ECDC — EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

TECHNICAL REPORT

External quality assurance scheme for *Haemophilus influenzae*

2012

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This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Adoracion Navarro Torne, and produced by Dr Mary Slack (Public Health England, London, UK) on behalf of the IBD-labnet consortium participants.

Suggested citation: European Centre for Disease Prevention and Control. External quality assurance scheme for *Haemophilus influenzae* - 2012. Stockholm: ECDC; 2013.

Stockholm, July 2013

ISBN 978-92-9193-489-8

doi 10.2900/87301

Catalogue number TQ-01-13-358-EN-N

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Abbreviations

BLNAR	Beta-lactamase-negative ampicillin-resistant strain
BLPACR	Beta-lactamase positive amoxicillin-clavulanate resistant strain
BLPAR	Beta-lactamase positive ampicillin-resistant strain
CHLOR	Chloramphenicol
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal fluid
CTR	Ceftriaxone
CTX	Cefotaxime
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EQA	External quality assurance scheme
NTHi	Non-typable *Haemophilus influenzae*
Hib	*H. influenzae* type b
Hif	*H. influenzae* serotype f
HRU	Haemophilus Reference Unit
MIC	Minimum inhibitory concentration
OMP	Outer membrane protein
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
PHE	Public Health England
TET	Tetracycline

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 Assurance (EQA) schemes, in which laboratories are sent simulated clinical specimens or bacterial isolates for testing by routine and/or reference laboratory methods. EQA schemes or 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 May 2012 a collection of five strains of *Haemophilus spp* [two non-capsulated *H. influenzae* (NTHi), 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* serotype b (Hib), one containing non-capsulated *H. influenzae)* was sent to 28 participating reference laboratories in the IBD-labnet surveillance network for quality assurance testing. The laboratories were asked to characterise the five strains by performing 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 Beta-lactamase testing was also requested for those laboratories that perform antimicrobial susceptibility testing of the isolates on a routine basis.

This EQA exercise has shown several improvements over the 2011 EQA distribution. All European Haemophilus Reference Laboratories routinely serotype isolates compared to 27/29 (93%) in 2011. Nineteen laboratories (68%) perform PCR-based capsular genotyping compared to 15 (52%) in 2011. Twenty four laboratories routinely perform antimicrobial susceptibility testing. The number of laboratories following European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines has increased from eight in 2011 to 14 in 2012.

The EQA scheme identified a few problems with speciation of strains, slide agglutination for the serotyping of strains and antimicrobial susceptibility testing. However, the phenotypic identification and phenotypic serotyping of viable isolates has improved since the last EQA distribution in 2011. All four strains of *H. influenzae* were correctly identified by all participants and the strain of *H. parainfluenzae* was correctly identified by 26/28 laboratories (93%) compared to 18/29 laboratories (62%) in 2011. The number of errors in phenotypic serotyping also declined from 17% in 2011 to 10% in 2012. 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 Beta-lactamase production in strains of *Haemophilus influenzae* and the results are excellent. The detection of Beta-lactamase- negative- ampicillin- resistance (BLNAR) proved more challenging for several reasons. 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.

Fourteen laboratories are using the EUCAST criteria whilst ten are still using clinical and laboratory standards institute (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.* The results submitted were very good, although 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. Future EQA panels will be designed to examine this aspect of reporting in more detail.

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.[i]'

External quality assurance (EQA) is part of quality management systems 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 EQA 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[ii] 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')
- the assessment of the effects of analytical procedures (method principle, instruments, reagents, calibration)
- the evaluation of individual laboratory performance
- the identification and justification of problem areas
- the provision of continuing education
- the 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, soft tissue, bone, joint and other body site infections. 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*. EUIBIS was coordinated by the Public Health England (PHE) in London, United Kingdom from 1999-2006. 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, has been outsourced 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, from the 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.

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[i] 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.

[ii] Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community.

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.
- To improve the quality of data, assisting in the standardisation of techniques and thereby facilitating consistent epidemiological data for submission to the ECDC European Surveillance System 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 anonymous 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 Public Health England's (PHE) Haemophilus Reference Unit (HRU, Microbiology Services Division: Colindale, PHE. London, UK) together with the expert knowledge of Dr Vivienne James (UK NEQAS for Microbiology) and facilities in the External Quality Assurance Department (eQAD) PHE: 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 PHE 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	
	Beta-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[i]. 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.

[i]United Kingdom National External Quality Assessment Service for Microbiology www.ukneqasmicro.org.uk

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	March 2012
Assessment of material	April 2012
Transfer of material to eQAD NEQAS	April 2012
Pre-despatch checks (HRU and eQAD NEQAS)	April 2012
Distribution of EQAC panel UK NEQAS EQA Distribution 2802	8 May 2012
Deadline for receipt of results	22 June 2012
Analysis and collation of results	July–August 2012
Interim report to participants	September 2012
Individual results released on UKNEQAS website https:results.ukneqas.org.uk	September 2012
Interim report at EUROVAC meeting, Barcelona, Spain	November 2012
Summary report and recommendations	December 2012

1.4 The EQA panel material

The EQA panel comprised five 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

Four 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 Beta-lactamase production and Beta-lactamase negative ampicillin resistance (BLNAR), and to demonstrate a range of MICs to other commonly used antimicrobials. The fifth 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 PHE'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. 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 a non-capsulated strain of *Haemophilus influenzae* DNA. (This would allow laboratories capable of determining the capsular type of a strain to report this information.)

Suspensions of live bacterial cultures were prepared in PBS. Viable counts were performed and the cultures were killed by heating to 100°C for 10 minutes. They were then diluted to a concentration equivalent to 100cfu/µl 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 returned to NEQAS by 28 laboratories.

A summary of consensus results was released to participants via the UK NEQAS for Microbiology website in September 2012. 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 September 2012. 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).

A summary of the results of the EQA exercise was presented at the EUROVAC meeting which was held in Barcelona in November 2012.

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

Laboratory Identifier	Viable isolates							Non-culture detection
	Phenotypic identification					Genotypic identification		
	Species ID	Serotype	Biotype	Antimicrobial susceptibility	Beta-lactamase production	Species ID	Capsule type	*H. influenzae* detection
NM02	+	+	+	+	+			+
NM09	+	+	+		+		+	
NM10	+	+		+	+	+	+	+
NM16	+	+	+	+	+	+	+	+
NM17	+	+	+	+	+	+	+	+
NM20A	+	+	+	+	+	+	+	+
NM23	+	+		+	+	+	+	+
NM26	+	+	+	+	+	+	+	+
NM27	+	+	+	+	+	+	+	+
NM29	+	+	+	+		+	+	
NM32A	+	+	+	+	+	+	+	+
NM33A	+	+			+	+	+	
NM34A	+	+	+	+	+	+	+	+
NM35A	+	+	+		+			
NM36	+	+		+	+			
NM37A	+			+	+	+	+	+
NM39	+	+	+	+	+	+	+	+
NM40	+	+		+	+			
NM41	+		+	+	+	+	+	+
NM47	+	+	+	+	+	+	+	+
NM48	+	+	+	+	+	+	+	+
NM51	+	+	+		+			
NM52	+	+		+	+			
NM53	+	+	+	+	+	+	+	+
NM54	+	+		+	+			
NM55	+	+	+	+	+	+	+	+
NM57	+	+	+	+	+		+	+
NM59	+	+	+	+	+			+
Total	29	26	20	24	27	18	20	19

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

2.1 Part 1. Characterisation of viable isolates

The intended results for Part 1 of the analysis are shown in Table 4. In the case of the genotypic species determination of sample 1389, two results ('*H. parainfluenzae*' or 'Not *H. influenzae*') were deemed acceptable, since some laboratories employ genotypic species determination simply to decide whether or not an isolate is *H. influenzae*.

All participants confirmed that the five 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 for each sample by method is shown in Table 5.

Table 5 shows the proportion 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 1389 and incorrect serotype for phenotypic serotyping results). In the case of sample 1389 (*H. parainfluenzae* isolate), the phenotypic serotyping and genotypic capsule typing tests were not appropriate. In the case of biotyping of sample 1389, 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 II according to the *H. parainfluenzae* scheme, but biotype IV if erroneously scored according to the *H. influenzae* scheme.

