly encouraged to report their results via the internet into a specially designed web-based report form on the UK NEQAS website (www.ukneqasmicro.org.uk). Each laboratory was given a unique username and password for secure reporting of their results.

1.2 Participants

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

All participants were contacted prior to the EQA distribution to confirm the address and contact details for despatch of the potentially hazardous material. It was envisaged that the reference laboratories would wish to store the viable cultures and retain any unused material for their own quality processes. It was hoped that the distribution of the well-characterised material would become a resource within and between the reference laboratories.

1.3 Timelines

The timelines for this EQA distribution are summarised in Table 2.

Table 2. Timelines for the EQA exercise

Event	Dates
Selection of EQA strains	01 December 2010
Assessment of material	22 December 2010
Building participants list	January 2011
Transfer of material to eQAD NEQAS	05 January 2011 to 10 February 2011
Freeze-dry panel (eQAD NEQAS)	12 January 2011 (simulated CSF samples: 10 February 2011)

Event	Dates
Pre-despatch checks (HRU and eQAD NEQAS)	13 January 2011 (non-culture samples tested 09.02.2011 before aliquoting by eQAD)
Distribution of EQAC panel UK NEQAS EQA Distribution 2802	14 February 2011
Reference lab testing	17 February 2011
Final return of results	30 March 2011
Analysis and collation of consensus results	April 2011
Producing reports	June 2011
Consensus summary	April 2011
Interim report at EMGM meeting, Ljubljana, Slovenia	May 2011
Individual results released on UKNEQAS website at https://results.uknegas.org.uk	July 2011

1.4 The EQA panel material

The EQA panel comprised six viable bacterial isolates (to test participating laboratories' abilities to identify and characterise live cultures) plus two non-viable simulated CSF samples (to test their ability to detect *H. influenzae* in clinical specimens using non-culture detection methods).

1.4.1 Bacterial isolates

Five viable isolates of *H. influenzae* were selected for the panel. These were selected to be representative of the major disease-causing serotypes (Hib, Hif and non-capsulated *H. influenzae*), to include strains demonstrating both β-lactamase production and β-lactamase-negative ampicillin resistance (BLNAR), and to demonstrate a range of MICs to other commonly used antimicrobials. The sixth isolate was a strain of *H. parainfluenzae*. This was included to test identification methods for *Haemophilus spp*. Further details on each strain are included in the Results section.

The isolates were selected and pre-screened by staff at the HPA's Haemophilus Reference Unit (HRU) and Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL). They were then grown up, aliquoted, freeze-dried and distributed at ambient temperature by UK NEQAS for Microbiology (eQAD NEQAS). The samples were accompanied by instructions for their revival.

1.4.2 Non-culture simulated meningitis samples

The two simulated CSF (non-culture) samples for PCR were prepared from heat-killed suspensions of isolates obtained from the UK National Collection of Type Cultures (NCTC). One sample contained *Haemophilus influenzae* type b DNA. The other contained *Streptococcus pneumoniae* DNA. (This would act as a negative control, but would also allow laboratories capable of determining its identity to report this information.)

Stock solutions of the bacterial cultures were prepared containing ≈ $2x10^8$ cfu/ml. The cultures were killed by heating to 100 °C for 10 minutes and then diluted 1/100 in simulated CSF solution. The simulated CSF contained 6% sucrose and 1.1% bovine serum albumin. These simulated CSF samples were also distributed by UK NEQAS for Microbiology at ambient temperature, with instructions to handle them in the same way as clinical specimens.

2 Results

The strains were processed as requested and the results were returned to UK NEQAS by 29 laboratories. One laboratory reported their results after the deadline for submission of results, because the laboratory was being reorganised during the distribution period and did not receive the specimens in time for testing. Because of these extenuating circumstances, their results were included in the data analysis.

A summary of consensus results was released to participants via the UK NEQAS for Microbiology website in April 2011. A semi-automated analysis of results from all participants was subsequently generated by UK NEQAS for Microbiology and HRU. This was released to all participants via the UK NEQAS for Microbiology website in July 2011. Each participant received a customised report containing an analysis of their own results plus a summary of the overall results from all participants. An example of this report is included in Annex 3. The summary of overall results contained in Annex 3 is intended to complement the analysis of data in the following sections. The participation of each laboratory in the various parts of the EQA procedure is shown in Table 3. It must be noted that each laboratory did not necessarily submit a result for all samples for a given test. Hence, the total participants for a given test varies by sample (see Table 5).

Table 3. Summary of tests for which each laboratory submitted results[a]

Laboratory identification	Viable isolates							Non-culture detection
	Phenotypic identification					Genotypic identification		
	Species ID	Serotype	Biotype	Antimicrobial susceptibility	β-lactamase production	Species ID	Capsule type	*H. influenzae* detection
NM02	+	+	+	+	+		+	+
NM09	+	+	+		+		+	
NM10	+	+			+	+	+	+
NM15	+	+	+	+	+			
NM16	+	+	+	+	+	+	+	+
NM17	+	+	+		+	+		+
NM20A	+	+	+	+	+	+	+	+
NM23	+	+	+	+	+	+	+	+
NM25	+	+			+		+	+
NM26	+	+	+	+	+	+	+	+
NM27	+	+	+	+	+	+	+	+
NM28	+	+	+	+	+		+	
NM29	+	+	+	+		+	+	+
NM32A	+		+	+	+	+	+	+
NM33A	+	+				+	+	+
NM34A	+	+	+	+	+	+	+	+
NM35A	+	+	+		+			
NM36	+	+		+	+			
NM37A	+	+		+	+	+	+	+
NM38A	+	+		+	+			
NM39	+	+	+	+	+			
NM40	+	+		+	+	+	+	
NM41	+	+	+	+	+	+	+	+
NM47	+	+	+	+	+	+	+	+
NM51	+	+	+	+	+			
NM52	+	+		+	+			
NM53	+	+	+	+	+		+	+
NM54	+	+		+	+			
NM55	+	+	+	+	+			+
Total	**29**	**28**	**20**	**23**	**27**	**15**	**19**	**18**

[a] *Laboratories did not necessarily submit a result for all samples for a given test.*

2.1 Part 1: Characterisation of viable isolates

All participants confirmed that the six bacterial isolates were viable following the revival procedure. Not all methods (tests) were performed on the isolates by all laboratories. A summary of the number of laboratories reporting results per method is shown in Table 3.

The intended results for Part 1 of the analysis are shown in Table 4. In the case of the genotypic species determination of sample 0263, two results ('*H. parainfluenzae*' or 'not *H. influenzae*') were deemed acceptable, since most laboratories employ genotypic species determination simply to decide whether or not an isolate is *H. influenzae*.

Table 5 shows the ratio of laboratories who successfully reported the intended result for each test. It also lists the results that did not match the intended result. In some cases these were incorrect results (e.g. phenotypic species identification of sample 0263). In others they were non-standard results which were consistent with the intended result, but were incomplete (e.g. 'Not Hib' or 'Not Hib, Hic or Hid' for phenotypic serotyping of sample 0265).

In the case of sample 0263 (*H. parainfluenzae* isolate), the phenotypic serotyping and genotypic capsule typing tests were not appropriate. Unfortunately, the web reporting form did not contain the option to select 'Not applicable'. Hence, participants may have declined to submit a result, or selected the response 'NE' (not evaluated) on the reporting form for these individual tests as a statement that this test was not applicable, but this could not be determined.

In the case of biotyping of sample 0263, the web reporting form did not explicitly ask the participants to select whether they had interpreted their results according to the scoring system for *H. influenzae* or *H. parainfluenzae* (shown in Table 8). The correct biochemical results would be interpreted as biotype V according to the *H. parainfluenzae* scheme, but biotype VIII if erroneously scored according to the *H. influenzae* scheme.

The percentage of participants reporting the intended result for each test is shown in Figures 1 to 5. In all tests for Part 1 of the study, the consensus of the submitted results matched the intended result. The percentage match varied between 62% and 100%. A detailed description of the results broken down by test is given below.

Table 4. Intended results for Part 1: Characterisation of viable isolates

EQA sample	Phenotypic species ID	Phenotypic serotype	Biotype	Genotypic species ID	Genotypic capsule type
0262	*H. influenzae*	Hinc	IV	*H. influenzae*	Hinc
0263	*H. parainfluenzae*	NA	V[a]	*H. parainfluenzae* or Not *H. influenzae*[b]	NA
0264	*H. influenzae*	Hinc	V	*H. influenzae*	Hinc
0265	*H. influenzae*	Hif	I	*H. influenzae*	Hif
0266	*H. influenzae*	Hib	IV	*H. influenzae*	Hib
0267	*H. influenzae*	Hinc	III	*H. influenzae*	Hinc

Abbreviations: ID, identification; Hinc, non-capsulated Haemophilus influenzae*; Hib*, H. influenzae *type b; Hif*, H. influenzae *type f; NA, not applicable.*

[a] *Biotype V according to the* H. parainfluenzae *scheme. If scored according to the* H. influenzae *biotyping scheme, the erroneous result of VIII would be generated.*

[b] *Because many laboratories perform genotypic testing to determine only whether an isolate is* H. influenzae *or not, a result of 'not* H. influenzae' *was deemed acceptable for this test.*

Table 5. Results for Part 1: Characterisation of viable isolates

Sample number	Intended result Phenotypic species identification	Ratio of labs reporting the intended result (%)	Results not matching intended result (frequency)
0262	*H. influenzae*	29/29 (100%)	NA
0263	*H. parainfluenzae*	18/29 (62%)	*H.ducreyi* (1) *H. paraphrophilus* (3) *H. aphrophilus* (2) *A. segnis* (4) Not *H. influenzae* (1)
0264	*H. influenzae*	28/29 (97%)	*H. ducreyi* (1)
0265	*H. influenzae*	29/29 (100%)	NA
0266	*H. influenzae*	29/29 (100%)	NA
0267	*H. influenzae*	29/29 (100%)	NA

Sample number	Intended result Phenotypic species identification	Ratio of labs reporting the intended result (%)	Results not matching intended result (frequency)
Phenotypic serotyping			
0262	Hinc	18/27[a] (67%)	Hia (1) Hib (1) Hid (5) Not Hib, Hic or Hid (1) Non-specific agglutination (1)
0263	NA	0/5 (NA)[b]	Hinc (3) Autoagglutination (1) Non-specific agglutination (1)
0264	Hinc	22/26[a] (85 %)	Hid (2) Hie (1) Not Hib, Hic or Hid (1)
0265	Hif	22/26 (85 %)	Hinc (1) Not Hib (1) Not Hib, Hic or Hid (1) Non-specific agglutination (1)
0266	Hib	26/27 (96%)	Non-specific agglutination (1)
0267	Hinc	22/27[a] (82 %)	Hib (1) Hic (1) Auto-agglutination (1) Non-specific agglutination (2)
Biotyping			
0262	IV	18/20 (90 %)	III (2)
0263	V[c]	6/8 (75%)	VIII[b] (2)
0264	V	17/19 (90 %)	IV (1) VII (1)
0265	I	19/20 (95%)	II (1)
0266	IV	18/20 (90%)	I (1) VI (1)
0267	III	15/20 (75%)	IV (5)
Genotypic species identification			
0262	*H. influenzae*	13/13 (100%)	NA
0263	*H. parainfluenzae* Not *H .influenzae*	4/13 9/13 (100% combined)	NA
0264	*H. influenzae*	14/14 (100%)	NA
0265	*H. influenzae*	13/13 (100%)	NA
0266	*H. influenzae*	13/13 (100%)	NA
0267	*H. influenzae*	13/14 (93%)	Not *H. influenzae* (1)
Genotypic capsular typing			
0262	Hinc	18/18 (100%)	
0263	NA	0/2 (NA)[d]	Hinc (1) Negative (1)
0264	Hinc	18/18 (100%)	
0265	Hif	18/18 (100%)	
0266	Hib	19/19 (100%)	
0267	Hinc	18/18 (100%)	

Abbreviations: Hinc, non-capsulated Haemophilus influenzae*; Hib*, H. influenzae *type b; Hif*, H. influenzae *type f; NA, not applicable.*

[a] *Includes one laboratory that only performed phenotypic serotyping using anti-serotype b antiserum and reported a negative result as Hinc.*

[b] *Phenotypic serotyping with* H. influenzae *antisera is not appropriate for this strain of* H. parainfluenzae*.*

[c] *The correct biochemical results would be interpreted as biotype V according to the* H. parainfluenzae *scheme. If scored according to the* H. influenzae *biotyping scheme, the erroneous result of VIII would be generated. Because raw data was not available, the result of V has been interpreted as a correct laboratory result interpreted according to the* H. parainfluenzae *biotyping scheme.*

[d] *Genotypic capsular typing is not appropriate for this strain of* H. parainfluenzae*.*

Figure 1. Strain identification

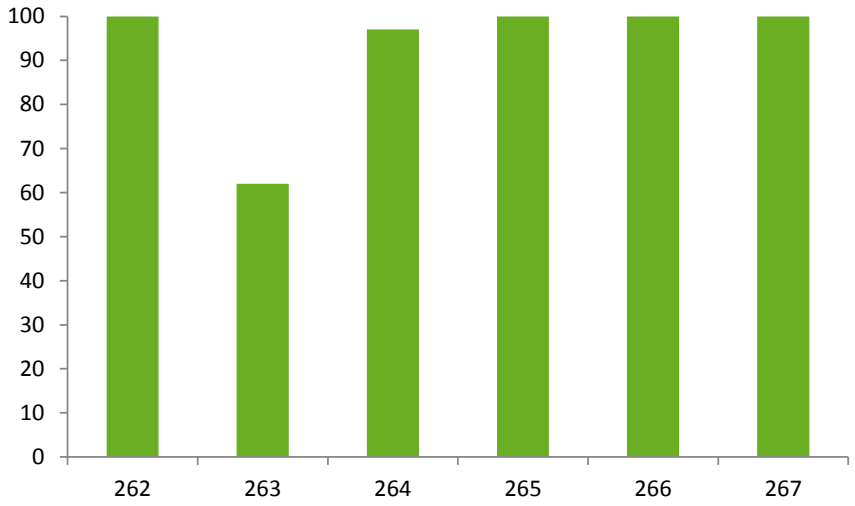


Figure 2. Phenotypic serotyping

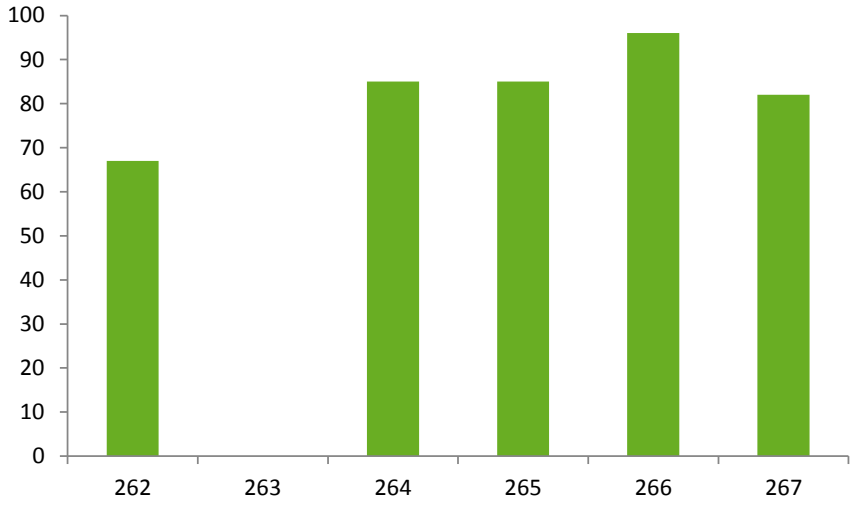


Figure 3. **Biotype identification**

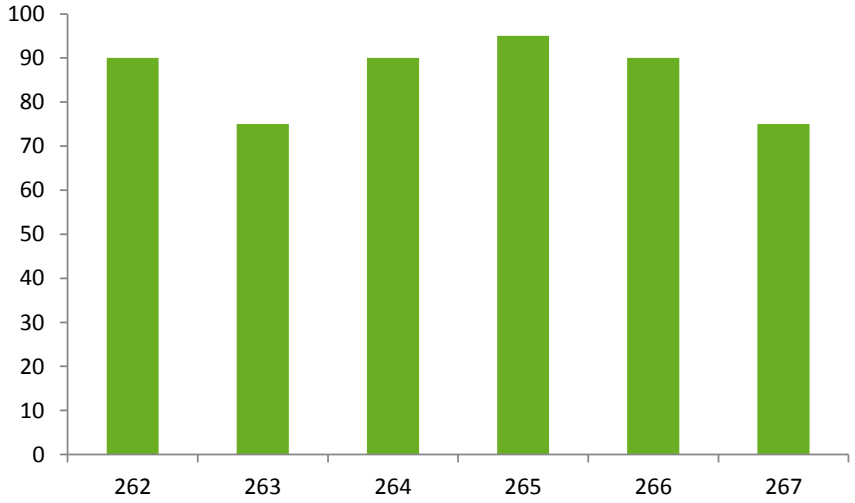


Figure 4. **Genotypic species identification**

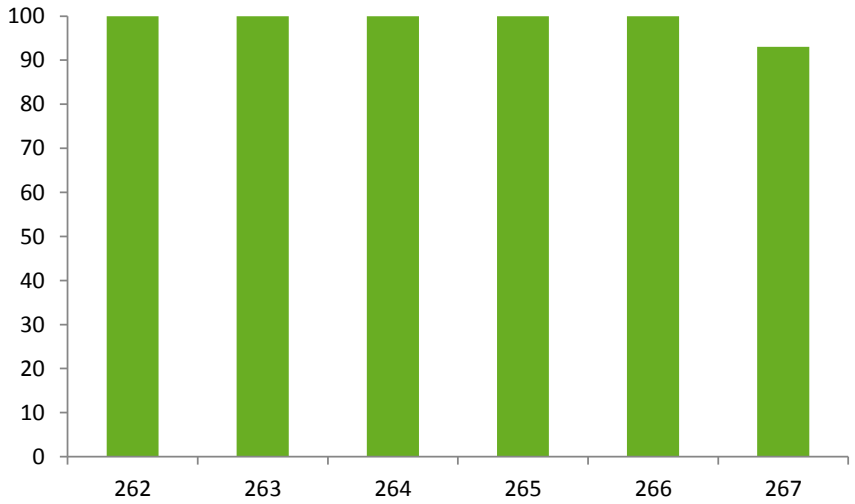
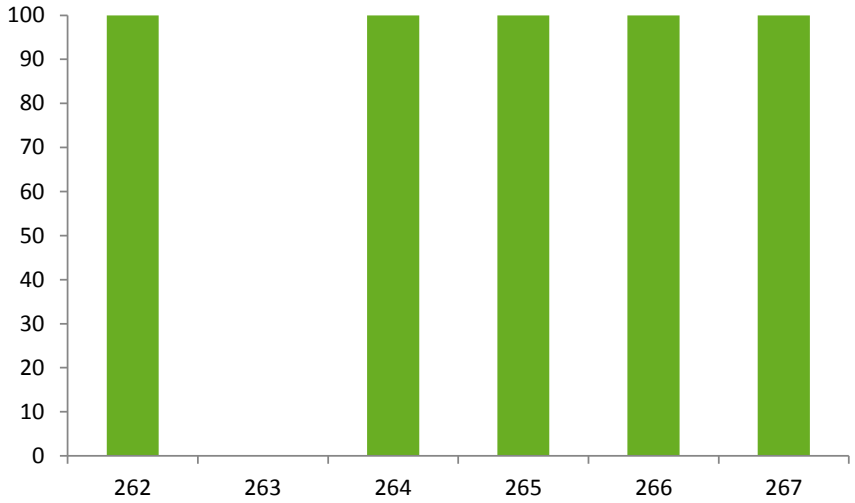


Figure 5. **Genotypic capsular typing**



2.1.1 Phenotypic species identification

Samples 0262, 0265, 0266 and 0267 were correctly identified as *H. influenzae* by all participants. One laboratory identified strain 0264 as *H. ducreyi*, using an unspecified method. Sample 0263 proved more problematic, with 10 laboratories giving identifications other than *H. parainfluenzae*. These other identifications were: *Aggregatibacter segnis (4)*, *H. paraphrophilus (3)*, *H. aphrophilus (2)* and *H. ducreyi (1).* A number of different methods were used to identify this strain, including API NH, RapID NH, Vitek and other unspecified methods. *Haemophilus ducreyi* is a fastidious organism that grows poorly and slowly on ordinary chocolate agar and therefore this identification should immediately be questioned by the laboratory staff.