The percentage of participants reporting the intended result for each test is also 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 74% 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
1386	*H. influenzae*	NTHi	I	*H. influenzae*	Hib-
1387	*H. influenzae*	NTHi	II	*H. influenzae*	NTHi
1388	*H. influenzae*	Hib	I	*H. influenzae*	Hib
1389	*H. parainfluenzae*	NA	II[a]	*H. parainfluenzae*[b] or not *H. influenzae*	NA
1390	*H. influenzae*	Hif	I	*H. influenzae*	Hif

Abbreviations: ID, identification; NTHi, non-capsulated (non-typable) Haemophilus influenzae; Hib, H. influenzae type b; Hib-capsule-deficient H. influenzae type b; Hif, H. influenzae type f; NA, not applicable.

[a]Biotype II according to the H. parainfluenzae scheme. If scored according to the H. influenzae biotyping scheme, the erroneous result of IV 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	Proportion of labs reporting the intended result (%)	Results not matching intended result (frequency)
Phenotypic species identification			
1386	*H. influenzae*	28/28 (100%)	NA
1387	*H. influenzae*	28/28 (100%)	NA
1388	*H. influenzae*	28/28 (100%)	NA
1389	*H.parainfluenzae*	26/28 (93%)	*H. influenzae* (1) Not *H. influenzae* (1)[a]
1390	*H. influenzae*	28/28 (100%)	NA
Phenotypic serotyping			
1386	NTHi	19/26 (73%)	Hib (5) Hid (1) Non-specific agglutination (1)
1387	NTHI	23/24 (96%)	Hia (1)
1388	Hib	25/26 (96%)	Hie (1)
1389	NA[b]	14/17 (82%)	NTHi (3)
1390	Hif	23/24 (96%)	NTHi (1)
Biotyping			
1386	I	19/20 (95%)	II (1)
1387	II	15/20 (75%)	I (4) IV (1)
1388	I	19/20 (95%)	II (1)
1389	II[c]	15/16 (94%)	IV (1)[c]
1390	I	19/20 (95%)	IV (1)
Genotypic species identification			
1386	*H. influenzae*	15/15 (100%)	NA
1387	*H. influenzae*	15/15 (100%)	NA
1388	*H. influenzae*	15/15 (100%)	NA
1389	*H. parainfluenzae*	12/13 (92%): *H. parainfluenzae* (5) Not *H. influenzae* (7)	*H. influenzae* (1)
1390	*H. influenzae*	15/15 (100%)	NA
Genotypic capsular typing			
1386	Hib-	15/19 (79%)	Hib (1) NTHi (3)
1387	NTHi	18/19 (95%)	Hib- (1)
1388	Hib	18/19 (95%)	NTHi (1)
1389	NA[d]	11/12 (92%)	Hib- (1)
1390	Hif	18/19 (95%)	Hif- (1)

Abbreviations: NTHi, non-typable Haemophilus influenzae; Hib, H influenzae type b; Hib- capsule-deficient strain of H. influenzae type b Hif, H. influenzae type f; NA, not applicable.

[a] *One laboratory did not attempt to fully identify non-H. nfluenzae strains.*

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

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

[d] *H. influenzae specific genotypic capsular typing is not appropriate for strains of H.parainfluenzae.*

Figure 1. Phenotypic species identification

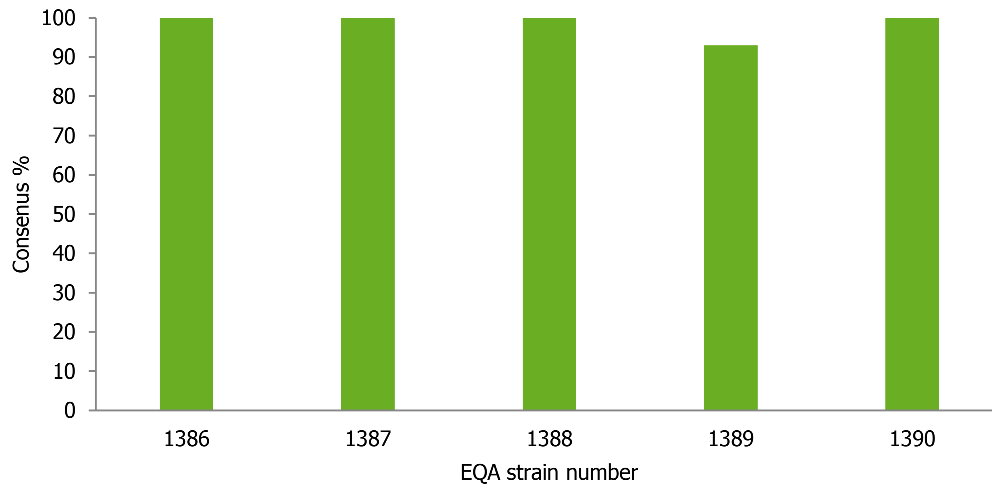


Figure 2. Phenotypic serotyping

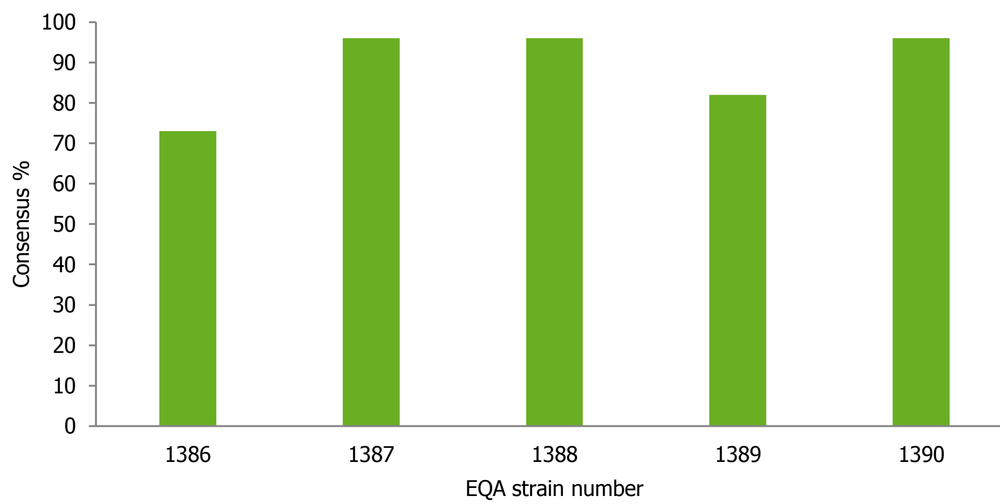


Figure 3. Biotype identification

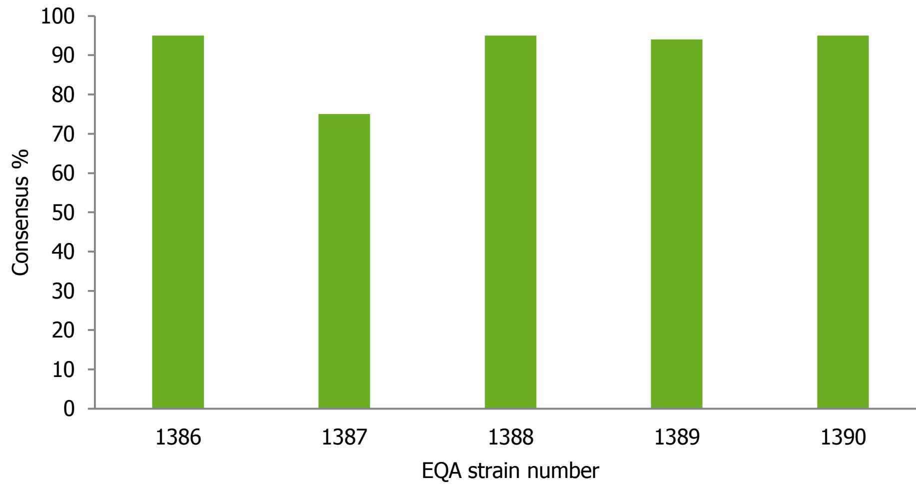


Figure 4. Genotypic species identification

Figure 5. Genotypic capsular typing

2.1.1 Phenotypic species identification

Samples 1386,1387,1388,1390 were correctly identified as *H. influenzae* by all participants. Sample 1389 was correctly identified as *H. parainfluenzae* by 26 laboratories. One laboratory mis-identified the strain as *H. influenzae* (but stated that they used X and V factors in their phenotypic identification) and one laboratory stated that it was 'not *H. influenzae'* which is a correct statement, though not a full speciation.

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

Table 6. Phenotypic species identification methods reported by participating laboratories

	Phenotypic identification method									
Lab ID	Gram stain	X,V factors	Biochemical profile	API NH	RapID NH	Oxidase	Catalase	Satellitism	Porphyrin test	Other
NM02	Y					Y	Y	Y	Y	MALDI-TOF
NM09	Y					Y	Y		Y	
NM10		Y			Y					
NM16	Y	Y	Y	Y					Y	
NM17						Y		Y	Y	
NM20A	Y		Y					Y	Y	
NM26		Y		Y						Vitek
NM27	Y	Y			Y			Y		Other (not specified)
NM29	Y	Y		Y		Y		Y		
NM32A	Y			Y		Y	Y			
NM33A		Y			Y			Y	Y	
NM34A		Y		Y						
NM35A		Y	Y							
NM36	Y	Y				Y		Y		Other (not specified)
NM37A									Y	
NM39				Y						
NM40		Y			Y					
NM41		Y			Y	Y	Y	Y		
NM47				Y						MALDI-TOF
NM48	Y	Y		Y						Vitek
NM51		Y			Y			Y		
NM53		Y								MALDI-TOF
NM54	Y							Y		Vitek
NM 55	Y	Y		Y				Y		Vitek
NM57		Y								MALDI-TOF
NM59	Y	Y	Y			Y		Y		

Note: The web reporting form asked participants to select five methods from predefined menus and then add further methods to a comments field.

2.1.2 Phenotypic serotyping

The number of laboratories reporting serotype varied between 19 and 26, according to the different samples. Twenty one laboratories used slide agglutination, four used latex agglutination and four used co-agglutination (some laboratories used more than one method). The results indicated that two 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). Neither of the two laboratories having problems with conventional serotyping reported PCR-based typing of isolates.

One laboratory reported non-specific agglutination with strain number 1386. Problems encountered with conventional phenotypic serotyping, including non-specific agglutination, cross-reactions can be resolved by using a PCR-based method of capsular genotyping (see below) [1].

As described above, *H. influenzae* serotyping is not appropriate for sample 1389 *(H. parainfluenzae).*

2.1.3 Biotyping

Twenty one 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 5).

Incorrect results did not appear to be linked to a particular method or one of the three biochemical reactions (see Annex 3). Apart from the occasional discrepancy, four laboratories reported strain number 1387 as biotype I, whereas it was in fact biotype II. These two biotypes vary in their reaction to ornithine decarboxylase (Table 8). Three of these laboratories used RapID NH for biotyping and the fourth used API NH.

Sixteen laboratories reported a biotype result for strain 1389 (the *H. parainfluenzae* isolate). The consensus result was biotype II, but one laboratory identified the strain as biotype IV. 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 II had scored the correct biochemical results according to the *H. parainfluenzae* system and the laboratory reporting biotype IV had applied the *H. influenzae* system.

Table 7. Summary of biotyping methods used by 21 participating laboratories

Method	Number of laboratories
Individual biochemical tests	8
Individual biochemical tests + API NH kit	1
API NH kit	8
RapID NH kit	4

Table 8. Biotyping scheme for *Haemophilus influenzae* and Haemophilus parainfluenzae (Kilian 1976)

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. With only one exception, all of these methods produced the intended result (Table 4). In the case of sample 1389, 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 1389 (*H. parainfluenzae*) was designated '*H. influenzae*' using a PCR to detect *H. influenzae* specific sequences in the *ompP2* gene. 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, other genetic typing and capsular typing on viable isolates

	Species identification				Other genetic typing		Capsular typing
DNA extraction procedure	*ompP2 PCR*	*ompP6* PCR	16S gene PCR	Other PCR (unspecified)	BLNAR detection	MLST	Variation of Falla *et al* (1994)
Manual procedure + commercial kit	5				1		6[a]
Automated procedure + commercial kit	1	1	1	1		1	3
Manual procedure + in-house method	4	1			1	3	9
Other (unspecified)	1				1		2[b]
Total	11	2	1	1	3	4	19

[a] *Includes one laboratory that didn't state that they performed capsule typing, but submitted genetic capsule typing results.*

[b] *Includes one laboratory that didn't submit any information on their genotypic ID or serotyping methods, but submitted genetic capsule typing results.*

2.1.5 Genotypic capsule typing

Nineteen laboratories performed a PCR-based capsular capsule typing procedure on the strains. Their DNA extraction procedures are also shown in Table 9. All of the participants used a PCR method based on that of Falla *et al* [1].

The majority of the submitted results matched the intended result, with only a few exceptions (Table 4). Three laboratories identified the Hib- strain as a NTHi. It was not clear whether this was merely a misunderstanding of the convention of reporting Hib- strains in this study or an artefact of the hierarchy of testing performed in their laboratories (none of them provided extra information to suggest they detected the Hib-specific PCR target). A fourth laboratory identified the Hib- strain as Hib (by both serotyping and genetic capsule typing). The reason for this is not clear. Capsule production in *H. influenzae* depends on a cluster of genes in the 18Kb *cap* locus. The *bexA* gene within the *cap* locus is essential for the export of capsular polysaccharide to the cell surface. The majority of Hib strains contain a tandem repeat of the *capb* locus with one complete copy of *bexA* and one truncated copy of *bexA.* Capsule-deficient mutants of type b strains (Hib-) have a single copy of the *bexA* locus possessing a deletion in the *bexA* gene and these strains are unable to export capsular polysaccharide to the cell surface. Such strains will often appear non-capsulated (NTHi) by conventional phenotypic serotyping. One laboratory identified strain 1390 as Hif- (capsule-deficient strain of Hif).

It is recommended that a PCR-based procedure for the confirmation, identification and capsular typing of H. influenzae should include methods for the detection of three genes: a universally carried gene (e.g. *ompP2),* which confirms the strain as *H. influenzae*, the 'Van Ketel gene' *(bexA)* which detects the strain's ability to export the capsule to the cell surface [2] and a capsular type-specific gene (for types a-f) which confirms the strain's capsular serotype [1].

One laboratory reported strain 1389 (a strain of *H.parainfluenzae)* as a Hib- strain (due to a weak positive in the Hib specific PCR). All of the other laboratories either reported this sample as 'not evaluated or' 'not applicable'.

2.1.6 Other molecular typing

Although not a requirement of the EQA study, four laboratories submitted multilocus sequence typing (MLST) results for the strains [3]. The results were all in agreement (Table 10).

Table 10. Multilocus sequence types (ST) of samples 1386 to 1390

Sample number	ST
1386	6
1387	14
1388	6
1389	NA
1390	124

NA: not applicable

2.2 Part 2. Antimicrobial susceptibility testing

2.2.1 Beta-lactamase activity testing

Twenty seven laboratories reported Beta-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 somewhat problematic. Up to twenty four laboratories reported the results of antimicrobial susceptibility testing. Fourteen laboratories used EUCAST guidelines and 10 used CLSI 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.

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

Sample number	Beta-lactamase activity	Antimicrobial susceptibility (S)/ resistance (R)
1386	Absent	All S
1387	Absent	AMP R, CO-AM S, BLNAR
1388	Present	AMP R, CHLOR R, TET R, CO-AM S , CTX S, CTR S
1389	Absent	All S
1390	Absent	All S

Abbreviations: AMP, ampicillin; CHLOR, chloramphenicol; TET; tetracycline; CO-AM, co-amoxiclav; CTX, cefotaxime, CTR, ceftriaxone, BLNAR, Beta-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 1386, 1389, 1390 see Table 12).