The identification methods used by the participants are shown in Table 6.

Table 6. Phenotypic species identification methods reported by participating laboratories

Laboratory identification	ID Method 1	ID Method 2	ID Method 3	Additional Methods
NM02	Biochemical profile	Porphyrin test	Other (not specified)	
NM09	Gram stain	Catalase	Oxidase	Porphyrin test
NM10	X, V factors	RapID NH		
NM15	API NH	Vitek	X, V factors	
NM16	API NH	Biochemical profile	X, V factors	Porphyrin test
NM17	X, V factors	Porphyrin test	Satellitism	
NM20A	Satellitism	Porphyrin test	Biochemical profile	
NM23	Other (not specified)			
NM25	X, V factors	RapID NH	API NH	
NM26	API NH	Vitek	XV factors	
NM27	X, V factors	Satellitism	RapID NH	
NM28	X, V factors	Other (not specified)		
NM29	API NH	X, V factors		
NM32A	API NH	Gram stain	Catalase	Oxidase
NM33A	X, V factors	Satellitism	Porphyrin test	modified Hodge test
NM34A	API NH	X, V factors		
NM35A	Only confirmation on heated blood agar plate (+ growth) and blood agar plate (no growth). Rest is done in primary laboratory			
NM36	Satellitism	X, V factors	Gram stain	
NM37A	Porphyrin test	X, V factors	Biochemical profile	
NM38A	Biochemical profile	X, V factors	Satellitism	
NM39	API NH	X, V factors		
NM40	Satellitism	X, V factors	Vitek	
NM41	X, V factors	Vitek	RapID NH	Hemolysis on horse blood medium, oxidase test, catalase test
NM47	API NH	XV factors		
NM51	RapID NH	Satellitism	X, V factors	
NM52	Not specified			
NM53	MALDI-TOF MS			
NM54	Vitek	Satellitism	Cefinase (Biomerieux)	
NM55	API NH	RapID NH	X, V factors	Satellitism, haemolysis on blood agar, biochemical profile

Note: The web reporting form asked participants to select three methods from predefined menus and then add further methods to a comments field (listed under Additional Methods).

2.1.2 Phenotypic serotyping

The number of laboratories reporting serotype varied between 26 and 28, according to the different samples. Twenty-two laboratories used slide agglutination, three used latex agglutination and three used co-agglutination. The results showed that some laboratories are experiencing some problems with conventional serotyping. A breakdown by method revealed that the discrepant results were confined to slide agglutination (see Annex 3).

Sample 0262 was included in the panel as an example of a non-capsulated strain of *H. influenzae* that shows cross-reaction with type d antiserum. Hence, an incorrect result for this isolate is not surprising. Such cross-reactions can be resolved by using a PCR-based method of capsular genotyping (see below and Falla et al. 1994). Non-specific auto-agglutination can be resolved in the same way.

As described above, *H. influenzae* serotyping is not appropriate for sample 0263 *(H. parainfluenzae).*

2.1.3 Biotyping

Twenty laboratories carried out biotyping on the strains, using a mixture of individual biochemical tests, the API NH kit and the RapID NH kit (Table 7). The results were generally very good (Table 4).

Incorrect results did not appear to be linked to a particular method or one of the three biochemical reactions (see Annex 3). However, in our laboratory, the biotyping of strain 0267 consistently varied by method. Individual biochemical tests or the API NH kit repeatedly generated the result of biotype III, whereas the RapID NH kit gave

biotype IV. These two biotypes differ in their reaction to ornithine decarboxylase (ODC; see Table 8). We have noted that the RapID NH may give a false positive result for ODC as a result of carryover of volatile products from urea well. This can be avoided by overlaying the urea well with mineral oil (as is recommended when using the API NH kit). Interestingly, 14 laboratories stated that this isolate was biotype III, whereas five stated that the strain was biotype IV. Three of the five laboratories reporting biotype IV used the RapID NH kit.

Eight laboratories reported a biotype result for strain 0263 (the *H. parainfluenzae* isolate). The consensus result was biotype V, but two laboratories identified the strain as biotype VIII. There is a scheme for biotyping *H. parainfluenzae* isolates that uses the same biochemical reactions, but a different scoring system to the *H. influenzae* scheme (Table 8). As mentioned above (Section 2.1), it was assumed that participants reporting biotype V had scored the correct biochemical results according to the *H. parainfluenzae* system and those reporting biotype VIII had scored the correct biochemical results incorrectly using the *H. influenzae* system.

Table 7. Summary of biotyping methods used by 20 participating laboratories

Method	Number of laboratories
Individual biochemical tests	9
Individual biochemical tests + API NH kit	1
Individual biochemical tests + RapID NH kit	1
API NH kit	6
RapID NH kit	3
API NH kit + RapID NH kit	1

Table 8. Biotyping scheme for *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Kilian 1976, Oberhofer and Back 1979, Gratten 1983, Sottnek and Albritton 1984)

a) Biotypes of *Haemophilus influenzae*

Biotype	Indole	Urea	Ornithine decarboxylase
I	+	+	+
II	+	+	-
III	-	+	-
IV	-	+	+
V	+	-	+
VI	-	-	+
VII	+	-	-
VIII	-	-	-

b) Biotypes of *Haemophilus parainfluenzae*

Biotype	Indole	Urea	Ornithine decarboxylase
I	-	-	+
II	-	+	+
III	-	+	-
IV	+	+	+
V	-	-	-
VI	+	-	+
VII	+	+	-
VIII	+	-	-

2.1.4 Genotypic species identification

Fifteen laboratories used a PCR-based method to identify the strains (Table 9). This comprised either a PCR to detect *H. influenzae*-specific sequences in genes such as *ompP2*, *ompP6*, or the 16S rRNA gene, or PCR amplification and sequencing of some part of the 16S rRNA gene. With only one exception, all of these methods produced the intended result (Table 4). These results indicate that genotypic methods are less error prone than phenotypic methods of bacterial speciation. In the case of sample 0263, a result of 'Not *H. influenzae*' or '*H. parainfluenzae*' was accepted as correct in order to accommodate participants who used a method that could simply confirm whether the target was *H. influenzae* or not.

In the single case that did not match the intended result, sample 0267 (a non-capsulated *H. influenzae*) was designated 'not *H. influenzae*', using 16S rRNA gene sequencing. As raw data is not available, the reason for this discrepancy is not known.

The 15 laboratories used a range of DNA extraction procedures, all of which were associated with good results (Table 9).

Table 9. Number or participants using various combinations of DNA extraction procedure and detection method for genotypic species identification and capsular typing on viable isolates

DNA extraction procedure	Method for species identification						Capsular typing
	16S rDNA PCR	*ompP2* PCR	*ompP6* PCR	16S gene sequencing	16S gene sequencing + *ompP2* PCR	Other PCR (not specified)	Variation of Falla et al (1994)
Manual procedure + commercial kit		2			1	2	7
Automated procedure + commercial kit	1					1	2[a]
Manual procedure + in-house method		3	1	2		1	7
Automated procedure + in-house method							1
Other (unspecified)						1	1
No details given							1
Total	**15**						**19**

[a] *Includes one laboratory that only performed PCR for the bexA and Hib-specific targets.*

2.1.5 Genotypic capsule typing

Nineteen laboratories performed a PCR-based capsular typing procedure on the strains. Their DNA extraction procedures are also shown in Table 9. Eighteen of the participants used a PCR method based on that of Falla et al. (1994). The remaining laboratory restricted its detection to the *bexA* and Hib-specific targets.

All of the submitted results matched the intended result, with only two exceptions (Table 4). Both related to strain 0263 (the *H. parainfluenzae* strain), for which capsular typing is not appropriate. One laboratory reported this as a non-capsulated *H. influenzae*. This participant had not performed genotypic speciation, but had correctly identified the sample as *H. parainfluenzae* in their phenotypic characterisation. The second laboratory reported this sample as 'negative', which could also have been interpreted as 'non-capsulated *H. influenzae*'. This laboratory had also correctly identified the strain as 'not *H. influenzae*' by phenotypic characterisation. All of the other 17 laboratories either reported this sample as 'NE' (not evaluated) or did not report a result. As mentioned earlier, there was no opportunity for the participants to select 'Not applicable' in the web reporting form.

2.1.6 Other molecular typing

Although not a requirement of the EQA exercise, three laboratories submitted multilocus sequence typing (MLST) results for the strains (see Meats et al., 2003). The results were all in agreement with the sequence types established for these isolates prior to the EQA distribution (Table 10).

Table 10. Multilocus sequence types (ST) of samples 0262 to 0267

EQA number	ST
0262	47
0263	NA
0264	849
0265	124
0266	6
0267	155

NA: not applicable

2.2 Part 2: Antimicrobial susceptibility testing

2.2.1 β-lactamase activity testing

Twenty-seven laboratories reported β-lactamase activity results. All of the results were correct for all strains.

2.2.2 Antimicrobial susceptibility testing

The intended results for the antimicrobial susceptibility testing are shown in Table 11. Detailed analysis of results from participants is given in Annex 3.

The antimicrobial susceptibility testing proved rather problematic. Twenty-three laboratories reported the results of antimicrobial susceptibility testing. Thirteen laboratories used CLSI guidelines, while eight have adopted EUCAST guidelines. Some laboratories reported zone sizes and their interpretation and others reported MIC values. The use of different methodologies, different disc strengths and different breakpoints makes it difficult to compare the results from laboratories in any meaningful way.

Table 11. Intended results for antimicrobial susceptibility testing of bacterial isolates

EQA number	β-lactamase activity	Antimicrobial susceptibility (S)/resistance (R)[a]
0262	Absent	All S
0263	Absent	All S
0264	Absent	AMP R, CHLOR R, TET R, TRIM R, CO-AM R, CXM R, CEC R, BLNAR
0265	Absent	All S
0266	Present	AMP R, CHLOR R, TET R
0267	Absent	AMP R CO-AM R CXM R Low BLNAR

[a] *Based on EUCAST breakpoints*

Abbreviations: AMP, ampicillin; CHLOR, chloramphenicol; TET, tetracycline; TRIM, trimethoprim; CO-AM, co-amoxiclav; CXM, cefuroxime; CEC, cefaclor; BLNAR, β-lactamase-negative ampicillin-resistant

In general there were few problems with the antimicrobial susceptibility testing of the strains that were susceptible to a wide range of antibiotics (samples 0262, 0263 and 0265; see Annex 3).

There were also few problems with the testing for sample 0266, which exhibited β-lactamase-mediated resistance to ampicillin and amoxicillin (see Annex 3). The most important mechanism of ampicillin resistance in *H. influenzae* is the production of TEM-1 β-lactamase (Medeiros and Bryan 1975). A second β-lactamase, ROB-1 (Medeiros et al 1986) is less frequently implicated.

This strain also exhibited chloramphenicol and tetracycline resistance, both of which were detected by the majority of participants. The most common mechanism of chloramphenicol resistance in *H. influenzae* is plasmid-mediated production of chloramphenicol acetyl transferase (CAT) encoded by the cat gene (van Klingeren et al. 1977). The cat gene is carried on conjugative plasmids ranging in size from 34 x 10^6 to 46 x 10^6. Genes encoding resistance to tetracycline and ampicillin are frequently carried on these plasmids as well, which can be incorporated into the bacterial chromosome (Powell and Livermore 1988). Less commonly, strains are resistant to chloramphenicol due to the loss of an outer membrane protein, resulting in a permeability barrier (Burns et al. 1985).

Two of the samples, 0264 and 0267, were β-lactamase negative, but showed reduced susceptibility to ampicillin, amoxicillin, co-amoxyclav and cefuroxime. *Haemophilus influenzae* may be resistant to aminopenicillins through the production of a plasmid-mediated β-lactamase or alterations in penicillin-binding proteins (PBP) (Parr and Bryan 1984), which leads to a reduced affinity to penicillins and cephalosporins. *Haemophilus influenzae* has five penicillin-binding proteins (1A, 1B, 2, 3 and 4). PBP 3 is encoded by the ftsI gene and mutations in the transpeptidase domain of ftsI are correlated with resistance (Clairoux et al. 1992, Ubukata et al., 2001). Strains which are ampicillin resistant because of alterations in PBP3 are termed β-lactamase-negative ampicillin-resistant (BLNAR) strains. Some BLNAR strains (High-BLNAR) have ampicillin MICs in the range 8–16 µg/ml. Such strains can be readily detected by conventional disc diffusion methods, but are rarely encountered in Europe, though they are increasingly observed in the Far East. High BLNAR strains have mutations in the acr gene, which encodes the AcrAB efflux pump, in addition to mutations in ftsI (Kaczmarek et al., 2004). Low-BLNAR strains usually have ampicillin MICs in the range 0.5 to 2µg/ml and such strains may be difficult to identify by conventional susceptibility testing even when low-strength ampicillin (2µg/ml) and co-amoxyclav (2+1µg/l) discs are used. Definitive identification of such strains relies on PCR and partial sequencing of the ftsI gene, but this is impractical as a routine test. The clinical significance of ampicillin resistance at this low level is, however, far from clear.

Samples 0264 and 0267 were both BLNAR strains. MICs for sample 0264 ranged between 1.5–4µg/ml for ampicillin and 2–8µg/ml for co-amoxyclav, and this strain was scored as resistant against each antibiotic by the majority of participants (12/23 and 9/14 participants, respectively). Sample 0267 was more difficult to define, however. Its consensus ampicillin MIC was 1 µg/ml with reported MICs ranging from 0.19–4µg/ml. This consensus MIC would be deemed susceptible by both CLSI and EUCAST guidelines. The co-amoxyclav MIC results ranged from 1.5–3 µg/ml, indicating resistance to this agent and suggesting the strain is BLNAR. Only a minority of participants scored sample 0267 as resistant to ampicillin or co-amoxyclav (5/23 or 4/14 respectively; see Annex 3).

Sequencing of the PBP3 transpeptidase domain of ftsI (encoding amino acids 327 and 540; Dabernat et al., 2002) reveals that sample 0264 contains mutations that would cause the amino acid substitutions Val511Ala and Asn526Lys. Similarly, sample 0267 contains changes resulting in the substitutions Asp350Asn, Ser357Asn, Met377Ile, Ser385Thr and Arg517His. These would classify sample 0264 as a group IIa BLNAR strain and sample 0267 as either a group I or group III strain, depending on interpretation of the classification scheme (Ubukata et al., 2001; Dabernat, 2002; Garcia-Cobos, 2007). Information on the BLNAR status of the samples was not explicitly elicited from the participants. However, five laboratories volunteered the information that 0264 was a BLNAR strain and four laboratories that 0267 was a BLNAR strain. One of these laboratories had stated that they offered 'BLNAR detection' in their list of methods, but did not clarify whether this involved sequencing the ftsI gene.

Sample 0264 was also resistant to cefuroxime, chloramphenicol, tetracycline and trimethoprim. This was correctly identified by the majority of participants (8/15, 13/15, 13/17 and 2/2 respectively).

Sample 0267 was also resistant to cefuroxime, with which approximately half the laboratories (7/15) were in agreement. The reason for the discrepancy in cefuroxime susceptibility testing relates to the use of different testing guidelines. The EUCAST guidelines states that a cefuroxime MIC of ≤1µg/ml = susceptible; >2µg/ml indicates resistance. CLSI guidelines state that a cefuroxime MIC of ≤4µg/ml = susceptible, ≥16 µg/ml = resistant and strains with an MIC = 8µg/ml should be regarded as being of intermediate susceptibility.

It should also be noted that CLSI guidelines state that BLNAR strains should be regarded as resistant to co-amoxyclav, cefaclor and cefuroxime, despite apparent in vitro susceptibility to these antimicrobials (CLSI, 2011).

Some strains of *H. influenzae* are resistant to aminopenicillins through both mechanisms, that is, they produce a β-lactamase and have altered PBP3. Such strains are termed β-lactamase-positive amoxicillin/clavulanate-resistant (BLPACR) strains. Such a strain was not included in the EQA panel.

2.3 Part 3: Non-culture detection of *H. influenzae*

Two simulated CSF samples (0268 and 0269) were included in the EQA panel to test participants' ability to extract DNA from the clinical samples and assay for the presence of *H. influenzae* DNA. They were also encouraged to offer any further information that their assay was capable of elucidating about the samples. Sample 0268 was a strain of *H. influenzae* serotype b and 0269 was a strain of *Streptococcus pneumoniae*. The intended results and breakdown of submitted data are shown in Table 12.

Seventeen participants correctly detected *H. influenzae* DNA in sample 0268. The remaining laboratory was only able to define the target as *Haemophilus sp.*, using a method of 16S rRNA gene-specific PCR plus gel electrophoresis. Some laboratories included additional PCR targets; of those correctly identifying *H. influenzae*, three confirmed that the isolate was capsulated and six identified it as Hib. One laboratory identified it as either Hib or Hic, according to the published specificity of their chosen assay (Corless et al., 2001). However, another misidentified the capsule type as f. One further laboratory confirmed that the sample was also positive, using a PCR against the *fucK* gene (one of the MLST gene targets (Meats et al., 2003)).

For sample 0269, the result 'not *H. influenzae*', 'negative' or 'other – *S. pneumoniae*' were all accepted as correct, in order to accommodate the different detection methods and reporting conventions of the participants. All 18 laboratories successfully reported the absence of *H. influenzae* DNA. While the participants were only required to detect the presence or absence of *H. influenzae*, two correctly identified *S. pneumoniae* and another detected streptococcal DNA.

The 18 laboratories used a variety of methods for DNA extraction and *H. influenzae*-specific gene target detection (Table 13), all of which gave good results with these two samples.