Table 12. Antimicrobial susceptibility testing results submitted by participating laboratories

Antimicrobial agent:	Specimen 1386				
	MIC range (n)	MIC (µg/ml) mode	Consensus interpretation	Ratio reporting consensus	Non consensus results (n)
Ampicillin	0.032-0.5	0.125	S	18/18	
Co-amoxyclav	0.125-0.5	0.25	S	17/17	
Beta-lactamase			negative	27/27	
Chloramphenicol	0.094-1	0.5	S	18/18	
Ciprofloxacin	<0.002-0.01	0.004	S	22/22	
Ceftriaxone	<0.002-0.003	<0.016	S	18/18	
Cefotaxime	<0.016-0.38	<0.016	S	20/20	
	Specimen 1387				
Ampicillin	1.5-8	2	R	14/23	S (3), I (6)
Co-amoxyclav	1.5-12	4	S	11/17	R (6)
Beta-lactamase			negative	26/26	
Chloramphenicol	0.38-2	1	S	18/18	
Ciprofloxacin	<0.03-0.064	0.016	S	21/21	
Ceftriaxone	0.023-0.5	0.032	S	19/19	
Cefotaxime	0.047-8	0.094	S	19/19	
	Specimen 1388				
Ampicillin	2- >256		R	23/23	
Co-amoxyclav			S	17/17	
Beta-lactamase			positive	28/28	
Chloramphenicol	3-32	16	R	17/17	
Ciprofloxacin	0.004-0.016	0.008	S	22/22	
Ceftriaxone	<0.003-<0.25	<0.016	S	19/19	
Cefotaxime	<0.016- 0.5		S	20/20	
	Specimen 1389				
Ampicillin	<0.015-1	0.25	S	21/22	R (1)
Co-amoxyclav	0.023-1	0.5	S	15/16	R (1)
Beta-lactamase			negative	25/25	
Chloramphenicol	0.25-1	0.75	S	18/18	
Ciprofloxacin	0.002-0.25	0.016	S	21/21	
Ceftriaxone	0.004-0.25	<0.016	S	17/17	
Cefotaxime	<0.002-0.5	<0.016	S	19/19	
	Specimen 1390				
Ampicillin	0.19-1	0.25	S	22/23	R (1)
Co-amoxyclav	0.25-1	1	S	16/16	
Beta-lactamase			negative	25/26	Positive (1)
Chloramphenicol	0.38-1.5	1	S	17/18	R (1)
Ciprofloxacin	0.01-0.032	0.016	S	22/22	
Ceftriaxone	0.006-0.25	0.008	S	18/18	
Cefotaxime	<0.16-0.75	0.023	S	20/20	

Abbreviations: S, sensitive; R, resistant; I, intermediate resistance.

There were also few problems with the testing for sample 1388, which exhibited Beta-lactamase-mediated resistance to ampicillin and amoxicillin (see Table12). Generally the most important mechanism of ampicillin resistance in *H. influenzae* is the production of TEM-1 Beta-lactamase [4]. A second Beta-lactamase, ROB-1 [5] is less frequently implicated. A significant increase in Beta-lactamase-negative ampicillin resistance in *H. influenzae* has been reported in Sweden and Norway [6]. Strain 1388 also exhibited chloramphenicol and tetracycline resistance, both of which were detected by the participants who tested for these agents. The most common mechanism of chloramphenicol resistance in *H. influenzae* is plasmid-mediated production of chloramphenicol acetyl transferase (CAT) encoded by the cat gene [7]. The cat gene is carried on conjugative plasmids ranging in size from 34×10^6 to 46×10^6. Genes encoding resistance to tetracycline and ampicillin are frequently carried on these plasmids as well, which can be incorporated into the bacterial chromosome [8]. Less commonly, strains are resistant to chloramphenicol due to the loss of an outer membrane protein, which results in a permeability barrier [9].

One of the samples, 1387 was Beta-lactamase negative, but showed reduced susceptibility to ampicillin, amoxicillin and co-amoxyclav. *Haemophilus influenzae* may be resistant to aminopenicillins through the production of a plasmid-mediated Beta-lactamase or from alterations in penicillin-binding proteins (PBP) [10], leading 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 [11, 12]. Strains which are ampicillin-resistant because of alterations in PBP3 are termed 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 [13]. 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 (2 µg/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 Nordic countries have agreed on the use of a screening test for detection of such strains (see Figure 6 below). The clinical significance of ampicillin resistance at this low level is, however, far from clear. However, if a strain is found to be a low-level BLNAR, it would be prudent to avoid the use of these antimicrobials to treat a serious invasive infection.

Sample 1387 was a low BLNAR strain. MICs for sample 1387 ranged between 1.5–8µg/ml for ampicillin and 1.5–12µg/ml for co-amoxyclav, and this strain was scored as resistant to ampicillin by 14/23 participants (60%), but 11/17 participants (65%) scored it as susceptible to co-amoxiclav. These results serve to highlight the different interpretations provided by the EUCAST and CLSI guidelines. According to EUCAST guidelines the breakpoint for ampicillin is 1µg/ml and for co-amoxyclav 2 µg/ml. The interpretative standards for CLSI state that strains with an ampicillin MIC of ≤1 µg/ml should be regarded as susceptible, those with an MIC of ≥4 µg/ml are resistant and an MIC of 2 µg/ml indicates intermediate susceptibility. For co-amoxyclav, CLSI guidelines specify strains with an MIC of ≤f LSI guidare susceptible and those with an MIC of ≥ 8/4 µg/ml are resistant. All 11 laboratories using EUCAST guidelines reported the sample as ampicillin resistant and 4/7 laboratories using EUCAST guidelines reported the sample as co-amoxyclav-resistant. The three laboratories using EUCAST guidelines that reported this strain as susceptible recorded MICs of 1.5 µg/ml, 2 µg/ml and 2 µg/ml which are all at or near to the breakpoint. Two of the seven laboratories using CLSI guidelines reported the sample as being ampicillin-resistant and five reported it as being of intermediate susceptibility. For co-amoxyclav, six laboratories gave results according to CLSI guidelines - two stated that the strain was resistant and four found it to be susceptible. It should be noted that CLSI recommends: 'BLNAR strains should be considered resistant to co-amoxyclav despite apparent in vitro susceptibility of some BLNAR strains'. Using EUCAST guidelines will reduce the problem of interpretation of the susceptibility of low BLNAR strains. A comparison of EUCAST and CLSI interpretative standards for MIC determination of a number of antimicrobial agents is shown in table 13.

Table 13. Comparison of interpretative standards for MIC determinations (µg/ml) with H. influenzae in EUCAST and CLSI guidelines

Antimicrobial agent	EUCAST MIC breakpoint (µg/ml)[1]		CLSI MIC Interpretative standard (µg/ml)		
	S≤	R>	S≤	I	R≥
Ampicillin	≤ 1	> 1	≤ 1	2	≥ 4
Co-amoxyclav	≤ 2	> 2	≤ 4/2		≥ 8/4
Ceftriaxone	≤ 0.12	> 2	≤ 2		
Cefotaxime	≤ 0.12	.> 0.12	≤ 2		
Ciprofloxacin	≤ 0.5	> 0.5	≤ 1		
Chloramphenicol	≤ 2	> 2	≤ 2	4	≥ 8

[1] *In order to simplify the EUCAST tables, the intermediate category is not listed. It is readily interpreted as the values between the S and the R breakpoint. For example, for MIC breakpoints listed as S ≤ 1 mg/L and R > 8 mg/L, the intermediate category is 2-8 (technically >1-8) mg/L, and for zone diameter breakpoints listed as S ≥ 22 mm and R < 18 mm, the intermediate category is 18-21 mm.*

Information on the BLNAR status of the samples was not explicitly elicited from the participants. However, three laboratories stated that they used a molecular technique to detect BLNAR strains.

Some strains of H. influenzae are resistant to aminopenicillins through both mechanisms, that is, they produce a Beta-lactamase and have altered PBP3. Such strains are termed Beta-lactamase positive amoxicillin-clavulanate positive (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 (1391 and 1392) 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 1391 contained 100cfu/µl of a heat-killed suspension of a non-typable strain of *H. influenzae* in simulated CSF. Sample 1392 contained 100cfu/µl of a heat-killed suspension of a strain of *H. influenzae* serotype b. The intended results and breakdown of submitted data are shown in Table 14.

Seventeen of 18 participants correctly detected *H. influenzae* DNA in sample 1391. The remaining laboratory stated that it was 'not *H. influenzae*' using *ompP2* PCR and gel electrophoresis. Several laboratories characterised the sample further by performing additional PCRs against the *bexA* or serotype-specific targets: one stated that it was 'non-typable' (no details); another that it was *bexA* and Hib negative; and another that it was negative for *bexA* plus the Hia, Hib, Hie and Hif-specific targets. However, one laboratory erroneously stated that the sample was bexA positive. (This did not appear to be a transposition of the two samples, because they correctly detected *bexA* in sample 1392, too.)

Seventeen of 18 laboratories also correctly detected H. influenzae DNA in sample 1392. The remaining laboratory erroneously stated that it was 'not *Haemophilus influenzae*' using a real-time PCR against *ompP6*. Hence, different laboratories and PCR methods failed to detect the *H. influenzae* DNA in the two non-culture samples. Some laboratories characterised the sample further by including additional PCR targets: one stated that the sample was Hib (no details); two that it was *bexA*-positive; and two that it was positive for *bexA* and a Hib-specific target. One participant erroneously stated that the sample was non-typable (no details).

The 18 laboratories used a variety of methods for DNA extraction and *H. influenzae*-specific gene target detection (Table 15), all of which gave good results with these two samples.

Table 14. Intended and submitted results for Part 3: Non-culture detection of H. influenzae

Sample Number	Intended results	Ratio of labs reporting the intended result (%)	Results not matching intended result (frequency)
1391	*H. influenzae* (optional further information = non-typable)	17[a,b] /18 (94%)	Not *H. influenzae*. (1)
1392	*H. influenzae* (optional further information = serotype b)	17/18[b] (94%)	Not *H. influenzae* (1)

[a] *Includes one laboratory that didn't select 'H. influenzae', but reported 'Other – not Hib' after using a method of 16S rDNA PCR and gel electrophoresis. It is assumed that they did correctly identify H. influenzae, but carried out additional PCR(s).*

[b] *See main text for results on additional characterisation.*

Table 15. 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*	*fucK*	Other (not specified)
Manual procedure + commercial kit	PCR and sequencing	3				
	PCR and gel electrophoresis	1	3			2
	Real-time PCR platform		2	1	1	
Automated procedure + commercial kit	PCR and sequencing					
	PCR and gel electrophoresis					
	Real-time PCR platform	1	2	1		
Manual procedure + in-house method	PCR and sequencing					
	PCR and gel electrophoresis					1
	Real-time PCR platform					

Overall comments

The laboratory EQA has shown that the 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 whilst others do not.

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* [14] 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 over-reporting of genotypically non-capsulated strains of *H. influenzae* as encapsulated strains. The results of this EQA exercise clearly indicate that PCR-based capsular genotyping gives more reliable results for the capsular typing of strains of *H. influenzae* than the results obtained by conventional serotyping methods.

The antimicrobial susceptibility testing results proved difficult to assess as some laboratories gave MIC values, whilst others gave zone sizes with or without interpretation of the results. Some laboratories are using EUCAST guidelines whilst others are still using CLSI guidelines. There are major differences between the EUCAST and CLSI both in terms of media, and defines 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 Beta-lactamase production. However the evaluation of 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 Beta-lactamase positive ampicillin-resistant strains, whereas the prevalence of BLNAR strains is relatively stable [15]. 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 Beta-lactam antibiotics, particularly some of the cephalosporins. Livermore et al [16] suggested that cefaclor resistance is a better indicator of a BLNAR strain than ampicillin resistance and James et al [17] used cefuroxime resistance (MIC > 4.0 µg/ml) to screen for BLNAR strains. CLSI recommends that BLNAR strains are considered resistant to amoxicillin-clavulanate, cefaclor and cefuroxime despite apparent susceptibility of some strains to these antimicrobials.

Nørskov-Lauritsen *et al* [18] 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 ($MIC \leq 1$ 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.

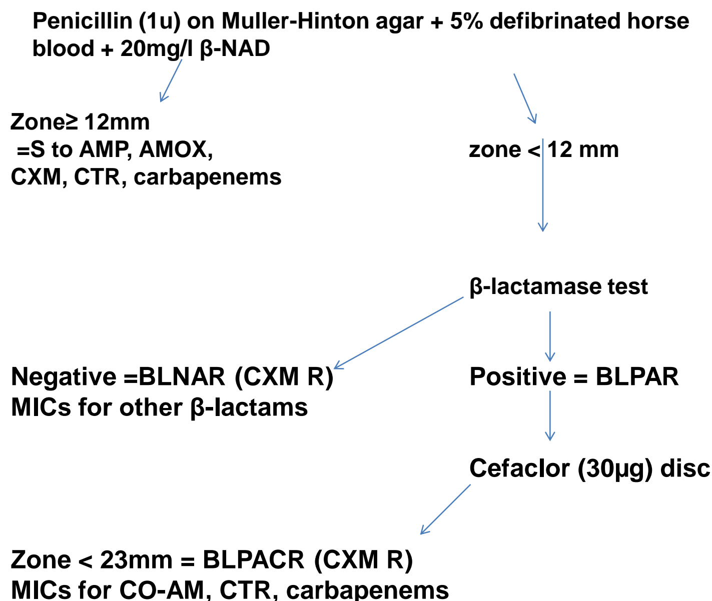
Some laboratories used low strength ampicillin disks (2µg) as recommended by EUCAST guidelines, whilst others used higher concentration ampicillin disks (10µg). The use of low dose ampicillin disks is recommended as it will increase the ability to identify low-BLNAR [18, 19]. The screening method outlined by Nordic AST (as described below) should improve the ability of laboratories to detect low level BLNAR and BLPACR.

The method for screening for BLNAR and BLPACR strains recommended by the Nordic Committee on Antimicrobial Susceptibility Testing (NordicAST, 2012) is shown in Figure 6.

In this procedure the strain of *H. influenzae* is plated onto Mueller-Hinton agar supplemented with 5% defibrinated horse blood and 20 mg/L Beta-NAD. A 1u penicillin disc is placed on the surface of the plate and the culture is incubated overnight. If the zone of inhibition around the penicillin disc is ≥ 12mm the strain can be assumed to be susceptible to ampicillin, amoxicillin, cefuroxime, ceftriaxone and carbapenems. If the zone of inhibition is < 12 mm a Beta-lactamase test should be performed. If the strain is Beta-lactamase negative the strain is a BLNAR and can be assumed to be resistant to cefuroxime. MIC determinations should be carried out to establish susceptibility to other Beta-lactams

If the strain is Beta-lactamase positive the strain is a BLPAR strain. The strain should then be tested with a 30µg cefaclor disc. If the zone of inhibition around the cefaclor disc <23mm, the strain is both Beta-lactamase positive and intrinsically resistant to ampicillin (BLPACR). BLPACR strains can be assumed to be resistant to cefuroxime. MIC determinations should be carried out to determine the susceptibility of BLPACR strain to amoxicillin-clavulanate, ceftriaxone and carbapenems.

Figure 6. Disc diffusion screening method for the detection of BLNAR and BLPACR strains of *H. influenzae*



AMP = ampicillin, AMOX = amoxicillin, CO-AM = amoxicillin-clavulanate, CXM = cefuroxime, CTR = ceftriaxone

Two simulated CSF samples were included in this EQA panel to assess laboratories' methods and expertise in non-culture detection of *H. influenzae*. The first sample was a non-typable *H. influenzae* (which may not be detected in some PCRs; see below), the second a Hib. The results were generally very good. Only one laboratory failed to detect *H. influenzae* in each sample. In the first sample, the detection method was *ompP2* PCR with gel electrophoresis; in the second it was a real-time PCR against the *ompP6* target. The reason for these false negatives is not known, but could have been a primer sequence mismatch in the isolate, or a technical failure during DNA extraction or PCR. It is unlikely to be an inherent problem with these PCR targets; although the details of each PCR were not requested from participants, other PCRs targeting *ompP2* and *ompP6* were successful.

A number of participants performed further PCRs (*bexA* and capsule type-specific PCRs) and correctly identified whether the bacterial sample was capsulated or, indeed, a Hib. This is a useful tool for surveillance purposes and we would encourage its use.