Table 12. Intended and submitted results for Part 3: Non-culture detection of *H. influenzae*

EQA number	Intended results	Ratio of labs reporting the intended result (%)	Results not matching intended result (frequency)
00268	*H. influenzae*	17/18[a] (94%)	*Haemophilus sp.* (1)
00269	Not *H. influenzae* Negative Other – *Streptococcus pneumoniae*	9/18[a,b] 8/18[c] 1/18 (100% combined)	

[a] *Includes data from one laboratory that did not formally report the result on the web form, but entered their results in a comments field.*

[b] *One laboratory clarified in a comments field that they had detected S. pneumoniae.*

[c] *One laboratory clarified in a comments field that they had detected streptococcal DNA.*

Table 13. Methods used for preparation and detection of *H. influenzae* DNA in simulated CSF samples

DNA extraction	Amplification	*H. influenzae* gene target[a]				
		16S rDNA	***ompP2***	***ompP6***	***bexA***	**Other (not specified)**
Manual procedure + commercial kit	PCR and sequencing	3				
	PCR and gel electrophoresis	2	1		2	
	Real-time PCR platform		1		1	
Automated procedure + commercial kit	PCR and sequencing					
	PCR and gel electrophoresis				1[b]	1[c]
	Real-time PCR platform	1	1	2		
Manual procedure + in-house method	PCR and sequencing					
	PCR and gel electrophoresis		1			
	Real-time PCR platform					
Automated procedure + in-house procedure	PCR and sequencing					
	PCR and gel electrophoresis					
	Real-time PCR platform		1			

DNA extraction	Amplification	*H. influenzae* gene target[a]				
		16S rDNA	*ompP2*	*ompP6*	*bexA*	Other (not specified)
Other (no details given)	PCR and sequencing					
	PCR and gel electrophoresis					
	Real-time PCR platform				1	

[a] *Additional targets used by some laboratories are not included in this table.*

[b] *Sample 0268 only.*

[c] *Sample 0269 only.*

2.4 Part 4: Summary comparison of IBD-Labnet *H. influenzae* EQA panels 2009 and 2011

Results	2009	2011
Phenotypic species identification		
H. parainfluenzae	24/26 (92%)	18/29 (62%)
H. influenzae-1	25/26 (96%)	28/29 (97%)
H. influenzae-2	26/26 (100%)	29/29 (100%)
H. influenzae-3	26/26 (100%)	29/29 (100%)
H. influenzae-4	26/26 (100%)	29/29 (100%)
H. influenzae-5	24/26 (92%)	28/29 (97%)
Phenotypic serotyping		
N/A	N/A	0/5[a]
Hinc	22/23 (95%)	18/27 (67%) 22/26 (85%) 22/27 (82%)
Hie	21/22[a] (95%)	-
Hinc/Hia	19/22 (86%)	-
Hif	-	22/26 (85 %)
Hib	-	26/27 (96%)
Biotyping		
Biotype I	12/14(86%)	19/20 (95%)
Biotype I	14/14(100%)	
Biotype I	14/14(100%)	
Biotype I	12/14(86%)	
Biotype II	8/9 (89%)	-
Biotype III	-	15/20(75%)
Biotype IV	-	18/20(90%)
Biotype IV	-	18/20(90%)
Biotype V	6/8(75%)[b]	
Biotype V		
Biotype VI	13/14(93%)	
Genotypic capsular typing		
N/A	N/A	0/2 (NA)[c]
Hinc	15[e]/15 (100%)	18/18(100%)
Hinc	16/16 (100%)	18/18(100%)
Hib[-]	14/16 (87%)	-
Hie	15/16 (93%)	-
Hia[-]	11/16 (68%)	-
Hif	-	18/18(100%)
Hib	-	19/19(100%)

[a] *Phenotypic serotyping with* H. influenzae *antisera is not appropriate for this strain of* H. parainfluenzae. *Five laboratories attempted phenotypic serotyping of this strain.*

[b] *The correct biochemical results would be interpreted as biotype V according to the* H. parainfluenzae *scheme. If scored according to the* H. influenzae *biotyping scheme, the erroneous result of VIII would be generated. Because raw data was not available, the result of V has been interpreted as a correct laboratory result interpreted according to the* H. parainfluenzae *biotyping scheme.*

[c] *Genotypic capsular typing is not appropriate for this strain of* H. parainfluenzae.

The second IBD-Labnet EQA panel was distributed to 29 laboratories in 2011, whereas it was sent to 28 in 2009. In 2011, 29 laboratories returned reports compared to 26 in 2009.

With regard to phenotypic species identification of isolates, overall the identification of the *H. influenzae* strains improved in 2011 compared with 2009. However, overall identification of *H. parainfluenzae* in 2011 was not as good as in 2009.

Five laboratories attempted the phenotypic serotyping of *H. parainfluenzae* in 2011, which was not appropriate, whereas in 2009 it was clear for the participants that this method was not applicable to this strain.

The phenotypic serotyping of non-capsulated *H. influenzae* strains (Hinc) rendered poorer results in 2011 than in 2009. In 2011, slide agglutination was revealed as the method causing the discrepant results.

The evaluation of biotyping was good in 2011 and improved in the biotyping of biotype 1 when compared with 2009.

In 2011, genotypic capsular typing was very good, with only two laboratories attempting to genotype *H. parainfluenzae*, which was not appropriate.

In 2011, the antimicrobial susceptibility testing proved rather problematic, as it was in 2009. Laboratories used different guidelines (CLSI or EUCAST); some reported zone sizes while others reported MIC values, making comparison of results difficult, as observed in 2009. Again in 2011, identification of BLNAR (β-lactamase-negative ampicillin-resistant) strains proved challenging.

Overall comments

The laboratory EQA has shown that European Haemophilus reference laboratories vary in the level to which they characterise strains referred to them, ranging from simple speciation to full identification. Similarly, some laboratories perform PCR-based capsular based genotyping and antimicrobial susceptibility testing.

This EQA distribution identified some problems with the use of conventional serotyping by slide agglutination. The results can be misinterpreted when there are problems such as non-specific agglutination, cross-reactions and auto-agglutination. Satola et al. (2007) found that *H. influenzae* isolates were misidentified by conventional *H. influenzae* serotyping in 17.5% of cases.

Discrepancies varied by serotype and usually resulted in overreporting of genotypically non-capsulated strains of *H. influenzae* as encapsulated strains. The results of this EQA exercise clearly indicate that PCR-based speciation and capsular genotyping gives more reliable results for the identification and capsular typing of strains of *H. influenzae* than the results obtained by conventional phenotypic methods.

The antimicrobial susceptibility testing results proved difficult to assess as some laboratories gave MIC values, while others gave zone sizes, with or without interpretation of the results. Some laboratories are using EUCAST guidelines while others are still using CLSI guidelines. There are major differences between the EUCAST and CLSI both in terms of media and defined breakpoints for a number of antimicrobials. All EU reference laboratories should be moving towards using EUCAST guidelines. There were no problems with the detection of β-lactamase production. However the evaluation of β-lactamase-negative ampicillin resistance (BLNAR) proved more difficult.

There is some evidence that the prevalence of ampicillin resistance of *H. influenzae* in Europe may be decreasing due to a reduction in the number of β-lactamase-positive ampicillin-resistant strains, whereas the prevalence of BLNAR strains is relatively stable (Jansen et al., 2006). The level of ampicillin resistance exhibited by BLNAR strains may be low (MIC 0.5–2 µg/ml) and this may make their detection difficult, particularly if a breakpoint of 1µg/ml is used to define ampicillin susceptibility.

Using PCR and sequencing to detect specific mutations in the *ftsI* gene and associated PBP 3 substitutions, strains can be categorised as BLNAR. Low BLNAR usually have ampicillin MICs in the range 0.5 to 2.0 µg/ml, and high BLNAR have ampicillin MICs in the range 1.0 to 16.0 µg/ml. García-Cobos et al. (2008) suggest that low BLNAR strains are best detected by broth dilution methods rather than disc susceptibility testing.

BLNAR strains show reduced susceptibility not only to ampicillin but also to other β-lactam antibiotics, particularly some of the cephalosporins. Livermore et al. (2001) suggested that cefaclor resistance is a better indicator of a BLNAR strain than ampicillin resistance and James et al. (1996) used cefuroxime resistance (MIC >4.0 µg/ml) to screen for BLNAR strains. CLSI recommends that BLNAR strains are considered resistant to co-amoxyclav, cefaclor and cefuroxime, despite apparent susceptibility of some strains to these antimicrobials.

Nørskov-Lauritsen et al. (2011) evaluated the efficacy of disk diffusion methods for the detection of low-BLNAR. Forty-seven low-BLNAR strains of *H. influenzae*, identified by partial sequencing of the *ftsI* gene had low-level resistance to ampicillin (MIC ≤1 mg/l; MIC_{50} = 0.5 mg/l) which would be interpreted as susceptible by both EUCAST and CLSI interpretative criteria. The MIC of cefuroxime varied between 1 and 4 mg/l (MIC_{50} = 2 mg/l), which would be interpreted as resistant by EUCAST but susceptible by CLSI criteria. These authors found that disk diffusion with cefaclor (30µg disks) on Sensitivity Test Agar + 5% horse blood + NAD was able to discriminate low-BLNAR strains from wild-type strains with 98% sensitivity and 86–99% specificity.

Some laboratories used low strength ampicillin disks (2µg) as recommended by EUCAST guidelines, while others used higher concentration ampicillin disks (10µg). The use of low-dose ampicillin disks is recommended as it will increase the ability to identify low-BLNAR (Nørskov-Lauritsen et al., 2011; Kärpänoja et al., 2004).

Two simulated CSF samples were included in this EQA panel to assess laboratories' methods and expertise in non-culture detection of *H. influenzae*. The results were very good. However, with so few samples it was not possible to test the sensitivity of different methods or test whether participants were reporting results that were appropriate to the gene targets they had chosen for their PCRs.

Care must be taken in reporting PCR-derived results, particularly when used in non-culture detection on clinical specimens. Some PCR targets are designed to be species-specific (e.g. *ompP2*, *ompP6*, *16S rDNA)* and a positive result can be reported as *H. influenzae*. Other targets are specific for capsulated *H. influenzae* only (e.g. *bexA*) or are specific for a subset of capsular types (e.g. the *bexA* PCR of Corless et al. (2001) or the type-specific PCRs of Falla et al. (1994)). Hence, the precise meaning of a positive or negative PCR result must be explained (e.g. whether the test can only detect capsulated *H. influenzae* or only a subset of capsule types).

The questions posed in this EQA were not designed to determine whether each laboratory reported a result appropriate to the gene targets they used. However, it was noted that some laboratories were aware of this issue and clarified the meaning of their results for samples 0268 and 0269 in a comments field. An expanded panel of samples could be included in a future distribution to investigate this in more detail. A larger panel would also allow the sensitivity of different methods to be compared.

Conclusions

A certain degree of heterogeneity exists in the level of characterisation of strains of *Haemophilus influenzae* among EU countries. This emphasises the need for consensus and agreement in methods for characterising and accurately defining this organism. This is outside the remit of the EQA exercise and should be addressed by the IBD-Labnet together with the ECDC. Some countries still require some capacity building in this area.

There were a number of problems with the design of the web reporting system. For example, it did not include a 'not applicable' category for the tests. We will endeavour to improve the design of the web reporting scheme in future distributions.

The EQA exercise has again demonstrated the value of PCR-based genotyping methods in providing identification of *Haemophilus spp*. and a serotype/genotype for strains that give inconclusive results on slide agglutination. Ideally a genotyping method should be used for all H. influenzae isolates in order to confidently identify Hib and capsule deficient Hib$^-$ strains. This is of particular importance where routine Hib immunisation is used, since it is essential to be able to accurately identify Hib vaccine failures. It is of note that the Hib isolate included in the EQA was identified by the majority of participating laboratories. In addition, molecular based capsular typing can act as a quality control measure to monitor the accuracy of the results of conventional serotyping.

The results of antimicrobial susceptibility testing again proved difficult to interpret due to the use of different methods and breakpoints. It is recommended that all European laboratories adopt the EUCAST methods and clinical breakpoints of antimicrobial susceptibility testing which should facilitate better comparison of the results from different laboratories (http://www.EUCAST.org) and comply with the 2012 case definitions for EU surveillance of antimicrobial resistance.

For the first time, two simulated clinical samples were included in the EQA panel to assess non-culture detection methods. The results were very encouraging, but a larger number of this type of sample will be required in future distributions to assess participants' proficiency more rigorously.

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Annex 1. Participating reference laboratories

Country	Contact person	Institution
Austria	Dr Sigrid Heuberger	National Reference Centre for Meningococci, Pneumococci and *Haemophilus influenzae* Austrian Agency for food and Health Safety Beethovenstraße 6 8010 Graz, Austria
Bulgaria	Dr Dimitar Nashev	National center for infectious and parasitic diseases 26 Y. Sakazov Blvd 1504 Sofia, Bulgaria
Cyprus	Dr. Despo Pieridou Bagatzouni	Nicosia general hospital Microbiology Department 1450 Nicosia, Cyprus
Czech Republic	Dr Vera Lebedova	National Reference Laboratory for Haemophilus Infections Centre of 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. Statens Serum Institut, 5 Artillerivej, building 211/117B. 2300 Copenhagen, Denmark
Estonia	Laura Kunder	Central Laboratory of Communicable Diseases Health Board Kotka 2 11315 Tallinn, Estonia
Finland	Dr Anni Virolainen-Julkunen	National Institute for Health and Welfare (THL) PO Box 30 00271 Helsinki, Finland
France	Dr Olivier Gaillot	Dr. Olivier Gaillot Centre National de Référence des *Haemophilus influenzae* Laboratoire de Bactériologie-Hygiène Centre de Biologie Pathologie CHRU de Lille Boulevard du Professeur Jules Leclercq 59037 Lille
Germany	Prof Dr Matthias Frosch/Prof Dr Ulrich Vogel	Institute for Hygiene and Microbiology University of Würzburg Josef-Schneider-Straße 2 97080 Würzburg, Germany
Greece	Dr Georgina Tzanakaki	National Meningitis Reference Laboratory National School of Public Health 196 Alexandras Avenue 115 21 Athens, Greece
Hungary	Dr Ákos Tóth	Department of Bacteriology Johan Bela National Centre for Epidemiology Gyali ut 2-6 1097 Budapest, Hungary
Iceland	Dr Hjordis Hardardóttoir	Department of Clinical Microbiology Institute of Laboratory Medicine Landspitali University Hospital Baronsstigur, 101 Reykjavik, Iceland
Ireland	Dr. Robert Cunney	Irish Meningococcal and Meningitis Reference Laboratory Children's University Hospital Temple Street Dublin 1, Ireland
Italy	Dr Marina Cerquetti	Department of Infectious, Parasitic and Immunomediated Diseases Instituto Superiore di Sanitá Viale Regina Elena 299 00161 Rome, Italy
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Netherlands	Dr Lodewijk Spanjaard	Netherlands Reference Laboratory for Bacterial Meningitis Department of Medical Microbiology Academic Medical Canter, L-1-Z Meibergdreef 15 1105 AZ Amsterdam, Netherlands

Country	Contact person	Institution
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Sweden	Prof Dr Birgitta Henriques Normark	Department of Bacteriology Swedish Institute for Infectious Disease Control Nobels väg 18 SE-171 82 Solna, Sweden
UK	Dr Mary Slack	*Haemophilus* Reference Unit Specialist and Reference Microbiology Divison Health Protection Agency 61 Colindale Avenue London NW9 5HT, UK

Annex 2. Consensus results for *Haemophilus influenzae* identification, typing and antimicrobial susceptibility testing

EQA number		0262	0263	0264	0265	0266	0267	0268	0269
Phenotypic Identification	**Species**	*H. influenzae*	*H. parainfluenzae*	*H. influenzae*	*H. influenzae*	*H. influenzae*	*H. influenzae*		
	Serotype	Non typable		Non typable	f	b	Non typable		
	Biotype	IV	V	V	I	IV	III		
Genotypic Identification	**Species**	*H. influenzae*	Not *H. influenzae*	*H. influenzae*	*H. influenzae*	*H. influenzae*	*H. influenzae*	*H. influenzae*	Not *H. influenzae*/ negative
	Capsular type	Non typable		Non typable	f	b	Non typable		
	Other	ST-47		ST-849	St-124	ST-6	ST-155		

Antimicrobial susceptibility

Antimicrobial susceptibility testing results

Antimicrobial agent	EQA number					
Amoxicillin	S	S	R	S	R	R
Ampicillin	S	S	R	S	R	S
Azithromycin	S	S	S	S	S	S
Beta-lactamase	NEG	NEG	NEG	NEG	POS	NEG
Cefotaxime	S	S	S	S	S	S
Ceftriaxone	S	S	S	S	S	S
Cefuroxime	S	S	R	S	S	R
Chloramphenicol	S	S	R	S	R	S
Ciprofloxacin	S	S	S	S	S	S
Co-amoxiclav	S	S	R	S	S	S
Rifampicin	S	S	S	S	S	S
Tetracycline	S	S	R	S	R	S
Trimethoprim	S	S	R	S	S	S
Trimethoprim/Sulpha	S	S	R	S	S	S

S= susceptible

R – resistant

NEG = negative

POS = positive

Annex 3. Example of report generated by UK NEQAS

UK National External Quality Assessment Service for Microbiology

Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 1 of 53
Dispatch Date : 14-Feb-2011	

Intended Result			Your Report	Your Score
Specimen 0262				
	Phenotypic species ID	H. influenzae	Not returned	Not scored
	Phenotypic serotype	Hi non typable	Not returned	Not scored
	Phenotypic biotype	IV	Not returned	Not scored
	Genotypic species ID	H. influenzae	Not returned	Not scored
	Genotypic capsular type	Hi non typable	Not returned	Not scored
	Amoxicillin	susceptible	Not returned	Not scored
	Ampicillin	susceptible	Not returned	Not scored
	Azithromycin	susceptible	Not returned	Not scored
	Beta-lactamase	negative	Not returned	Not scored
	Cefotaxime	susceptible	Not returned	Not scored
	Ceftriaxone	susceptible	Not returned	Not scored
	Cefuroxime	susceptible	Not returned	Not scored
	Chloramphenicol	susceptible	Not returned	Not scored
	Ciprofloxacin	susceptible	Not returned	Not scored
	Co-amoxiclav	susceptible	Not returned	Not scored
	Rifampicin	susceptible	Not returned	Not scored
	Tetracycline	susceptible	Not returned	Not scored
	Trimethoprim	susceptible	Not returned	Not scored
	Trimethorpim/sulp	susceptible	Not returned	Not scored
Specimen 0263				
	Phenotypic species ID	H. parainfluenzae	Not returned	Not scored
	Phenotypic serotype	Not applicable	Not returned	Not scored
	Phenotypic biotype	V	Not returned	Not scored
	Genotypic species ID	Multiple - see comments	Not returned	Not scored
	Genotypic capsular type	Not applicable	Not returned	Not scored
	Amoxicillin	susceptible	Not returned	Not scored
	Ampicillin	susceptible	Not returned	Not scored
	Azithromycin	susceptible	Not returned	Not scored
	Beta-lactamase	negative	Not returned	Not scored
	Cefotaxime	susceptible	Not returned	Not scored
	Ceftriaxone	susceptible	Not returned	Not scored
	Cefuroxime	susceptible	Not returned	Not scored
	Chloramphenicol	susceptible	Not returned	Not scored
	Ciprofloxacin	susceptible	Not returned	Not scored
	Co-amoxiclav	susceptible	Not returned	Not scored
	Rifampicin	susceptible	Not returned	Not scored
	Tetracycline	susceptible	Not returned	Not scored
	Trimethoprim	susceptible	Not returned	Not scored
	Trimethorpim/sulp	susceptible	Not returned	Not scored
Specimen 0264				
	Phenotypic species ID	H. influenzae	Not returned	Not scored
	Phenotypic serotype	Hi non typable	Not returned	Not scored
	Phenotypic biotype	V	Not returned	Not scored
	Genotypic species ID	H. influenzae	Not returned	Not scored
	Genotypic capsular type	Hi non typable	Not returned	Not scored
	Amoxicillin	resistant	Not returned	Not scored
	Ampicillin	resistant	Not returned	Not scored
	Azithromycin	susceptible	Not returned	Not scored
	Beta-lactamase	negative	Not returned	Not scored
	Cefotaxime	susceptible	Not returned	Not scored
	Ceftriaxone	susceptible	Not returned	Not scored
	Cefuroxime	resistant	Not returned	Not scored
	Chloramphenicol	resistant	Not returned	Not scored
	Ciprofloxacin	susceptible	Not returned	Not scored
	Co-amoxiclav	resistant	Not returned	Not scored
	Rifampicin	susceptible	Not returned	Not scored
	Tetracycline	resistant	Not returned	Not scored
	Trimethoprim	resistant	Not returned	Not scored
	Trimethorpim/sulp	resistant	Not returned	Not scored

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Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 2 of 53
Dispatch Date : 14-Feb-2011	