The 15 of 18 participants that provided the information appeared to be using PCRs directed at gene targets present in all (or almost all) *H. influenzae* isolates, regardless of serotype (16S rDNA, *ompP2*, *ompP6*, *fucK*) as their primary PCR target. This is a great improvement on last year's EQA, in which some laboratories used PCRs directed against the *bexA* gene. The *bexA* target is either restricted to solely capsulated isolates or Hib and Hic isolates only (e.g. Corless *et al* 2001) and is not recommended as the sole PCR target in non-culture detection as it will not detect the increasing number of non-typable isolates causing disease (e.g. one of the samples in this EQA). Care must be taken in reporting PCR-derived results on clinical specimens if the PCR target is not universally present (e.g. bexA), and 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). When used in conjunction with a universally present gene target however, *bexA* or a capsule type-specific PCR provides useful additional information.

With only two samples in the panel it was not possible to test the sensitivity of different methods. This would require a larger number of non-culture samples to be included in the EQA panel. A generous concentration of killed bacteria was used in the samples in this EQA to avoid the relative sensitivities of different methods complicating the interpretation of results. A proposal to include more non-culture samples in the panel was put forward following the inclusion of only two non-culture samples in the previous EQA distribution. It is disappointing that it was not possible to increase the number of samples this year.

Conclusions

The level of characterisation of strains of *Haemophilus influenzae* varies between EU countries. This emphasises the need for consensus and agreement in methods for characterising and accurately defining this organism. Some countries still require some capacity building in this area.

The results of this EQA exercise have shown improvements in a number of areas compared to the results from the 2011 EQA distribution. All European Haemophilus Reference Laboratories now routinely serotype isolates compared to 27/29 (93%) in 2011. Nineteen laboratories (68%) perform PCR-based capsular genotyping compared to 15 (52%) in 2011.Twenty six laboratories routinely perform antimicrobial susceptibility testing and the number of laboratories following EUCAST guidelines has increased from 8 in 2011 to 14 in 2012.

The EQA exercise has again demonstrated the value of PCR-based genotyping methods in providing 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 has been implemented, 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 of antimicrobial susceptibility testing which should facilitate better comparison of the results from different laboratories. On a positive note the number of countries following EUCAST guidelines has increased from eight to fourteen since the 2011 EQA distribution.

Two simulated clinical samples were again included in the EQA panel to assess non-culture detection methods. This time a non-typable isolate was included, which would not have been detected by some of the PCRs used by last year's participants. Encouragingly, all participants used PCRs targeted against universal (or almost universally) carried genes and so this isolate did not cause the anticipated problems. It was encouraging that some participants also performed additional PCRs and so could determine the capsule type of the H. influenzae isolates. The future inclusion of a greater number of non-culture samples would allow the sensitivity of each participant's PCR to be compared.

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Annex 1 List of reference laboratories participating in ECDC project

Laboratory surveillance and External Quality Assurance (EQA) of invasive bacterial diseases in EU

Reference Laboratories for *H. influenzae*

Austria
Sigrid Heuberger
National Reference Centre for Meningococci,
Pneumococci and Haemophilus influenzae
Austrian Agency for Food and Health Safety
Beethovenstraße 6
8010 Graz, Austria

Belgium
Françoise Crokaert
Laboratoire de la Porte de Hal - CHU St-Pierre
Rue Haute 322 B-1000 Bruxelles, Belgique

Bulgaria
Dimitar Nashev
National center for infectious and parasitic diseases
26 Y. Sakazov Blvd
1504 Sofia, Bulgaria

Cyprus
Despo Pieridou Bagatzouni
Nicosia general hospital
Microbiology Department
1450 Nicosia, Cyprus

Czech Republic
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
Anni Virolainen-Julkunen
National Institute for Health and Welfare (THL)
PO Box 30
00271 Helsinki, Finland

France
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
Matthias Frosch & Ulrich Vogel
Institute for Hygiene and Microbiology
University of Wuerzburg
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
Ákos Tóth
Department of Bacteriology
Johan Bela National Centre for Epidemiology
Gyali ut 2-6
1097 Budapest, Hungary

Iceland
Hjordis Hardardóttoir
Department of Clinical Microbiology
Institute of Laboratory Medicine
Landspitali University Hospital
Baronsstigur
101 Reykjavik, Iceland

Ireland
Robert Cunney
Irish Meningococcal and Meningitis Reference Laboratory
Children's University Hospital
Temple Street
Dublin 1, Ireland

Italy
Marina Cerquetti
Department of Infectious, Parasitic and Immunomediated Diseases
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Rome, Italy

Latvia
Jelena Galajeva
Bacteriology Department
Laboratory of the State Agency
Infectology Center of Latvia
Bacteriology Department
3 Linezera street
Riga, LV 1006, Latvia

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

Luxembourg
Jos Even
Director, Laboratoire National de Santé
42 rue du Laboratoire
L-1911 Luxembourg, Luxembourg

Malta
Paul Caruna
Mater Dei hospital
Tal-Qroqq
Msida, MSD 2090, Malta

Netherlands
Lodewijk Spanjaard
Netherlands Reference Laboratory for Bacterial Meningitis
Department of Medical Microbiology
Academic Medical Center, L-1-Z
Meibergdreef 15
1105 AZ Amsterdam, Netherlands

Norway
Martin Steinbakk
National Institute of Public Health
Division of Infectious Disease Control
Dept. of Bacteriology and Immunology
PO Box 4404 Nydalen
0403 Oslo, Norway

Poland
Alicja Kuch & Anna Skoczynska
National Reference Centre for Bacterial Meningitis
Department of Epidemiology and Clinical Microbiology
National Medicines Institute
Chelmska Street 30/34
00-725 Warsaw, Poland

Portugal
Paula Lavado
Departamento de Doenças Infecciosas
Laboratorio Nacional de Referência de Infecções Respiratórias (agentes bacterianos)
Instituto Nacional de Saúde Dr Ricardo Jorge
Avenida Padre Cruz
1649-016 Lisboa, Portugal

Romania
Mihaela Giuca
National Institute for Microbiology and Immunology
Cantacuzino
Splaiul Independentei 103
050096 Sector 5, Bucuresti, Romania

Slovak Republic
Elana Nováková
National Reference Centre for Haemophilus Infections
Regional Public Health Authority
RUVZ - NRC HI V Spanyola 27
011 71 Žilina, Slovak Republic

Slovenia
Metka Paragi & Tamara Kastrin
Head of Laboratory for Immunology and Molecular Diagnostics
Institute of Public Health Slovenia
Grablovičeva 44
1000 Ljubljana, Slovenia

Spain
Jose Campos
Centro Nacional de Microbiologia
Instituto de Salud Carlos III
Ctra Majadahonda Pozuelo Km2
28220 Madrid, Spain

Sweden
Birgitta Henriques Normark
Department of Bacteriology
Swedish Institute for Infectious Disease Control
Nobels väg 18
171 82 Solna, Sweden

UK
Mary Slack
Haemophilus Reference Unit
Specialist and Reference Microbiology Division
Public Health England
61 Colindale Avenue
London NW9 5HT, UK

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

EQA number		1386	1387	1388	1389	1390	1391	1392
Phenotypic identification	Species	*H. influenzae*	*H. influenzae*	*H. influenzae*	*H.parainfluenzae*	*H. influenzae*		
	Serotype	Hib-	NTHi	Hib	NA	Hif		
	Biotype	I	II	I	II	I		
Genotypic identification	Species	*H. influenzae*	*H. influenzae*	*H. influenzae*	*H.parainfluenzae*	*H. influenzae*	*H. influenzae*	*H. influenzae*
	Capsular type	Hib-	NTHi	Hib	NA	Hif		
	other	ST-6	ST-14	ST-6	NA	ST-124		

Antimicrobial susceptibility

Antimicrobial agent	EQA number				
	1386	1387	1388	1389	1390
Ampicillin	S	R	R	S	S
Co-amoxyclav	S	R	S	S	S
Beta-lacatamase	Negative	Negative	Positive	Negative	Negative
Chloramphenicol	S	S	R	S	S
Ciprofloxacin	S	S	S	S	S
Ceftriaxone	S	S	S	S	S
Cefotaxime	S	S	S	S	S

S=susceptible

R=resistant

Annex 3 Example of report generated by UK NEQAS

UK National External Quality Assessment Service for Microbiology

Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 1 of 30
Dispatch Date : 08-May-2012	