Intended Result			Your Report	Your Score
Specimen 0265				
	Phenotypic species ID	H. influenzae	Not returned	Not scored
	Phenotypic serotype	Hif	Not returned	Not scored
	Phenotypic biotype	I	Not returned	Not scored
	Genotypic species ID	H. influenzae	Not returned	Not scored
	Genotypic capsular type	Hif	Not returned	Not scored
	Amoxicillin	susceptible	Not returned	Not scored
	Ampicillin	susceptible	Not returned	Not scored
	Azithromycin	susceptible	Not returned	Not scored
	Beta-lactamase	negative	Not returned	Not scored
	Cefotaxime	susceptible	Not returned	Not scored
	Ceftriaxone	susceptible	Not returned	Not scored
	Cefuroxime	susceptible	Not returned	Not scored
	Chloramphenicol	susceptible	Not returned	Not scored
	Ciprofloxacin	susceptible	Not returned	Not scored
	Co-amoxiclav	susceptible	Not returned	Not scored
	Rifampicin	susceptible	Not returned	Not scored
	Tetracycline	susceptible	Not returned	Not scored
	Trimethoprim	susceptible	Not returned	Not scored
	Trimethorpim/sulp	susceptible	Not returned	Not scored
Specimen 0266				
	Phenotypic species ID	H. influenzae	Not returned	Not scored
	Phenotypic serotype	Hib	Not returned	Not scored
	Phenotypic biotype	IV	Not returned	Not scored
	Genotypic species ID	H. influenzae	Not returned	Not scored
	Genotypic capsular type	Hib	Not returned	Not scored
	Amoxicillin	resistant	Not returned	Not scored
	Ampicillin	resistant	Not returned	Not scored
	Azithromycin	susceptible	Not returned	Not scored
	Beta-lactamase	positive	Not returned	Not scored
	Cefotaxime	susceptible	Not returned	Not scored
	Ceftriaxone	susceptible	Not returned	Not scored
	Cefuroxime	susceptible	Not returned	Not scored
	Chloramphenicol	resistant	Not returned	Not scored
	Ciprofloxacin	susceptible	Not returned	Not scored
	Co-amoxiclav	susceptible	Not returned	Not scored
	Rifampicin	susceptible	Not returned	Not scored
	Tetracycline	resistant	Not returned	Not scored
	Trimethoprim	susceptible	Not returned	Not scored
	Trimethorpim/sulp	susceptible	Not returned	Not scored
Specimen 0267				
	Phenotypic species ID	H. influenzae	Not returned	Not scored
	Phenotypic serotype	Hi non typable	Not returned	Not scored
	Phenotypic biotype	III	Not returned	Not scored
	Genotypic species ID	H. influenzae	Not returned	Not scored
	Genotypic capsular type	Hi non typable	Not returned	Not scored
	Amoxicillin	resistant	Not returned	Not scored
	Ampicillin	resistant	Not returned	Not scored
	Azithromycin	susceptible	Not returned	Not scored
	Beta-lactamase	negative	Not returned	Not scored
	Cefotaxime	susceptible	Not returned	Not scored
	Ceftriaxone	susceptible	Not returned	Not scored
	Cefuroxime	resistant	Not returned	Not scored
	Chloramphenicol	susceptible	Not returned	Not scored
	Ciprofloxacin	susceptible	Not returned	Not scored
	Co-amoxiclav	resistant	Not returned	Not scored
	Rifampicin	susceptible	Not returned	Not scored
	Tetracycline	susceptible	Not returned	Not scored
	Trimethoprim	susceptible	Not returned	Not scored
	Trimethorpim/sulp	susceptible	Not returned	Not scored

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Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 3 of 53
Dispatch Date : 14-Feb-2011	

Intended Result			Your Report	Your Score
Specimen 0268				
	Non-culture species ID (molecular)	H. influenzae	Not returned	Not scored
Specimen 0269				
	Non-culture species ID (molecular)	Multiple - see comments	Not returned	Not scored

Comments

Specimens were sent to laboratories in 30 countries: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, England and Wales, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Scotland, Slovak Republic, Slovenia, Spain and Sweden.
Results were returned from 28 laboratories within the time period allowed for examination of the specimens. One laboratory reported results late as their laboratory was being reorganised during the distribution period and they did not receive the specimens in time for testing. However their data has been included in this report.

For more detailed information and comments on this EQA exercise and strain information see pages 51 to 53.

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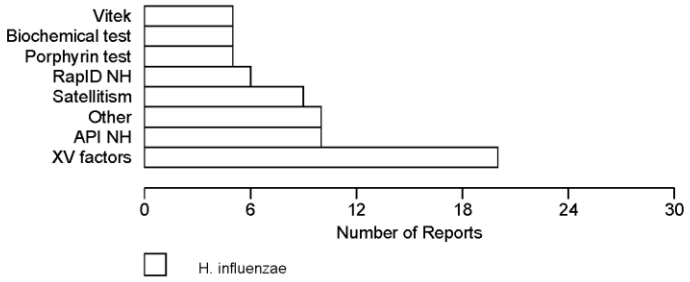
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 4 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0262 Phenotypic species ID
Intended result: *Haemophilus influenzae*

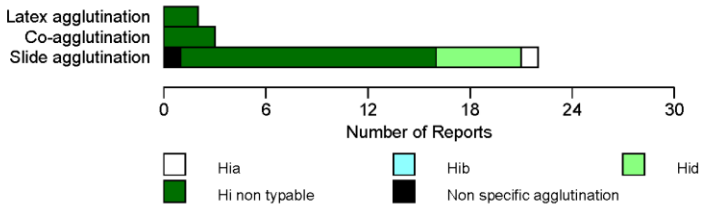


	All	(%)
Vitek	5	(7.1)
Biochemical test	5	(7.1)
Porphyrin test	5	(7.1)
RapID NH	6	(8.6)
Satellitism	9	(12.9)
Other	10	(14.3)
API NH	10	(14.3)
XV factors	20	(28.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	29	
Total	29	

Specimen : 0262 Phenotypic serotype
Intended result: Hi non typable

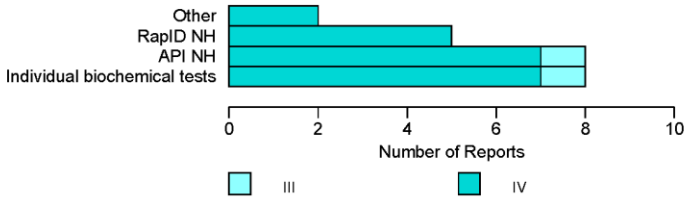


	All	(%)
Latex agglutination	2	(7.4)
Co-agglutination	3	(11.1)
Slide agglutination	22	(81.5)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hia	1	
Hib	1	
Hid	5	
Hi non typable	18	
Non specific agglutination	1	
Total	26	

Specimen : 0262 Phenotypic biotype
Intended result: Biotype IV



	All	(%)
Other	2	(8.7)
RapID NH	5	(21.7)
API NH	8	(34.8)
Individual biochemical tests	8	(34.8)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
III	2	
IV	18	
Total	20	

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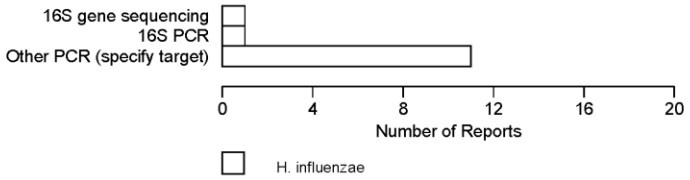
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 5 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0262 Genotypic species ID
Intended result: *Haemophilus influenzae*

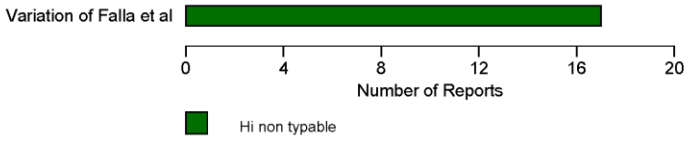


	All	(%)
16S gene sequencing	1	(7.7)
16S PCR	1	(7.7)
Other PCR (specify target)	11	(84.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	13	
Total	13	

Specimen : 0262 Genotypic capsular type
Intended result: Hi non typable



	All	(%)
Variation of Falla et al	17	(100)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hi non typable	18	
Total	18	

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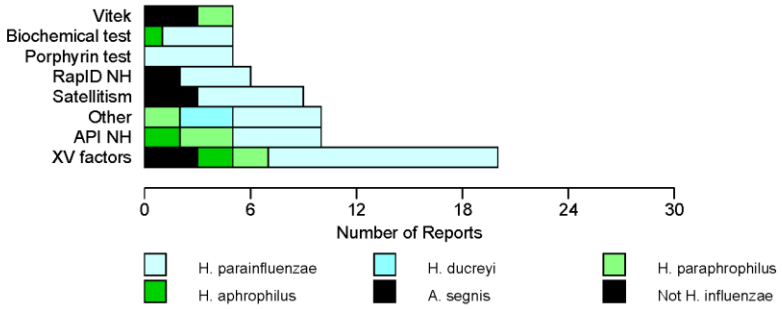
Published at 15:33:01 on Friday 22 July 2011

UK National External Quality Assessment Service for Microbiology

Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 6 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0263 Phenotypic species ID
Intended result: *Haemophilus parainfluenzae*

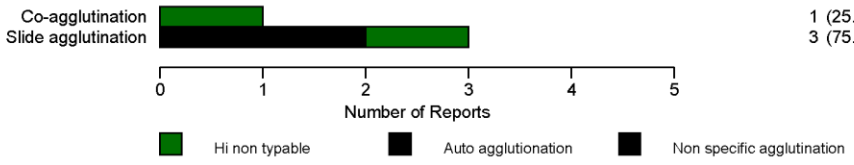


	All	(%)
Vitek	5	(7.1)
Biochemical test	5	(7.1)
Porphyrin test	5	(7.1)
RapID NH	6	(8.6)
Satellitism	9	(12.9)
Other	10	(14.3)
API NH	10	(14.3)
XV factors	20	(28.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. parainfluenzae	18	
H. ducreyi	1	
H. paraphrophilus	3	
H. aphrophilus	2	
A. segnis	4	
Not H. influenzae	1	
Total	29	

Specimen : 0263 Phenotypic serotype
Intended result: Not applicable

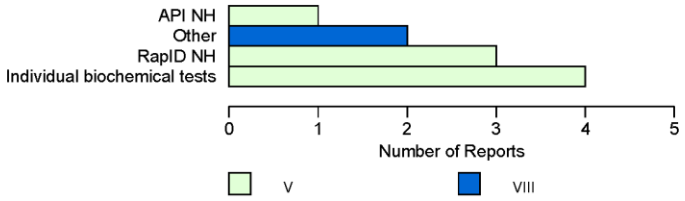


	All	(%)
Co-agglutination	1	(25.0)
Slide agglutination	3	(75.0)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hi non typable	3	
Auto agglutionation	1	
Non specific agglutination	1	
Total	5	

Specimen : 0263 Phenotypic biotype
Intended result: Biotype V



	All	(%)
API NH	1	(10.0)
Other	2	(20.0)
RapID NH	3	(30.0)
Individual biochemical tests	4	(40.0)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
V	6	
VIII	2	
Total	8	

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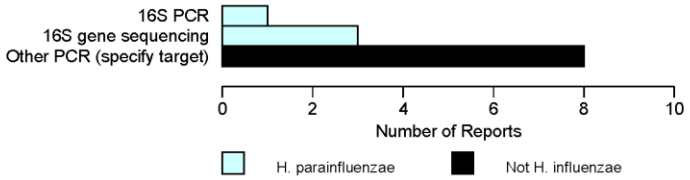
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 7 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0263 Genotypic species ID
Intended result: *Haemophilus parainfluenzae* / not *H. influenzae*

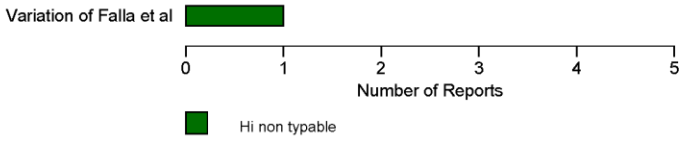


	All	(%)
16S PCR	1	(8.3)
16S gene sequencing	3	(25.0)
Other PCR (specify target)	8	(66.7)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. parainfluenzae	4	
Not H. influenzae	8	
Total	12	

Specimen : 0263 Genotypic capsular type
Intended result: Not applicable



	All	(%)
Variation of Falla et al	1	(100)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hi non typable	2	
Total	2	

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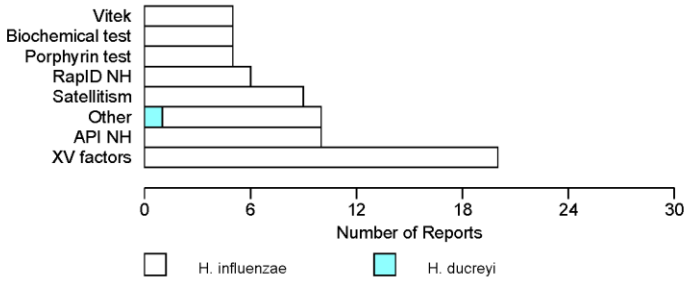
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 8 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0264 Phenotypic species ID
Intended result: *Haemophilus influenzae*

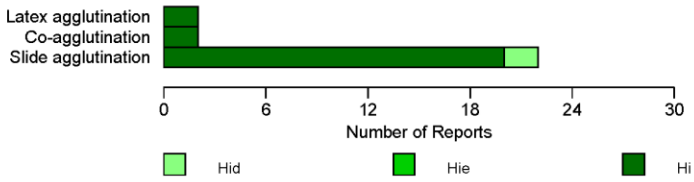


	All	(%)
Vitek	5	(7.1)
Biochemical test	5	(7.1)
Porphyrin test	5	(7.1)
RapID NH	6	(8.6)
Satellitism	9	(12.9)
Other	10	(14.3)
API NH	10	(14.3)
XV factors	20	(28.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	28	
H. ducreyi	1	
Total	29	

Specimen : 0264 Phenotypic serotype
Intended result: Hi non typable

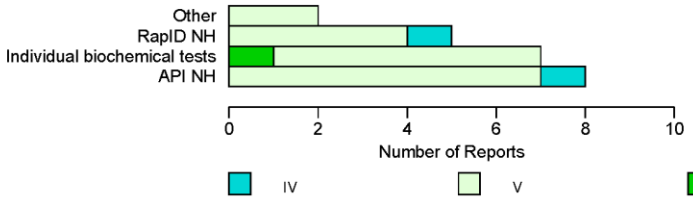


	All	(%)
Latex agglutination	2	(7.7)
Co-agglutination	2	(7.7)
Slide agglutination	22	(84.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hid	2	
Hie	1	
Hi non typable	22	
Total	25	

Specimen : 0264 Phenotypic biotype
Intended result: Biotype V



	All	(%)
Other	2	(9.1)
RapID NH	5	(22.7)
Individual biochemical tests	7	(31.8)
API NH	8	(36.4)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
IV	1	
V	17	
VII	1	
Total	19	

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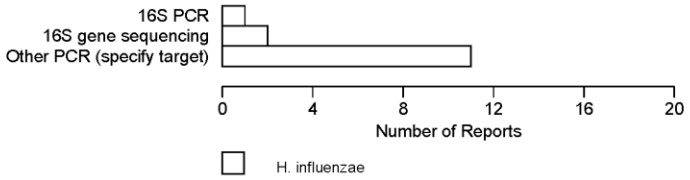
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 9 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0264 Genotypic species ID
Intended result: *Haemophilus influenzae*

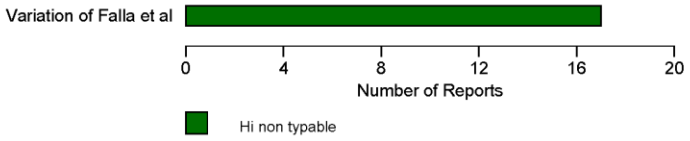


	All	(%)
	1	(7.1)
	2	(14.3)
	11	(78.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	14	
Total	14	

Specimen : 0264 Genotypic capsular type
Intended result: Hi non typable



	All	(%)
	17	(100)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hi non typable	18	
Total	18	

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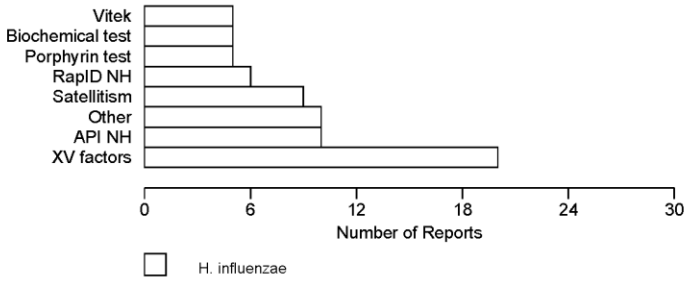
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 10 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0265 Phenotypic species ID
Intended result: *Haemophilus influenzae*

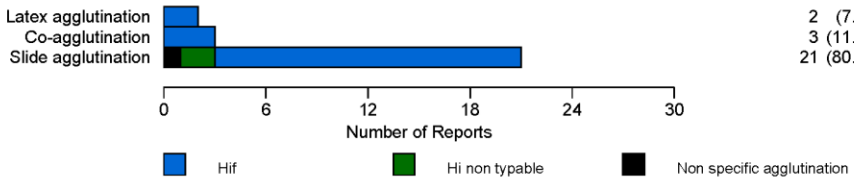


	All	(%)
Vitek	5	(7.1)
Biochemical test	5	(7.1)
Porphyrin test	5	(7.1)
RapID NH	6	(8.6)
Satellitism	9	(12.9)
Other	10	(14.3)
API NH	10	(14.3)
XV factors	20	(28.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	29	
Total	29	

Specimen : 0265 Phenotypic serotype
Intended result: Serotype Hif

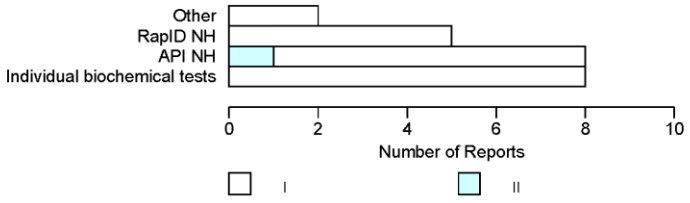


	All	(%)
Latex agglutination	2	(7.7)
Co-agglutination	3	(11.5)
Slide agglutination	21	(80.8)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hif	22	
Hi non typable	2	
Non specific agglutination	1	
Total	25	

Specimen : 0265 Phenotypic biotype
Intended result: Biotype I



	All	(%)
Other	2	(8.7)
RapID NH	5	(21.7)
API NH	8	(34.8)
Individual biochemical tests	8	(34.8)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
I	19	
II	1	
Total	20	

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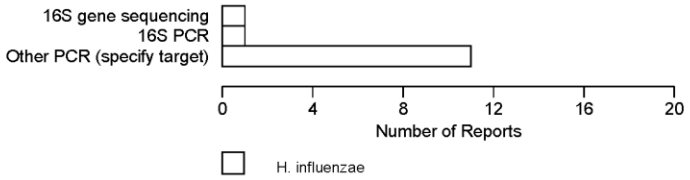
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 11 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0265 Genotypic species ID
Intended result: *Haemophilus influenzae*

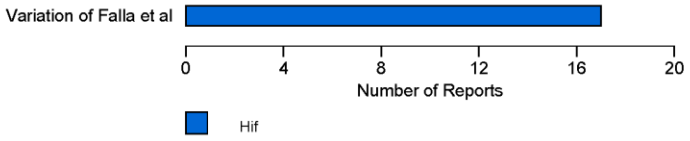


	All	(%)
16S gene sequencing	1	(7.7)
16S PCR	1	(7.7)
Other PCR (specify target)	11	(84.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	13	
Total	13	

Specimen : 0265 Genotypic capsular type
Intended result: Capsular type Hif



	All	(%)
Variation of Falla et al	17	(100)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hif	18	
Total	18	

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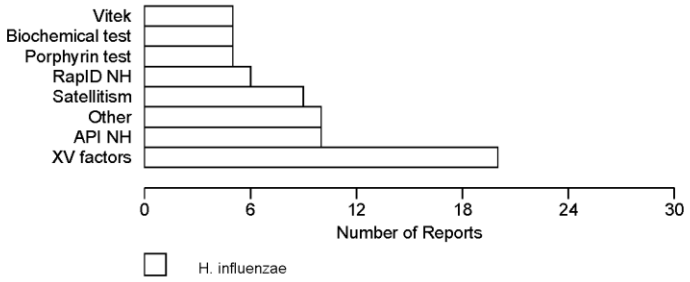
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 12 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0266 Phenotypic species ID
Intended result: *Haemophilus influenzae*

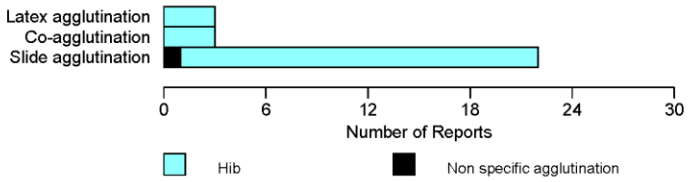


	All	(%)
Vitek	5	(7.1)
Biochemical test	5	(7.1)
Porphyrin test	5	(7.1)
RapID NH	6	(8.6)
Satellitism	9	(12.9)
Other	10	(14.3)
API NH	10	(14.3)
XV factors	20	(28.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	29	
Total	29	

Specimen : 0266 Phenotypic serotype
Intended result: Serotype Hib

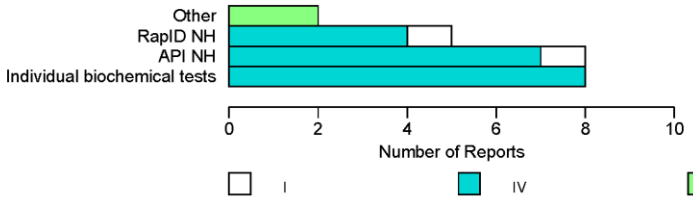


	All	(%)
Latex agglutination	3	(10.7)
Co-agglutination	3	(10.7)
Slide agglutination	22	(78.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hib	26	
Non specific agglutination	1	
Total	27	

Specimen : 0266 Phenotypic biotype
Intended result: Biotype IV



	All	(%)
Other	2	(8.7)
RapID NH	5	(21.7)
API NH	8	(34.8)
Individual biochemical tests	8	(34.8)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
I	1	
IV	18	
VI	1	
Total	20	

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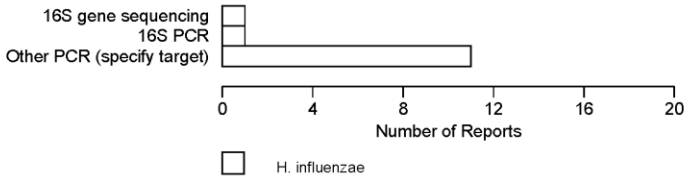
UK National External Quality Assessment Service for Microbiology

Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 13 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0266 Genotypic species ID
Intended result: *Haemophilus influenzae*

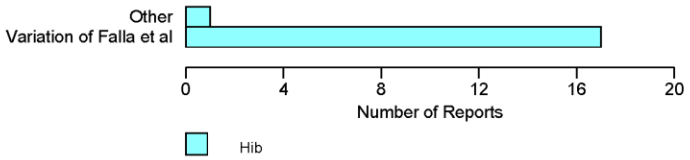


	All	(%)
16S gene sequencing	1	(7.7)
16S PCR	1	(7.7)
Other PCR (specify target)	11	(84.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	13	
Total	13	

Specimen : 0266 Genotypic capsular type
Intended result: Capsular type Hib



	All	(%)
Other	1	(5.6)
Variation of Falla et al	17	(94.4)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hib	19	
Total	19	

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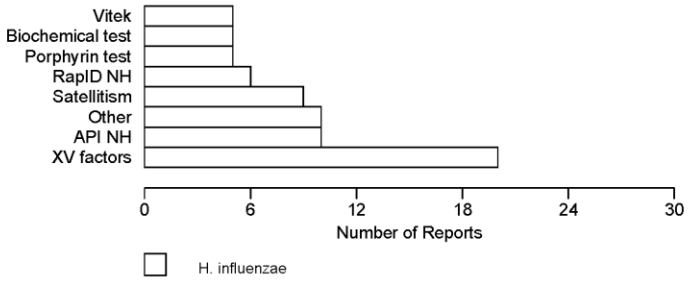
UK National External Quality Assessment Service for Microbiology

Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 14 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0267 Phenotypic species ID
Intended result: *Haemophilus influenzae*

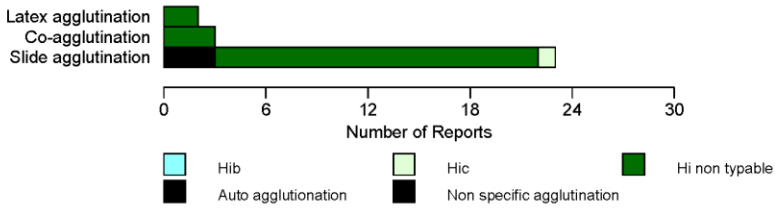


	All	(%)
Vitek	5	(7.1)
Biochemical test	5	(7.1)
Porphyrin test	5	(7.1)
RapID NH	6	(8.6)
Satellitism	9	(12.9)
Other	10	(14.3)
API NH	10	(14.3)
XV factors	20	(28.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	29	
Total	29	

Specimen : 0267 Phenotypic serotype
Intended result: Hi non typable

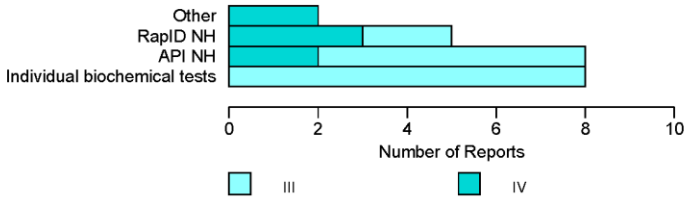


	All	(%)
Latex agglutination	2	(7.1)
Co-agglutination	3	(10.7)
Slide agglutination	23	(82.1)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hib	1	
Hic	1	
Hi non typable	22	
Auto agglutionation	1	
Non specific agglutination	2	
Total	27	

Specimen : 0267 Phenotypic biotype
Intended result: Biotype III



	All	(%)
Other	2	(8.7)
RapID NH	5	(21.7)
API NH	8	(34.8)
Individual biochemical tests	8	(34.8)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
III	15	
IV	5	
Total	20	

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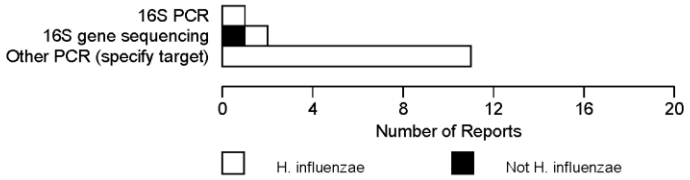
UK National External Quality Assessment Service for Microbiology

Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 15 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0267 Genotypic species ID
Intended result: *Haemophilus influenzae*

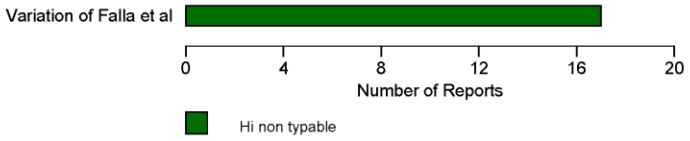


	All	(%)
16S PCR	1	(7.1)
16S gene sequencing	2	(14.3)
Other PCR (specify target)	11	(78.6)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	13	
Not H. influenzae	1	
Total	14	

Specimen : 0267 Genotypic capsular type
Intended result: Hi non typable



	All	(%)
Variation of Falla et al	17	(100)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
Hi non typable	18	
Total	18	

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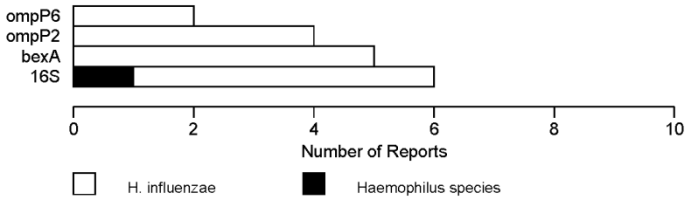
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 16 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0268 Non-culture species ID (molecular)
Intended result: *Haemophilus influenzae*



	All	(%)
ompP6	2	(11.8)
ompP2	4	(23.5)
bexA	5	(29.4)
16S	6	(35.3)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	16	
Haemophilus species	1	
Total	17	

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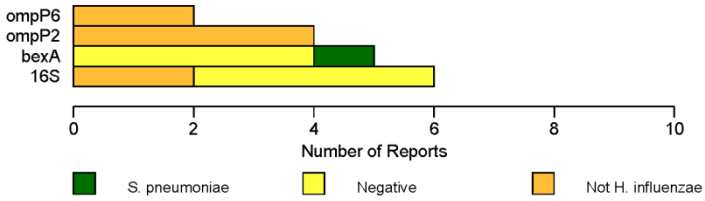
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 17 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0269 Non-culture species ID (molecular)
Intended result: Not *Haemophilus influenzae* / negative / *S. pneumoniae*



	All	(%)
ompP6	2	(11.8)
ompP2	4	(23.5)
bexA	5	(29.4)
16S	6	(35.3)

Your Report : Not returned
Your Score : **Not scored**

Overall Results	All	Score
S. pneumoniae	1	
Negative	8	
Not H. influenzae	8	
Total	17	

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Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 18 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0262 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
		All			All
Amoxicillin	susceptible	4	0	0	100
Ampicillin	susceptible	22	0	0	100
Co-amoxiclav	susceptible	14	0	0	100
Azithromycin	susceptible	10	1	1	83.3
Beta-lactamase	negative	27	0	0	100
Chloramphenicol	susceptible	15	0	0	100
Ciprofloxacin	susceptible	20	0	0	100
Ceftriaxone	susceptible	12	0	0	100
Cefotaxime	susceptible	17	0	0	100
Cefuroxime	susceptible	15	0	0	100
Rifampicin	susceptible	14	0	1	93.3
Tetracycline	susceptible	16	0	1	94.1
Trimethoprim	susceptible	2	0	0	100
Trimethorpim/sulp	susceptible	17	0	1	94.4

Specimen : 0263 H. parainfluenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
		All			All
Amoxicillin	susceptible	3	0	0	100
Ampicillin	susceptible	19	0	1	95.0
Co-amoxiclav	susceptible	13	0	0	100
Azithromycin	susceptible	9	2	0	81.8
Beta-lactamase	negative	25	0	0	100
Chloramphenicol	susceptible	12	1	0	92.3
Ciprofloxacin	susceptible	19	0	0	100
Ceftriaxone	susceptible	11	0	0	100
Cefotaxime	susceptible	16	0	0	100
Cefuroxime	susceptible	13	0	1	92.9
Rifampicin	susceptible	11	0	2	84.6
Tetracycline	susceptible	13	0	1	92.9
Trimethoprim	susceptible	1	0	0	100
Trimethorpim/sulp	susceptible	16	0	0	100

Specimen : 0264 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
		All			All
Amoxicillin	resistant	0	0	3	100
Ampicillin	resistant	2	9	12	52.2
Co-amoxiclav	resistant	5	0	9	64.3
Azithromycin	susceptible	10	1	1	83.3
Beta-lactamase	negative	27	0	0	100
Chloramphenicol	resistant	0	2	13	86.7
Ciprofloxacin	susceptible	21	0	0	100
Ceftriaxone	susceptible	13	0	1	92.9
Cefotaxime	susceptible	12	0	5	70.6
Cefuroxime	resistant	4	3	8	53.3
Rifampicin	susceptible	14	0	1	93.3
Tetracycline	resistant	2	2	13	76.5
Trimethoprim	resistant	0	0	2	100
Trimethorpim/sulp	resistant	4	2	12	66.7

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Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 19 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0265 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
		All			All
Amoxicillin	susceptible	4	0	0	100
Ampicillin	susceptible	23	0	0	100
Co-amoxiclav	susceptible	14	0	0	100
Azithromycin	susceptible	10	2	0	83.3
Beta-lactamase	negative	27	0	0	100
Chloramphenicol	susceptible	14	1	0	93.3
Ciprofloxacin	susceptible	20	0	0	100
Ceftriaxone	susceptible	13	0	0	100
Cefotaxime	susceptible	17	0	0	100
Cefuroxime	susceptible	15	0	0	100
Rifampicin	susceptible	14	0	1	93.3
Tetracycline	susceptible	15	1	1	88.2
Trimethoprim	susceptible	2	0	0	100
Trimethorpim/sulp	susceptible	18	0	0	100

Specimen : 0266 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
		All			All
Amoxicillin	resistant	0	0	2	100
Ampicillin	resistant	0	0	23	100
Co-amoxiclav	susceptible	13	0	1	92.9
Azithromycin	susceptible	10	2	0	83.3
Beta-lactamase	positive	0	0	27	100
Chloramphenicol	resistant	1	1	13	86.7
Ciprofloxacin	susceptible	20	0	0	100
Ceftriaxone	susceptible	13	0	0	100
Cefotaxime	susceptible	15	0	0	100
Cefuroxime	susceptible	15	0	0	100
Rifampicin	susceptible	15	0	0	100
Tetracycline	resistant	0	0	17	100
Trimethoprim	susceptible	2	0	0	100
Trimethorpim/sulp	susceptible	17	0	1	94.4

Specimen : 0267 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S	M/I	R	% of Laboratories with correct result
		All			All
Amoxicillin	resistant	0	1	2	66.7
Ampicillin	resistant	15	3	5	21.7
Co-amoxiclav	resistant	8	2	4	28.6
Azithromycin	susceptible	9	3	0	75.0
Beta-lactamase	negative	27	0	0	100
Chloramphenicol	susceptible	16	0	0	100
Ciprofloxacin	susceptible	21	0	0	100
Ceftriaxone	susceptible	15	0	0	100
Cefotaxime	susceptible	13	0	3	81.3
Cefuroxime	resistant	6	2	7	46.7
Rifampicin	susceptible	14	0	1	93.3
Tetracycline	susceptible	16	0	1	94.1
Trimethoprim	susceptible	2	0	0	100
Trimethorpim/sulp	susceptible	17	0	1	94.4

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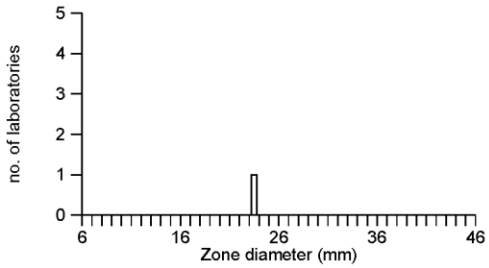
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 20 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0262

Amoxicillin - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 10



MIC results / counts

0.25	2

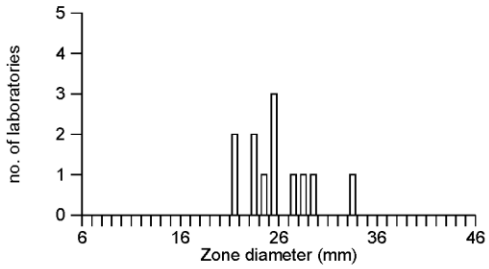
Amoxicillin

	S	I	R	% concordance
score				
Combined	1	0	0	100
CLSI	3	0	0	100
All	4	0	0	100.0
UK	0	0	0	

Ampicillin - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 10



MIC results / counts

0.064	1
0.12	1
0.125	2
0.19	1
0.25	5
0.50	1
0.75	1

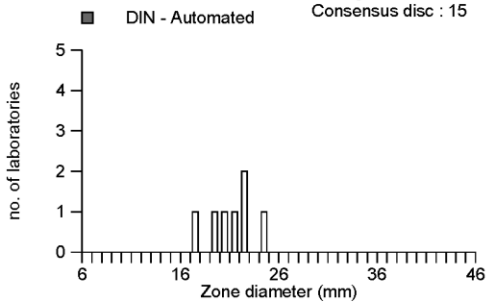
Ampicillin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	8	0	0	100
CLSI	13	0	0	100
All	22	0	0	100.0
UK	0	0	0	

Azithromycin - specimen 0262

Intended result : susceptible

Your guideline : **DIN**
Consensus disc : 15



MIC results / counts

1	1
2	1
4	1
8	1

Azithromycin

	S	I	R	% concordance
score				
EUCAST	2	1	1	50.0
CLSI	8	0	0	100
All	10	1	1	83.3
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 21 of 53
Dispatch Date : 14-Feb-2011	

Beta-lactamase - specimen 0262

Intended result : negative

Your guideline :
Consensus disc :

MIC results / counts

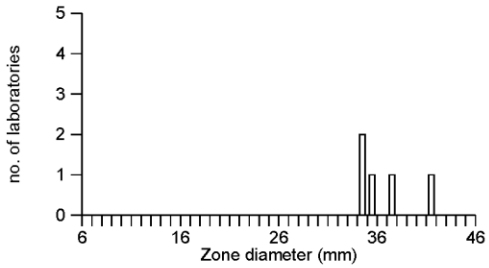
Beta-lactamase

	S	R	% concordance
score			
Combined	1	0	100
EUCAST	9	0	100
CLSI	14	0	100
NWGA	1	0	100
Other	1	0	100
All	27	0	100.0
UK	2	0	100.0

Cefotaxime - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.003	1
0.006	1
0.008	1
0.016	1
0.032	1
<=0.03	1
<0.016	5

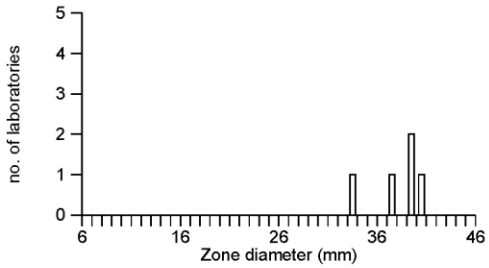
Cefotaxime

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	7	0	0	100
CLSI	9	0	0	100
All	17	0	0	100.0
UK	0	0	0	

Ceftriaxone - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.002	1
0.003	1
0.006	1
0.094	1
<0.016	4

Ceftriaxone

	S	I	R	% concordance
score				
EUCAST	4	0	0	100
CLSI	8	0	0	100
All	12	0	0	100.0
UK	0	0	0	

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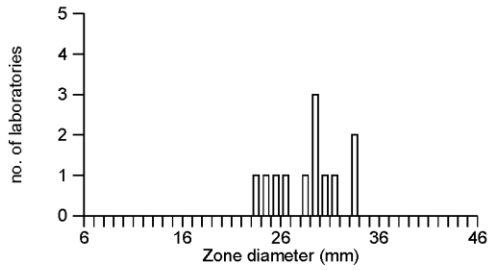
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 22 of 53
Dispatch Date : 14-Feb-2011	

Cefuroxime - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.5	2
0.50	1

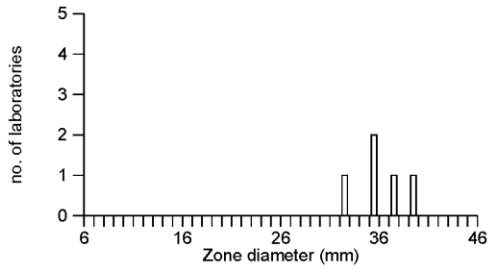
Cefuroxime

	S	I	R	% concordance
score				
EUCAST	3	0	0	100
CLSI	12	0	0	100
All	15	0	0	100.0
UK	0	0	0	

Chloramphenicol - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.19	1
0.25	2
0.5	1
0.50	1
1	2
1.0	2
<=1	1

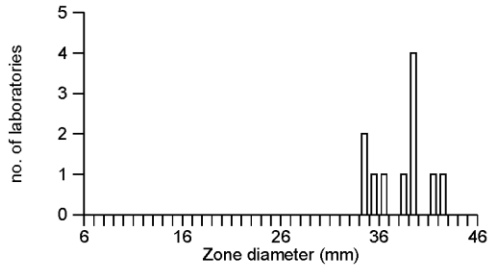
Chloramphenicol

	S	I	R	% concordance
score				
EUCAST	6	0	0	100
CLSI	9	0	0	100
All	15	0	0	100.0
UK	0	0	0	