Intended Result			Your Report	Your Score
Specimen 1386				
	Phenotypic species ID	H. influenzae	H. influenzae	Not scored
	Phenotypic serotype	Hi non typable	Non specific agglutination	Not scored
	Phenotypic biotype	I	Not examined	Not scored
	Genotypic species ID	H. influenzae	H. influenzae	Not scored
	Genotypic capsular type	Hib-	Hib-	Not scored
	Ampicillin	susceptible	susceptible	Not scored
	Beta-lactamase	negative	negative	Not scored
	Cefotaxime	susceptible	susceptible	Not scored
	Ceftriaxone	susceptible	susceptible	Not scored
	Chloramphenicol	susceptible	susceptible	Not scored
	Ciprofloxacin	susceptible	susceptible	Not scored
	Co-amoxiclav	susceptible	susceptible	Not scored
Specimen 1387				
	Phenotypic species ID	H. influenzae	H. influenzae	Not scored
	Phenotypic serotype	Hi non typable	Hi non typable	Not scored
	Phenotypic biotype	II	Not examined	Not scored
	Genotypic species ID	H. influenzae	H. influenzae	Not scored
	Genotypic capsular type	Hi non typable	Hi non typable	Not scored
	Ampicillin	resistant	resistant	Not scored
	Beta-lactamase	negative	negative	Not scored
	Cefotaxime	susceptible	susceptible	Not scored
	Ceftriaxone	susceptible	susceptible	Not scored
	Chloramphenicol	susceptible	susceptible	Not scored
	Ciprofloxacin	susceptible	susceptible	Not scored
	Co-amoxiclav	susceptible	resistant	Not scored
Specimen 1388				
	Phenotypic species ID	H. influenzae	H. influenzae	Not scored
	Phenotypic serotype	Hib	Hib	Not scored
	Phenotypic biotype	I	Not examined	Not scored
	Genotypic species ID	H. influenzae	H. influenzae	Not scored
	Genotypic capsular type	Hib	Hib	Not scored
	Ampicillin	resistant	resistant	Not scored
	Beta-lactamase	positive	positive	Not scored
	Cefotaxime	susceptible	susceptible	Not scored
	Ceftriaxone	susceptible	susceptible	Not scored
	Chloramphenicol	resistant	resistant	Not scored
	Ciprofloxacin	susceptible	susceptible	Not scored
	Co-amoxiclav	susceptible	susceptible	Not scored
Specimen 1389				
	Phenotypic species ID	H. parainfluenzae	H. parainfluenzae	Not scored
	Phenotypic serotype	Not applicable	Not applicable	Not scored
	Phenotypic biotype	II	Not examined	Not scored
	Genotypic species ID	Not H. influenzae	Not examined	Not scored
	Genotypic capsular type	Not applicable	Not applicable	Not scored
	Ampicillin	susceptible	susceptible	Not scored
	Beta-lactamase	negative	negative	Not scored
	Cefotaxime	susceptible	susceptible	Not scored
	Ceftriaxone	susceptible	susceptible	Not scored
	Chloramphenicol	susceptible	susceptible	Not scored
	Ciprofloxacin	susceptible	susceptible	Not scored
	Co-amoxiclav	susceptible	susceptible	Not scored

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UK National External Quality Assessment Service for Microbiology

Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 2 of 30
Dispatch Date : 08-May-2012	

Intended Result			Your Report	Your Score
Specimen 1390				
	Phenotypic species ID	H. influenzae	H. influenzae	Not scored
	Phenotypic serotype	Hif	Hif	Not scored
	Phenotypic biotype	I	Not examined	Not scored
	Genotypic species ID	H. influenzae	H. influenzae	Not scored
	Genotypic capsular type	Hif	Hif	Not scored
	Ampicillin	susceptible	susceptible	Not scored
	Beta-lactamase	negative	negative	Not scored
	Cefotaxime	susceptible	susceptible	Not scored
	Ceftriaxone	susceptible	susceptible	Not scored
	Chloramphenicol	susceptible	susceptible	Not scored
	Ciprofloxacin	susceptible	susceptible	Not scored
	Co-amoxiclav	susceptible	susceptible	Not scored
Specimen 1391				
	Non-culture species ID (molecular)	H. influenzae	H. influenzae	Not scored
Specimen 1392				
	Non-culture species ID (molecular)	H. influenzae	H. influenzae	Not scored

Comments
Specimens were sent to 29 laboratories and results were returned by 28. All 28 reported phenotypic results for the species ID with 26 reporting the serotype and 20 the biotype. Genotypic species identification results were reported by 15 laboratories with 19 reporting the capsular type. These results are presented in the histograms on pages 3 to 11 which display the results reported by method(s); your results are indicated by arrow(s). Where participants reported the use of more than one method all these methods are displayed. Where participants failed to provide method information the results data are not displayed in the histograms; results are included in the summary tables.

MLST results were reported by 3 laboratories with all reporting consensus results. Specimen 1386 was ST6, 1387 was ST14, 1389 was ST6 and 1390 was ST124.

Results for the non culture specimens were reported by 18 laboratories. The extraction, amplification and detection methods used are tabulated on page 30.

Beta-lactamase results were reported by all of the participating laboratories with 24 reporting susceptibility results: 23 tested for ampicillin, 17 for co-amoxiclav, 18 for chloramphenicol, 22 for ciprofloxacin, 19 for ceftriaxone and 20 for cefotaxime. These results are summarised on pages 14 and 15. A breakdown of the MIC results reported by method is displayed on pages 15 to 29 with the interpretation by guideline tabulated. The majority of participants reported using E test with one determining the MIC by broth dilution and another agar dilution. Participants were asked only to report MIC results however the table of susceptibility results by guideline includes results for other methods.

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 3 of 30
Dispatch Date : 08-May-2012	

Specimen : 1386 Phenotypic species ID

	All	(%)
RapID NH	6	(8.8)
XV factors	14	(20.6)
Catalase	2	(2.9)
Biochemical test	2	(2.9)
Oxidase	3	(4.4)
MALDI-TOF	3	(4.4)
Vitek	3	(4.4)
Porphyrin test	5	(7.4)
Gram	9	(13.2)
API NH	9	(13.2)
Satellitism	12	(17.6)

Number of Reports (0, 4, 8, 12, 16, 20)

H. influenzae

Your Report : H. influenzae
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	28	
Total	28	

Specimen : 1386 Phenotypic serotype

	All	(%)
Slide agglutination	22	(73.3)
Other	1	(3.3)
Latex agglutination	3	(10.0)
Co-agglutination	4	(13.3)

Number of Reports (0, 6, 12, 18, 24, 30)

Hib · Hid · Hi non typable · Non specific agglutination

Your Report : Non specific agglutination
Your Score : **Not scored**

Overall Results	All	Score
Hib	5	
Hid	1	
Hi non typable	19	
Non specific agglutination	1	
Total	26	

Specimen : 1386 Phenotypic biotype

	All	(%)
RapID NH	4	(18.2)
API NH	9	(40.9)
Individual biochemical tests	9	(40.9)

Number of Reports (0, 2, 4, 6, 8, 10)

I · II · III

Your Report : Not examined
Your Score : **Not scored**

Overall Results	All	Score
I	19	
II	1	
III	0	
Total	20	

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 4 of 30
Dispatch Date : 08-May-2012	

Specimen : 1386 Genotypic species ID

	All	(%)
ompP2 PCR	11	(78.6)
16S PCR	1	(7.1)
ompP6 PCR	2	(14.3)

Your Report : H. influenzae
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	15	
Total	15	