Ciprofloxacin - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.003	1
0.004	1
0.008	4
0.023	1
0.04	1
<=0.06	1

Ciprofloxacin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	7	0	0	100
CLSI	12	0	0	100
All	20	0	0	100.0
UK	0	0	0	

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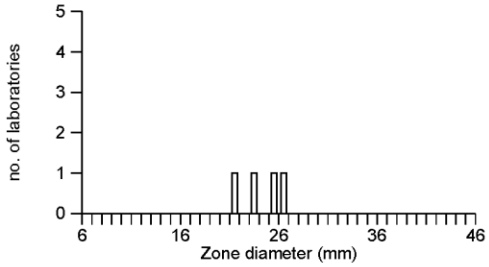
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 23 of 53
Dispatch Date : 14-Feb-2011	

Co-amoxiclav - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.25	2
0.38	1
0.5	1
<=0.25	1

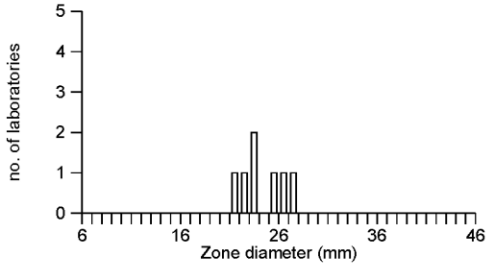
Co-amoxiclav

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	4	0	0	100
CLSI	9	0	0	100
All	14	0	0	100.0
UK	0	0	0	

Rifampicin - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.25	1
0.38	2
0.5	2
1.0	1
<=0.5	1

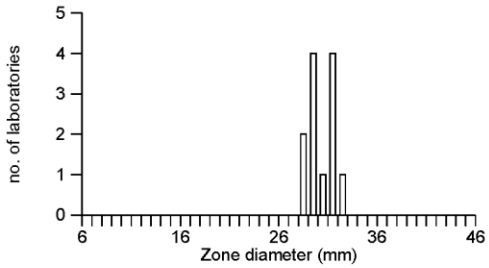
Rifampicin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	4	0	1	80.0
CLSI	9	0	0	100
All	14	0	1	93.3
UK	0	0	0	

Tetracycline - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.19	1
0.5	2
0.75	1
1.0	1
2	1
<=1	1

Tetracycline

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	3	0	1	75.0
CLSI	12	0	0	100
All	16	0	1	94.1
UK	0	0	0	

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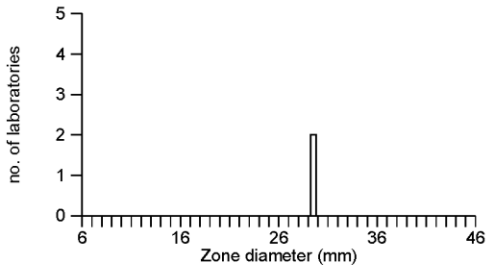
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 24 of 53
Dispatch Date : 14-Feb-2011	

Trimethoprim - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

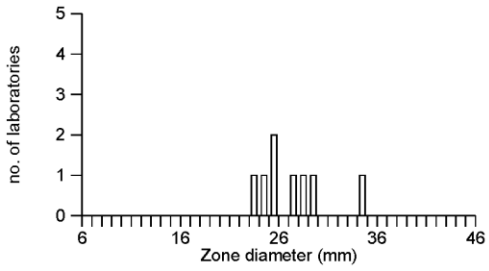
Trimethoprim

score	S	I	R	% concordance
Combined	1	0	0	100
CLSI	1	0	0	100
All	2	0	0	100.0
UK	0	0	0	

Trimethorpim/sulp - specimen 0262

Intended result : susceptible

Your guideline :
Consensus disc : 25



MIC results / counts

0.047	1
0.064	1
0.094	1
<=0.25	1
>32	1

Trimethorpim/sulp

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	2	0	1	66.7
CLSI	14	0	0	100
All	17	0	1	94.4
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 25 of 53
Dispatch Date : 14-Feb-2011	

Specimen : 0263

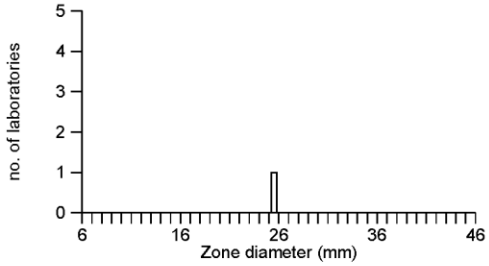
Amoxicillin - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 10

MIC results / counts

0.5	1



Amoxicillin

	S	I	R	% concordance
score				
CLSI	3	0	0	100
All	3	0	0	100.0
UK	0	0	0	

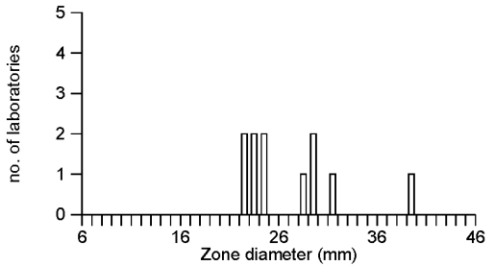
Ampicillin - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 10

MIC results / counts

0.125	1
0.25	4
0.38	3
0.5	1
1	1
1.5	1



Ampicillin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	6	0	1	85.7
CLSI	12	0	0	100
All	19	0	1	95.0
UK	0	0	0	

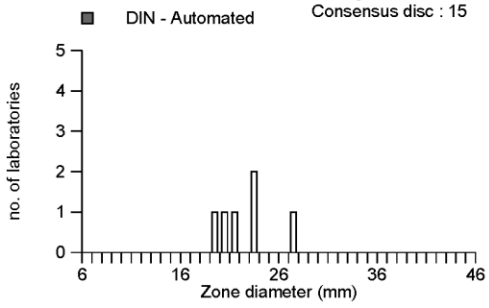
Azithromycin - specimen 0263

Intended result : susceptible

Your guideline : **DIN**
Consensus disc : 15

MIC results / counts

2	2
4	2



Azithromycin

	S	I	R	% concordance
score				
EUCAST	1	2	0	33.3
CLSI	8	0	0	100
All	9	2	0	81.8
UK	0	0	0	

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Beta-lactamase - specimen 0263

Intended result : negative

Your guideline :
Consensus disc :

MIC results / counts

Beta-lactamase

score	S	R	% concordance
Combined	1	0	100
EUCAST	8	0	100
CLSI	13	0	100
NWGA	1	0	100
Other	1	0	100
All	25	0	100.0
UK	2	0	100.0

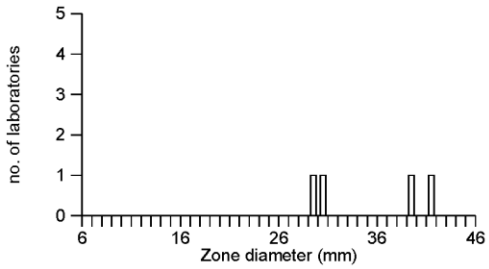
Cefotaxime - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.003	1
0.006	1
0.012	1
0.015	1
0.016	1
<=0.03	1
<0.016	4



Cefotaxime

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	6	0	0	100
CLSI	9	0	0	100
All	16	0	0	100.0
UK	0	0	0	

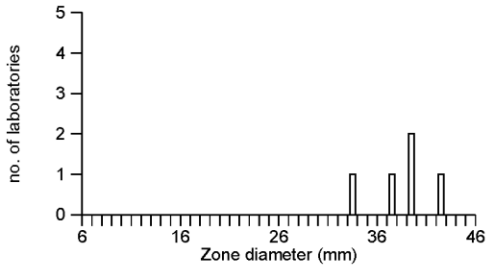
Ceftriaxone - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.002	1
0.003	1
<0.016	3
<0.16	1



Ceftriaxone

score	S	I	R	% concordance
EUCAST	4	0	0	100
CLSI	7	0	0	100
All	11	0	0	100.0
UK	0	0	0	

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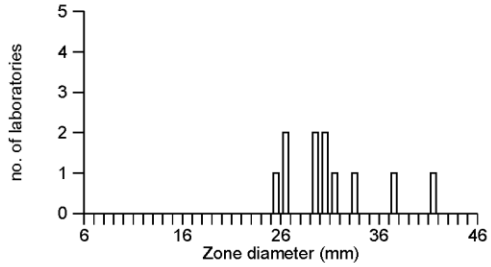
Cefuroxime - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.5	3
<=0.25	1

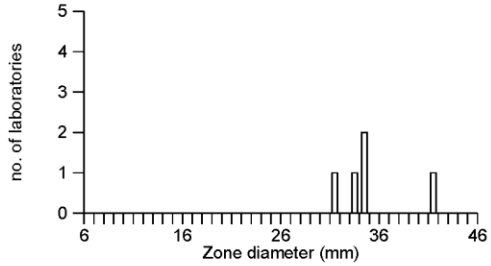
Cefuroxime

	S	I	R	% concordance
score				
EUCAST	1	0	1	50.0
CLSI	12	0	0	100
All	13	0	1	92.9
UK	0	0	0	

Chloramphenicol - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.25	1
0.38	1
0.5	2
1.0	1
1.5	2
<=1	1

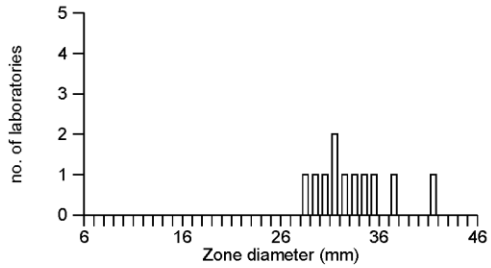
Chloramphenicol

	S	I	R	% concordance
score				
EUCAST	4	1	0	80.0
CLSI	8	0	0	100
All	12	1	0	92.3
UK	0	0	0	

Ciprofloxacin - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.012	1
0.015	1
0.016	3
0.023	1
0.08	1
<=0.06	1

Ciprofloxacin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	6	0	0	100
CLSI	12	0	0	100
All	19	0	0	100.0
UK	0	0	0	

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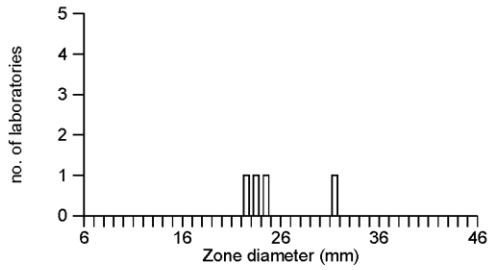
Co-amoxiclav - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.38	2
0.5	3
1	1

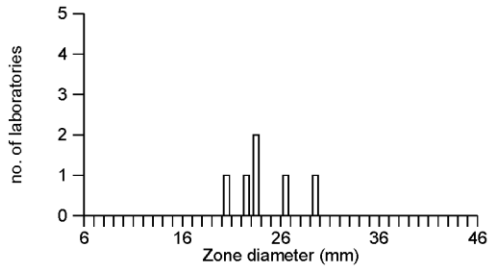
Co-amoxiclav

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	3	0	0	100
CLSI	9	0	0	100
All	13	0	0	100.0
UK	0	0	0	

Rifampicin - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.25	1
0.75	2
1	1
2	1

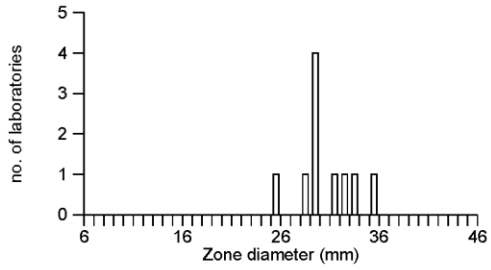
Rifampicin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	2	0	2	50.0
CLSI	8	0	0	100
All	11	0	2	84.6
UK	0	0	0	

Tetracycline - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.25	1
0.5	2
0.75	1
2	1
<=1	1

Tetracycline

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	2	0	1	66.7
CLSI	10	0	0	100
All	13	0	1	92.9
UK	0	0	0	

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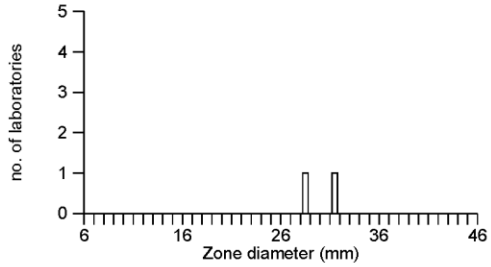
Trimethoprim - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

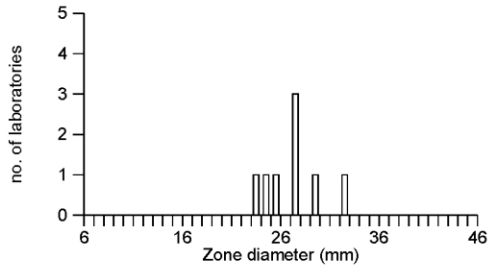
Trimethoprim

score	S	I	R	% concordance
CLSI	1	0	0	100
All	1	0	0	100.0
UK	0	0	0	

Trimethorpim/sulp - specimen 0263

Intended result : susceptible

Your guideline :
Consensus disc : 25



MIC results / counts

0.064	1
0.094	2
<=0.25	1

Trimethorpim/sulp

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	2	0	0	100
CLSI	13	0	0	100
All	16	0	0	100.0
UK	0	0	0	

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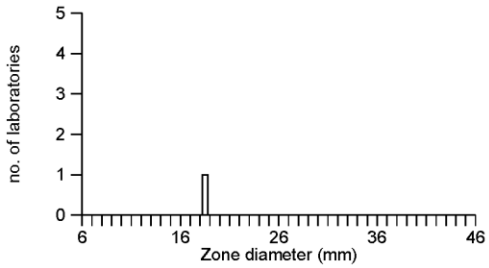
Specimen : 0264

Amoxicillin - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 10



MIC results / counts

8.0	1

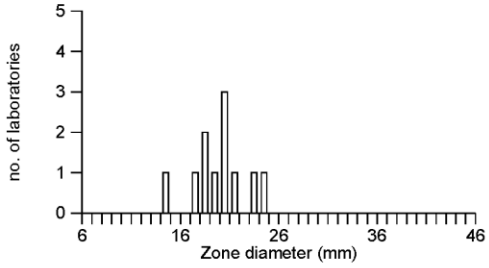
Amoxicillin

	S	I	R	% concordance
score				
Combined	0	0	1	100
CLSI	0	0	2	100
All	0	0	3	100.0
UK	0	0	0	

Ampicillin - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 10



MIC results / counts

1.5	4
2	5
2.5	1
3	1
3.0	1
4	3

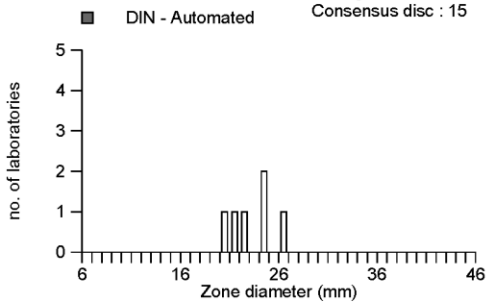
Ampicillin

	S	I	R	% concordance
score				
Combined	0	0	1	100
EUCAST	1	0	7	87.5
CLSI	1	9	4	28.6
All	2	9	12	52.2
UK	0	0	0	

Azithromycin - specimen 0264

Intended result : susceptible

Your guideline : **DIN**
Consensus disc : 15



MIC results / counts

2	2
4	1
6	1

Azithromycin

	S	I	R	% concordance
score				
EUCAST	2	1	1	50.0
CLSI	8	0	0	100
All	10	1	1	83.3
UK	0	0	0	

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Beta-lactamase - specimen 0264

Intended result : negative

Your guideline :
Consensus disc :

MIC results / counts

Beta-lactamase

score	S	R	% concordance
Combined	1	0	100
EUCAST	9	0	100
CLSI	14	0	100
NWGA	1	0	100
Other	1	0	100
All	27	0	100.0
UK	2	0	100.0

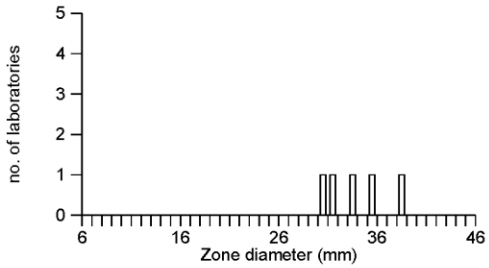
Cefotaxime - specimen 0264

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.064	1
0.12	1
0.125	1
0.19	1
0.25	4
0.38	2
0.5	1



Cefotaxime

score	S	I	R	% concordance
Combined	0	0	1	0.0
EUCAST	3	0	4	42.9
CLSI	9	0	0	100
All	12	0	5	70.6
UK	0	0	0	

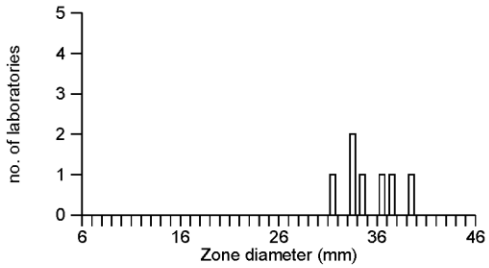
Ceftriaxone - specimen 0264

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.064	3
0.094	2
0.125	2
0.25	1
2.0	1



Ceftriaxone

score	S	I	R	% concordance
Combined	0	0	1	0.0
EUCAST	4	0	0	100
CLSI	9	0	0	100
All	13	0	1	92.9
UK	0	0	0	

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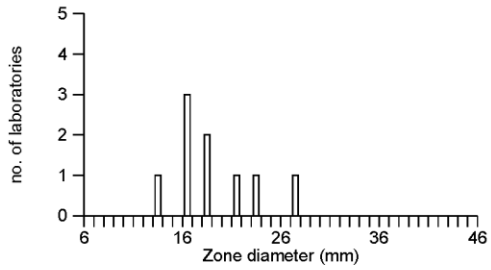
Cefuroxime - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results	counts
4	1
8	1
16	1
24	1
32	1

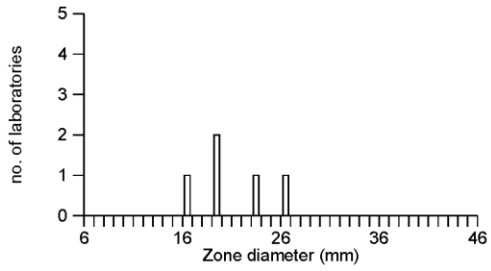
Cefuroxime

score	S	I	R	% concordance
EUCAST	1	0	2	66.7
CLSI	3	3	6	50.0
All	4	3	8	53.3
UK	0	0	0	

Chloramphenicol - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results	counts
4	3
8	2
8.0	1
12	1
16	2
24	1

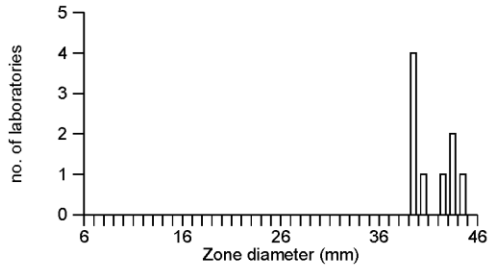
Chloramphenicol

score	S	I	R	% concordance
EUCAST	0	0	6	100
CLSI	0	2	7	77.8
All	0	2	13	86.7
UK	0	0	0	

Ciprofloxacin - specimen 0264

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results	counts
0.004	1
0.006	2
0.008	4
0.023	1
<=0.06	1

Ciprofloxacin

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	7	0	0	100
CLSI	13	0	0	100
All	21	0	0	100.0
UK	0	0	0	

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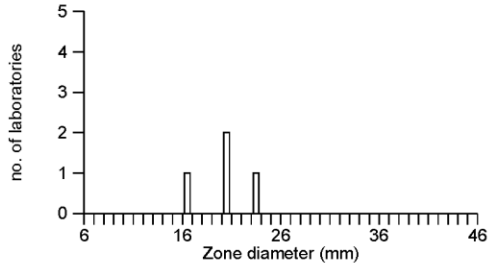
Co-amoxiclav - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results / counts

2	2
3	2
8	1

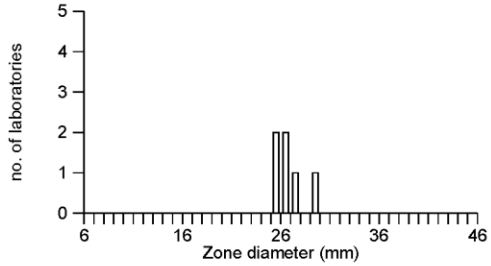
Co-amoxiclav

	S	I	R	% concordance
score				
Combined	0	0	1	100
EUCAST	1	0	3	75.0
CLSI	4	0	5	55.6
All	5	0	9	64.3
UK	0	0	0	

Rifampicin - specimen 0264

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.25	1
0.38	2
0.5	1
0.75	1
1.5	1
<=0.5	1

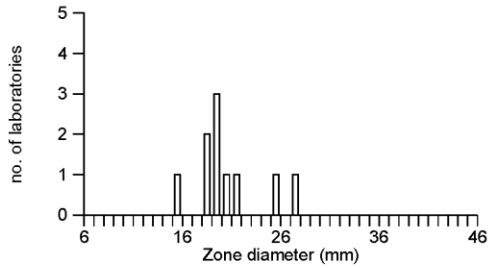
Rifampicin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	4	0	1	80.0
CLSI	9	0	0	100
All	14	0	1	93.3
UK	0	0	0	

Tetracycline - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results / counts

2	1
4	1
4.0	1
6	1
6.0	1
8	2
12	1

Tetracycline

	S	I	R	% concordance
score				
Combined	1	0	0	0.0
EUCAST	0	0	4	100
CLSI	1	2	9	75.0
All	2	2	13	76.5
UK	0	0	0	

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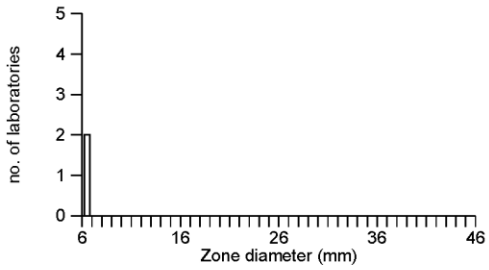
Trimethoprim - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 5



MIC results / counts

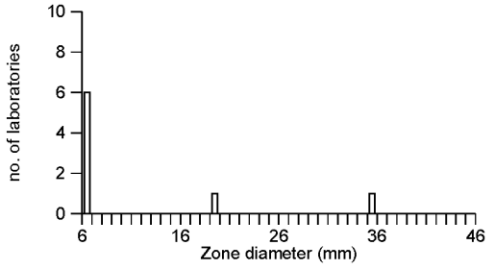
Trimethoprim

score	S	I	R	% concordance
Combined	0	0	1	100
CLSI	0	0	1	100
All	0	0	2	100.0
UK	0	0	0	

Trimethorpim/sulp - specimen 0264

Intended result : resistant

Your guideline :
Consensus disc : 25



MIC results / counts

1.0	1
8	1
16	1
>2	1
>32	2

Trimethorpim/sulp

score	S	I	R	% concordance
Combined	0	0	1	100
EUCAST	1	0	2	66.7
CLSI	3	2	9	64.3
All	4	2	12	66.7
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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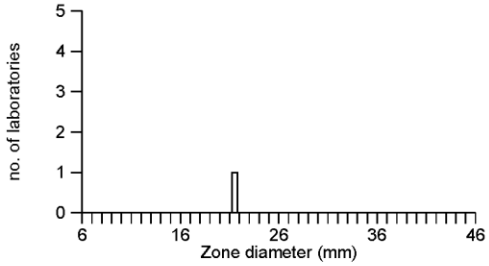
Dispatch Date : 14-Feb-2011	

Specimen : 0265

Amoxicillin - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 10



MIC results / counts

0.38	1

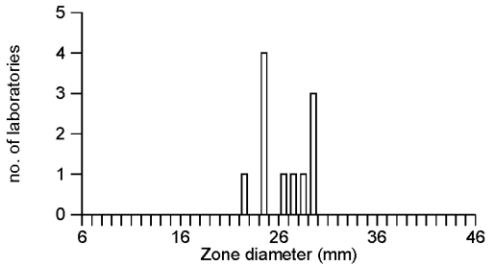
Amoxicillin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	1	0	0	100
CLSI	2	0	0	100
All	4	0	0	100.0
UK	0	0	0	

Ampicillin - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 10



MIC results / counts

0.125	2
0.19	3
0.25	3
0.38	2
0.5	1
0.50	1
1	1

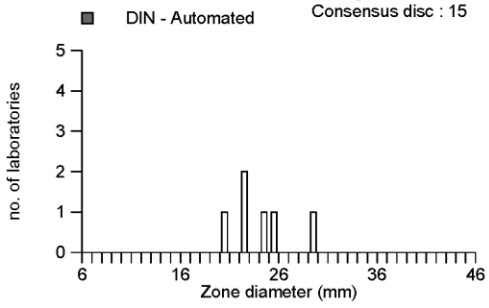
Ampicillin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	8	0	0	100
CLSI	14	0	0	100
All	23	0	0	100.0
UK	0	0	0	

Azithromycin - specimen 0265

Intended result : susceptible

Your guideline : **DIN**
Consensus disc : 15



MIC results / counts

1	1
1.5	1
3	1
4	1

Azithromycin

	S	I	R	% concordance
score				
EUCAST	2	2	0	50.0
CLSI	8	0	0	100
All	10	2	0	83.3
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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Beta-lactamase - specimen 0265

Intended result : negative

Your guideline :
Consensus disc :

MIC results / counts

Beta-lactamase

score	S	R	% concordance
Combined	1	0	100
EUCAST	9	0	100
CLSI	14	0	100
NWGA	1	0	100
Other	1	0	100
All	27	0	100.0
UK	2	0	100.0

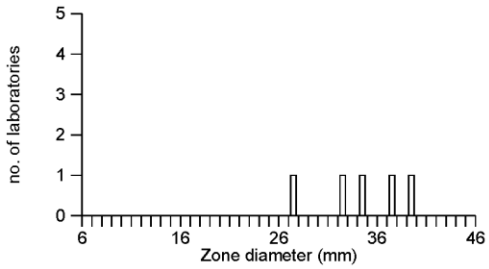
Cefotaxime - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.008	1
0.016	3
0.023	3
0.03	1
0.032	1
0.064	1
<=0.03	1



Cefotaxime

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	7	0	0	100
CLSI	9	0	0	100
All	17	0	0	100.0
UK	0	0	0	

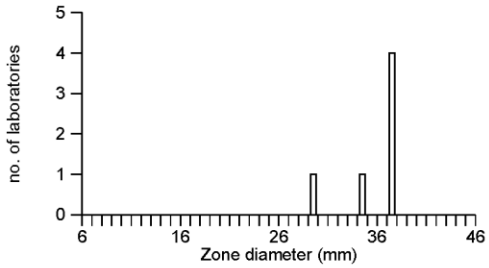
Ceftriaxone - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.006	2
0.008	1
0.25	1
<0.016	3



Ceftriaxone

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	4	0	0	100
CLSI	8	0	0	100
All	13	0	0	100.0
UK	0	0	0	

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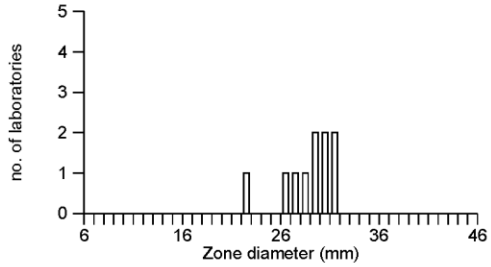
Cefuroxime - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.5	1
1	2

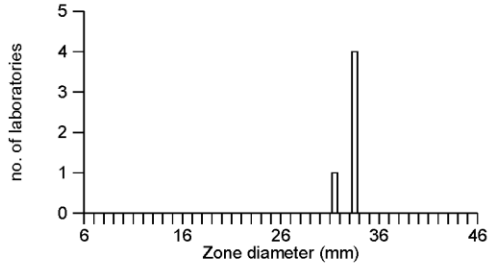
Cefuroxime

	S	I	R	% concordance
score				
EUCAST	3	0	0	100
CLSI	12	0	0	100
All	15	0	0	100.0
UK	0	0	0	

Chloramphenicol - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.38	2
0.5	2
0.75	2
1.5	3
<=1	1

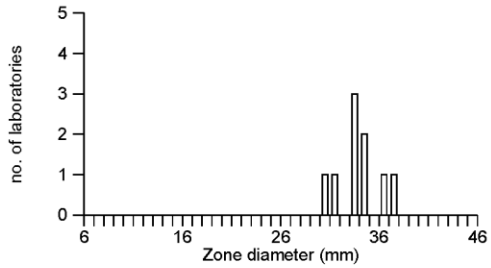
Chloramphenicol

	S	I	R	% concordance
score				
EUCAST	5	1	0	83.3
CLSI	9	0	0	100
All	14	1	0	93.3
UK	0	0	0	

Ciprofloxacin - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.008	2
0.015	1
0.016	4
0.064	1
<=0.06	1

Ciprofloxacin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	7	0	0	100
CLSI	12	0	0	100
All	20	0	0	100.0
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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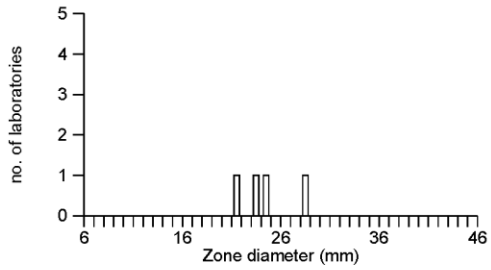
Co-amoxiclav - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.25	4
0.50	1
<=0.25	1

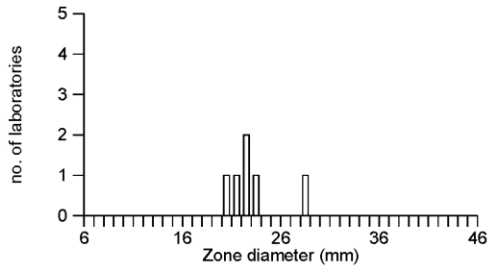
Co-amoxiclav

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	4	0	0	100
CLSI	9	0	0	100
All	14	0	0	100.0
UK	0	0	0	

Rifampicin - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.25	2
0.38	1
0.5	2
1.5	1
<=0.5	1

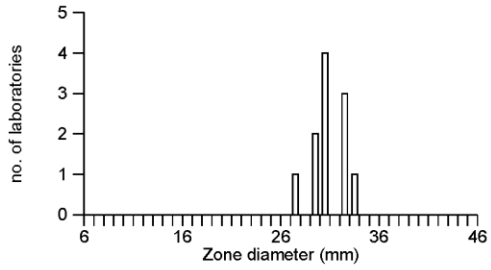
Rifampicin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	4	0	1	80.0
CLSI	9	0	0	100
All	14	0	1	93.3
UK	0	0	0	

Tetracycline - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.25	1
0.5	2
0.75	1
1.0	1
2	1
<=1	1

Tetracycline

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	3	0	1	75.0
CLSI	11	1	0	91.7
All	15	1	1	88.2
UK	0	0	0	

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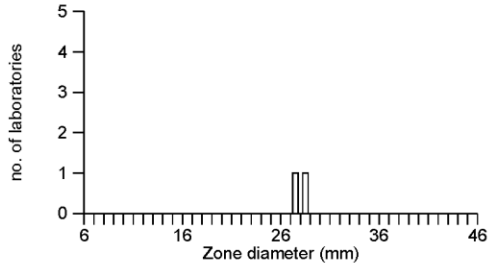
Trimethoprim - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

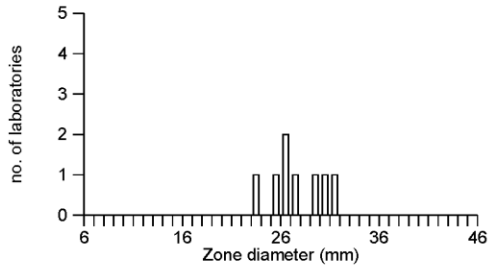
Trimethoprim

	S	I	R	% concordance
score				
Combined	1	0	0	100
CLSI	1	0	0	100
All	2	0	0	100.0
UK	0	0	0	

Trimethorpim/sulp - specimen 0265

Intended result : susceptible

Your guideline :
Consensus disc : 25



MIC results / counts

0.047	1
0.064	1
0.125	2
<=0.25	1

Trimethorpim/sulp

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	3	0	0	100
CLSI	14	0	0	100
All	18	0	0	100.0
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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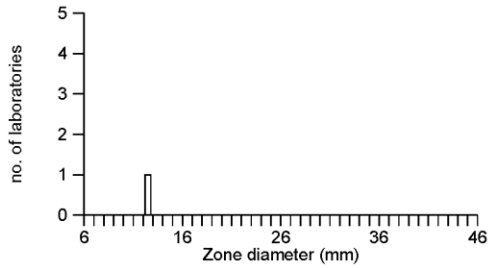
Specimen : 0266

Amoxicillin - specimen 0266

Intended result : resistant

Your guideline :
Consensus disc : 10



MIC results / counts

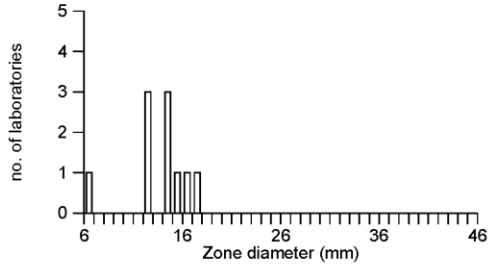
Amoxicillin

	S	I	R	% concordance
score				
CLSI	0	0	2	100
All	0	0	2	100.0
UK	0	0	0	

Ampicillin - specimen 0266

Intended result : resistant

Your guideline :
Consensus disc : 10



MIC results / counts

3	1
4	2
8	1
12	1
16	1
24.0	1
64	1
>256	3
>8	1

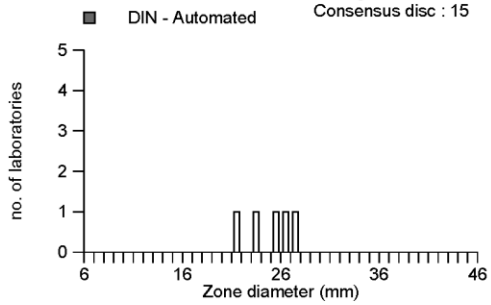
Ampicillin

	S	I	R	% concordance
score				
Combined	0	0	1	100
EUCAST	0	0	8	100
CLSI	0	0	14	100
All	0	0	23	100.0
UK	0	0	0	

Azithromycin - specimen 0266

Intended result : susceptible

Your guideline : **DIN**
Consensus disc : 15



MIC results / counts

0.5	1
1.5	1
2	2

Azithromycin

	S	I	R	% concordance
score				
EUCAST	2	2	0	50.0
CLSI	8	0	0	100
All	10	2	0	83.3
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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Beta-lactamase - specimen 0266

Intended result : positive

Your guideline :
Consensus disc :

MIC results / counts

Beta-lactamase

score	S	R	% concordance
Combined	0	1	100
EUCAST	0	9	100
CLSI	0	14	100
NWGA	0	1	100
Other	0	1	100
All	0	27	100.0
UK	0	2	100.0

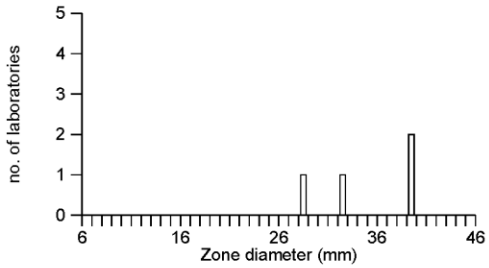
Cefotaxime - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.008	2
0.015	1
0.016	1
0.032	1
<=0.03	1
<0.016	4



Cefotaxime

score	S	I	R	% concordance
EUCAST	6	0	0	100
CLSI	9	0	0	100
All	15	0	0	100.0
UK	0	0	0	

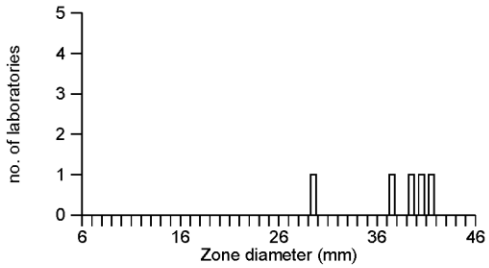
Ceftriaxone - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.002	1
0.003	1
0.004	1
0.064	1
<0.016	3



Ceftriaxone

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	4	0	0	100
CLSI	8	0	0	100
All	13	0	0	100.0
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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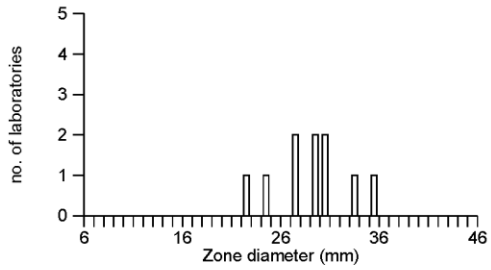
Cefuroxime - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.75	2
<=0.25	1

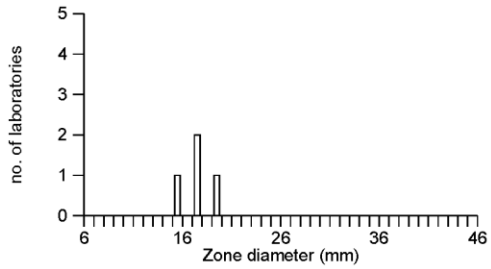
Cefuroxime

	S	I	R	% concordance
score				
EUCAST	3	0	0	100
CLSI	12	0	0	100
All	15	0	0	100.0
UK	0	0	0	

Chloramphenicol - specimen 0266

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results / counts

2	1
4	3
6	1
6.0	1
8	2
12.0	1
16	2

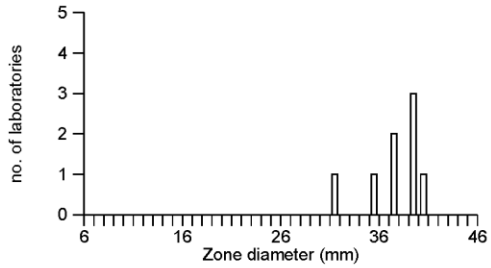
Chloramphenicol

	S	I	R	% concordance
score				
EUCAST	0	0	6	100
CLSI	1	1	7	77.8
All	1	1	13	86.7
UK	0	0	0	

Ciprofloxacin - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.004	2
0.006	2
0.008	2
0.023	1
0.12	1
<=0.06	1

Ciprofloxacin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	7	0	0	100
CLSI	12	0	0	100
All	20	0	0	100.0
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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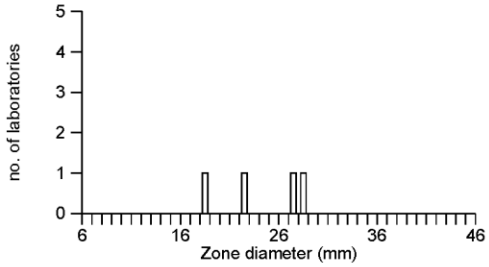
Co-amoxiclav - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts	
0.25	4
0.5	2
1	1

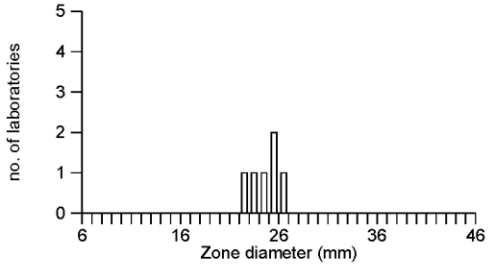
Co-amoxiclav

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	4	0	0	100
CLSI	8	0	1	88.9
All	13	0	1	92.9
UK	0	0	0	

Rifampicin - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts	
0.125	1
0.19	3
0.38	1
0.5	1
<=0.5	1

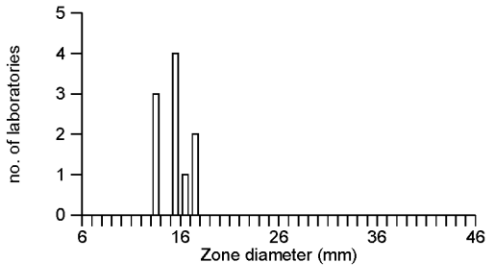
Rifampicin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	5	0	0	100
CLSI	9	0	0	100
All	15	0	0	100.0
UK	0	0	0	

Tetracycline - specimen 0266

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results / counts	
8	3
8.0	1
12	1
16.0	1
>4	1

Tetracycline

	S	I	R	% concordance
score				
Combined	0	0	1	100
EUCAST	0	0	4	100
CLSI	0	0	12	100
All	0	0	17	100.0
UK	0	0	0	

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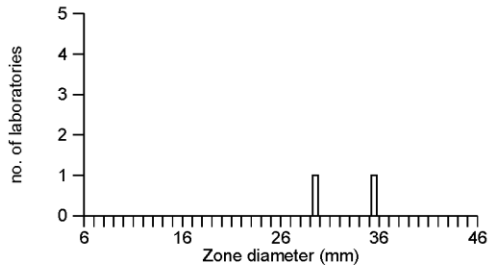
Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 44 of 53
Dispatch Date : 14-Feb-2011	

Trimethoprim - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

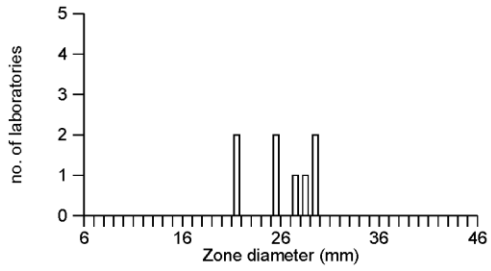
Trimethoprim

	S	I	R	% concordance
score				
Combined	1	0	0	100
CLSI	1	0	0	100
All	2	0	0	100.0
UK	0	0	0	

Trimethorpim/sulp - specimen 0266

Intended result : susceptible

Your guideline :
Consensus disc : 25



MIC results / counts

0.047	1
0.064	1
0.125	1
<=0.25	1
>32	1

Trimethorpim/sulp

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	2	0	1	66.7
CLSI	14	0	0	100
All	17	0	1	94.4
UK	0	0	0	

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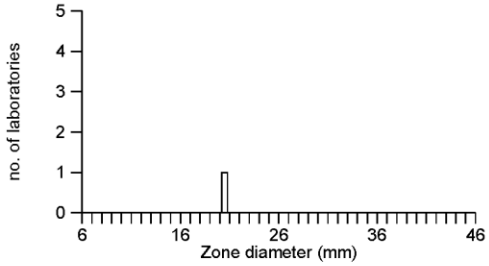
Specimen : 0267

Amoxicillin - specimen 0267

Intended result : resistant

Your guideline :
Consensus disc : 10



MIC results / counts

4.0	1

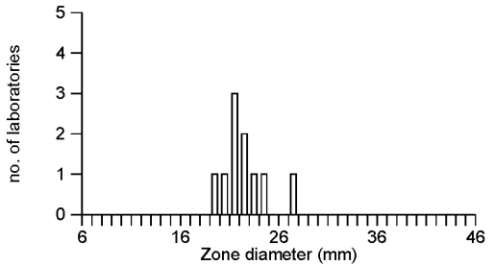
Amoxicillin

	S	I	R	% concordance
score				
Combined	0	0	1	100
CLSI	0	1	1	50.0
All	0	1	2	66.7
UK	0	0	0	

Ampicillin - specimen 0267

Intended result : resistant

Your guideline :
Consensus disc : 10



MIC results / counts

0.19	1
0.5	1
0.75	1
1	6
1.0	1
1.5	2
2	3
4	1

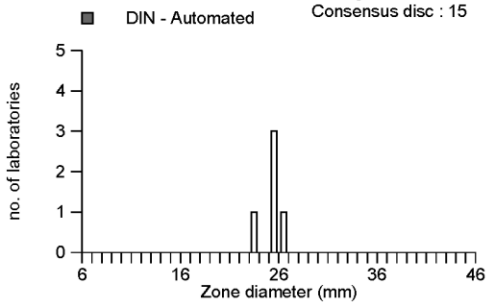
Ampicillin

	S	I	R	% concordance
score				
Combined	0	0	1	100
EUCAST	6	0	2	25.0
CLSI	9	3	2	14.3
All	15	3	5	21.7
UK	0	0	0	

Azithromycin - specimen 0267

Intended result : susceptible

Your guideline : **DIN**
Consensus disc : 15



MIC results / counts

1	1
2	2
4	1

Azithromycin

	S	I	R	% concordance
score				
EUCAST	2	2	0	50.0
CLSI	7	1	0	87.5
All	9	3	0	75.0
UK	0	0	0	

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Beta-lactamase - specimen 0267

Intended result : negative

Your guideline :
Consensus disc :

MIC results / counts

Beta-lactamase

score	S	R	% concordance
Combined	1	0	100
EUCAST	9	0	100
CLSI	14	0	100
NWGA	1	0	100
Other	1	0	100
All	27	0	100.0
UK	2	0	100.0

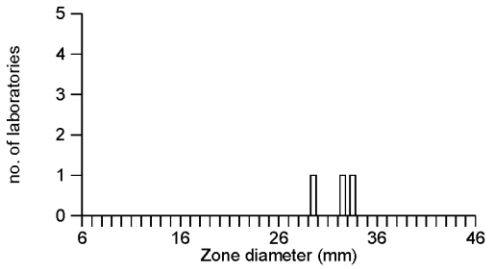
Cefotaxime - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.064	1
0.12	2
0.125	4
0.19	2
0.25	2
0.5	1



Cefotaxime

score	S	I	R	% concordance
Combined	0	0	1	0.0
EUCAST	5	0	2	71.4
CLSI	8	0	0	100
All	13	0	3	81.3
UK	0	0	0	

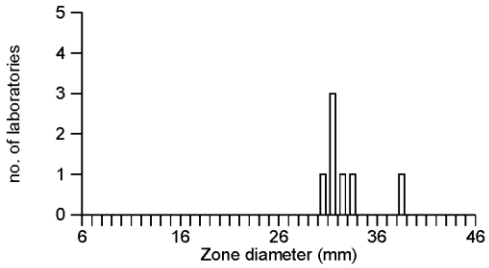
Ceftriaxone - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 30

MIC results / counts

0.047	1
0.06	1
0.064	3
0.094	2
0.125	1



Ceftriaxone

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	4	0	0	100
CLSI	10	0	0	100
All	15	0	0	100.0
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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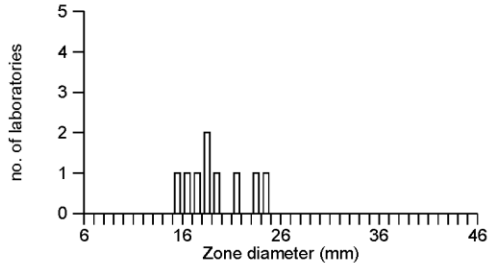
Cefuroxime - specimen 0267

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results / counts

24	2
32	2
>8	1

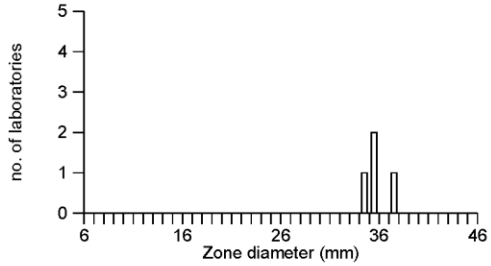
Cefuroxime

	S	I	R	% concordance
score				
EUCAST	1	0	2	66.7
CLSI	5	2	5	41.7
All	6	2	7	46.7
UK	0	0	0	

Chloramphenicol - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.38	2
0.5	3
0.75	1
1	2
2.0	1
<=1	1

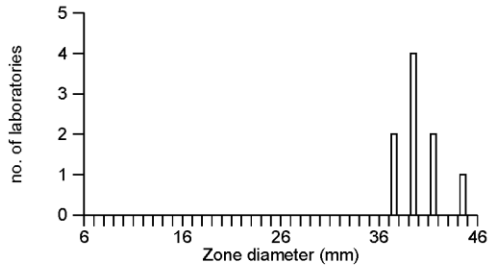
Chloramphenicol

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	6	0	0	100
CLSI	9	0	0	100
All	16	0	0	100.0
UK	0	0	0	

Ciprofloxacin - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.004	2
0.006	1
0.008	4
0.032	1
<=0.06	1

Ciprofloxacin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	7	0	0	100
CLSI	13	0	0	100
All	21	0	0	100.0
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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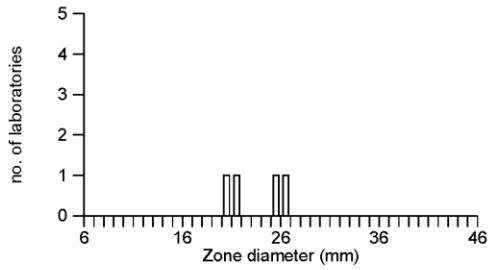
Co-amoxiclav - specimen 0267

Intended result : resistant

Your guideline :
Consensus disc : 30



MIC results / counts

1.5	1
2	2
3	3

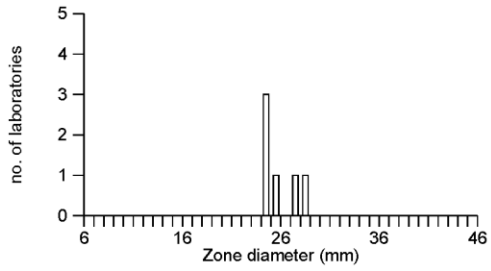
Co-amoxiclav

	S	I	R	% concordance
score				
Combined	0	0	1	100
EUCAST	1	1	2	50.0
CLSI	7	1	1	11.1
All	8	2	4	28.6
UK	0	0	0	

Rifampicin - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

0.25	3
0.5	2
1.5	1
<=0.5	1

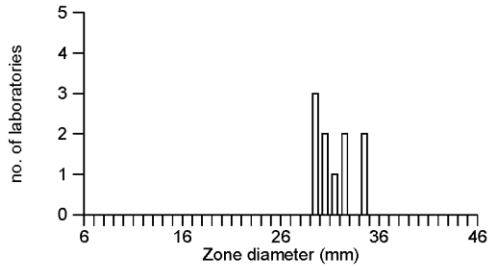
Rifampicin

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	4	0	1	80.0
CLSI	9	0	0	100
All	14	0	1	93.3
UK	0	0	0	

Tetracycline - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 30



MIC results / counts

0.25	1
0.38	1
0.5	1
0.75	1
1.5	1
2	1
<=1	1

Tetracycline

	S	I	R	% concordance
score				
Combined	1	0	0	100
EUCAST	3	0	1	75.0
CLSI	12	0	0	100
All	16	0	1	94.1
UK	0	0	0	

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Haemophilus influenzae	Laboratory : **9999**
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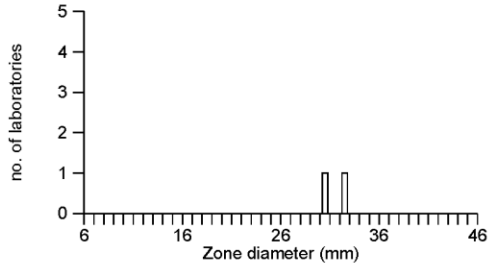
Trimethoprim - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 5



MIC results / counts

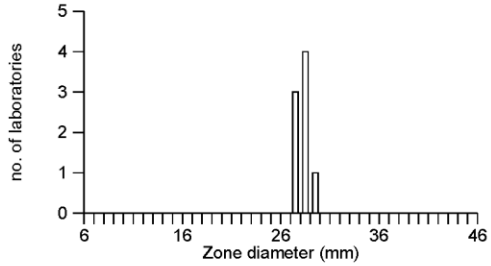
Trimethoprim

score	S	I	R	% concordance
Combined	1	0	0	100
CLSI	1	0	0	100
All	2	0	0	100.0
UK	0	0	0	

Trimethorpim/sulp - specimen 0267

Intended result : susceptible

Your guideline :
Consensus disc : 25



MIC results / counts

0.064	1
0.094	2
<=0.25	1
>32	1

Trimethorpim/sulp

score	S	I	R	% concordance
Combined	1	0	0	100
EUCAST	2	0	1	66.7
CLSI	14	0	0	100
All	17	0	1	94.4
UK	0	0	0	

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PART 3

Genotypic DNA extraction method	Non-culture DNA extraction method	Non-culture detection method	Combination count
Manual / in-house	Manual / in-house	PCR & gel electrophoresis	1
Manual / in-house	Manual / commercial kit	PCR & gel electrophoresis	2
Manual / in-house	Automated / commercial kit	Real-time PCR platform	2
Manual / in-house	Other	Real-time PCR platform	1
Manual / in-house	NE	NE	1
Manual / commercial kit	Manual / commercial kit	PCR & gel electrophoresis	2
Manual / commercial kit	Manual / commercial kit	Real-time PCR platform	2
Manual / commercial kit	Manual / commercial kit	PCR & sequencing	2
Manual / commercial kit	NE	NE	1
Automated / in-house	Automated / in-house	Real-time PCR platform	1
Automated / commercial kit	Automated / commercial kit	Real-time PCR platform	2
Other	Manual / commercial kit	PCR & sequencing	1
Other	Automated / commercial kit	PCR & gel electrophoresis	1

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COMMENTS for NEQAS Haemophilus influenzae EQA distribution 2802

General comments

The EQA panel comprised of 6 cultures and 2 simulated CSF samples. For the first part of the EQA, the cultures were tested for phenotypic species ID, phenotypic serotype, biotype, genotypic species ID and genotypic capsular type. For the second part, the cultures were tested for antimicrobial susceptibility and β-lactamase activity. For the third part of the EQA the simulated CSF samples were analysed for non-culture species identification.

Pages 1 to 3 of the report summarise the intended results and your results of each test for each sample. In some cases more than one result was acceptable, and "multiple - see comments" is given as the intended result. See comments below for further explanation. Some tests are not applicable to sample 0263 and "N/A" is given as the intended result. The final column headed "Your Score" is not in use (and all results are listed as "Not scored").
Your results are automatically generated from the data you entered via the web interface. If you entered a result in a non-standard way (e.g. putting it in a "comments" field) you may appear to have the wrong result in the summary. A more in-depth analysis of the results (including data in "comments" fields) will be published in the final report to ECDC.

Pages 4 to 15 summarise the results of part 1 of the EQA (species ID and typing). If you submitted a result for a particular test, the method you used is indicated on the summary graph using an arrow.
Please note that the summary graph for "Phenotypic species ID" (pages 4, 6, 8, 10, 12 and 14) appears to show the results by method used (e.g. Vitek, XV factors etc.) This is not a true representation of all the methods used, as the graph was automatically generated from only the three fields in which participants could enter their choice of method (further information submitted in a comments field was not included).
In a few of the other graphs, one of the answers in the graph legend does not appear in the actual graph. This occurs when a laboratory does not submit any details of the method they used for this test. Due to the way the graphs are automatically generated, their result is not included in the graph even though it appears in the legend. The associated table of results is not affected by this problem.

Pages 16 and 17 summarise the results of part 3 of the EQA (non-culture ID).

Pages 18 to 49 summarise the results of part 2 of the EQA (antimicrobial susceptibilities). For each test, disc diffusion zone sizes obtained by the participants are summarised in graphical form. Only the zone sizes for participants using the consensus disc concentration for that antibiotic are presented. Grey bars in the graph highlight the results from other laboratories following the same interpretation guideline as you, and an arrow shows your result (if applicable). MIC results are summarised in the small adjacent table. The table on the right hand side is a summary of Sensitive/Intermediate/Resistant scoring submitted by the laboratories, stratified by guideline followed.

Page 50 summarises the methods used in part 3 (non-culture ID).

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Haemophilus influenzae	Laboratory : **9999**
Distribution : **2802**	Page 52 of 53

Expected results and notes for the EQA panel cultures (0262 – 0267):

0262
This is a non-capsulated strain of *Haemophilus influenzae*. It is biotype IV, β-lactamase negative and is fully susceptible to amoxicillin, ampicillin, co-amoxyclav, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, cefotaxime, cefuroxime, rifampicin, tetracycline and trimethoprim.
This strain may cross-react with serotype d antiserum when tested by slide agglutination. Therefore, valid serotyping results would include "Hi non typable", "Hid" and "non specific agglutination". Capsular genotyping will confirm that this is a non-capsulated strain (i.e. not Hid).

0263
This is a strain of *Haemophilus parainfluenzae*. It is biotype V (according to the *H. parainfluenzae* scoring scheme), but may also be described as non-typable, depending on the method being used for the biochemical analysis. The strain is β-lactamase negative and fully susceptible to amoxicillin, ampicillin, co-amoxyclav, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, cefotaxime, cefuroxime, rifampicin, tetracycline and trimethoprim.
Phenotypic serotyping is not applicable if *H. influenzae* antisera are used. The genotypic ID results of "*Haemophilus parainfluenzae*" and "Not *H. influenzae*" are both valid.

0264
This is a non-capsulated strain of *Haemophilus influenzae*; biotype V and β-lactamase negative. This strain was resistant to amoxicillin, ampicillin, co-amoxyclav, chloramphenicol, cefuroxime, cefaclor, tetracycline and trimethoprim. This was a β-lactamase-negative ampicillin resistant (BLNAR) strain.

0265
This strain is *Haemophilus influenzae* serotype f (Hif), biotype I. It is β -lactamase negative and fully susceptible to amoxicillin, ampicillin, co-amoxyclav, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, cefotaxime, cefuroxime, rifampicin, tetracycline and trimethoprim.

0266
This is a *Haemophilus influenzae,* serotype b, biotype IV strain. It is β -lactamase positive and resistant to amoxicillin, ampicillin, chloramphenicol and tetracycline.

0267
This is a non-capsulated strain of *Haemophilus influenzae*, biotype III. It is β-lactamase negative and is a low-level β-lactamase negative ampicillin resistant (BLNAR) strain (which has been confirmed by partial sequencing of the *ftsI* gene). As the MIC of ampicillin is 1µg/ml it would be deemed susceptible by both EUCAST and CLSI guidelines. The MIC of co-amoxyclav is 2µg/ml and for Cefuroxime is 8µg/ml, both of which would be regarded as resistant by EUCAST but intermediate by CLSI guidelines.

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Haemophilus influenzae	Laboratory : **9999**
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Expected results and notes for the EQA panel simulated CSF samples (0268 – 0269):
These samples contained bacterial suspensions that had been killed by heating to 100°C for 10 minutes before addition to a simulated CSF buffer. Bacterial cfu/ml were calculated by plating out serial dilutions of each suspension before heat killing.

0268
This was a simulated CSF containing ~2 x10^5 cfu/ml of *H. influenzae* serotype b strain ATCC10211. Hence, the intended result was "*Haemophilus influenzae*" (plus the optional further information of "capsule type b").

0269
This was a simulated CSF containing ~2 x10^5 cfu/ml of *Streptococcus pneumoniae* strain NCTC7465. Hence, a reported result of "*Not H. influenzae*", "Negative" or "Other – *S. pneumoniae*" would be acceptable, depending on the method and reporting conventions used by individual laboratories.

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