Specimen : 1386 Genotypic capsular type

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

Your Report : Hib-
Your Score : **Not scored**

Overall Results	All	Score
Hib	1	
Hib-	14	
Hi non typable	4	
Total	19	

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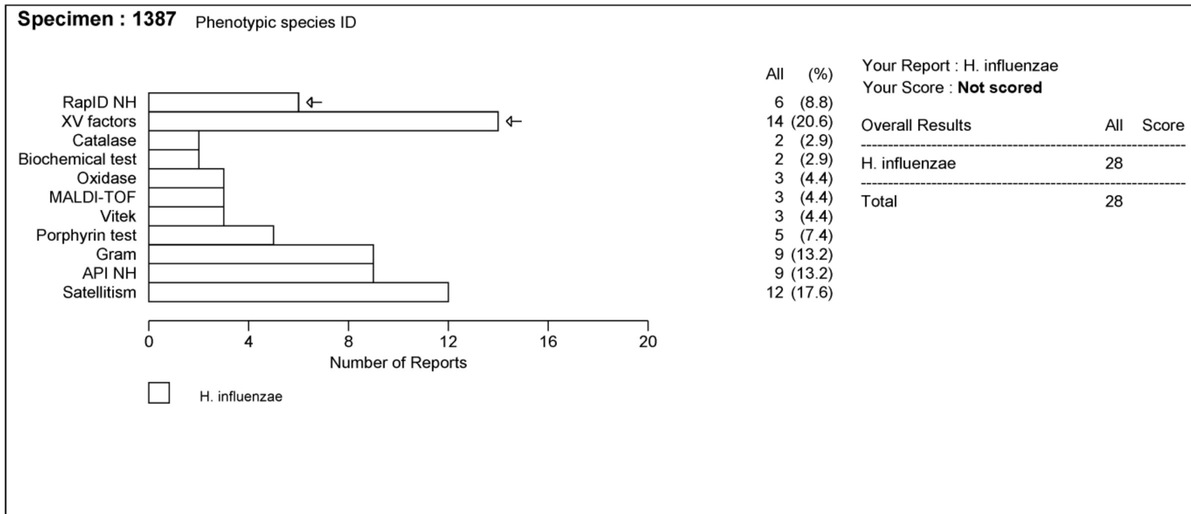
Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 5 of 30
Dispatch Date : 08-May-2012	

Specimen : 1387 Phenotypic species ID

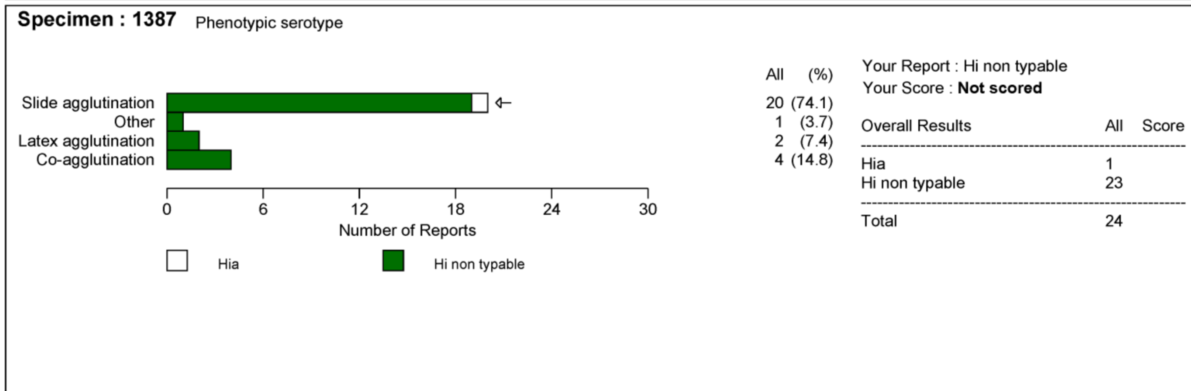


	All	(%)
RapID NH	6	(8.8)
XV factors	14	(20.6)
Catalase	2	(2.9)
Biochemical test	2	(2.9)
Oxidase	3	(4.4)
MALDI-TOF	3	(4.4)
Vitek	3	(4.4)
Porphyrin test	5	(7.4)
Gram	9	(13.2)
API NH	9	(13.2)
Satellitism	12	(17.6)

Your Report : H. influenzae
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	28	
Total	28	

Specimen : 1387 Phenotypic serotype

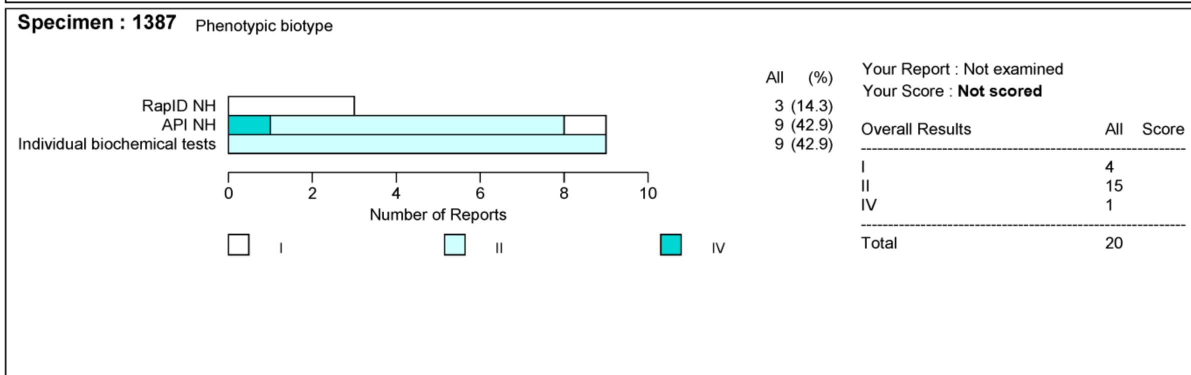


	All	(%)
Slide agglutination	20	(74.1)
Other	1	(3.7)
Latex agglutination	2	(7.4)
Co-agglutination	4	(14.8)

Your Report : Hi non typable
Your Score : **Not scored**

Overall Results	All	Score
Hia	1	
Hi non typable	23	
Total	24	

Specimen : 1387 Phenotypic biotype



	All	(%)
RapID NH	3	(14.3)
API NH	9	(42.9)
Individual biochemical tests	9	(42.9)

Your Report : Not examined
Your Score : **Not scored**

Overall Results	All	Score
I	4	
II	15	
IV	1	
Total	20	

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 6 of 30
Dispatch Date : 08-May-2012	

Specimen : 1387 Genotypic species ID

	All	(%)
ompP2 PCR	11	(78.6)
16S PCR	1	(7.1)
ompP6 PCR	2	(14.3)

Number of Reports (0, 4, 8, 12, 16, 20)

Legend: H. influenzae

Your Report : H. influenzae
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	15	
Total	15	

Specimen : 1387 Genotypic capsular type

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

Number of Reports (0, 4, 8, 12, 16, 20)

Legend: Hib-, Hi non typable

Your Report : Hi non typable
Your Score : **Not scored**

Overall Results	All	Score
Hib-	1	
Hi non typable	18	
Total	19	

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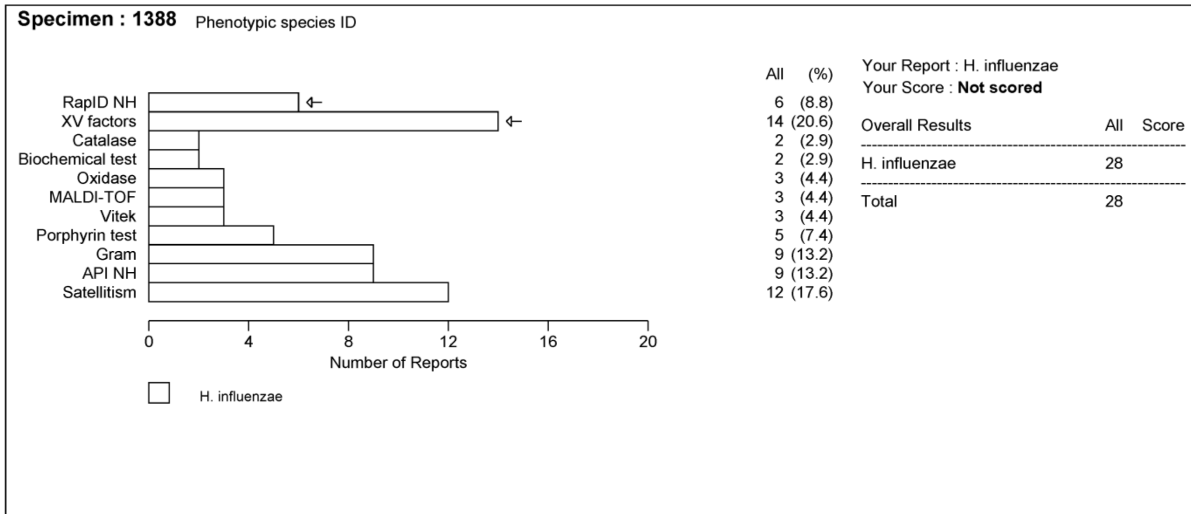
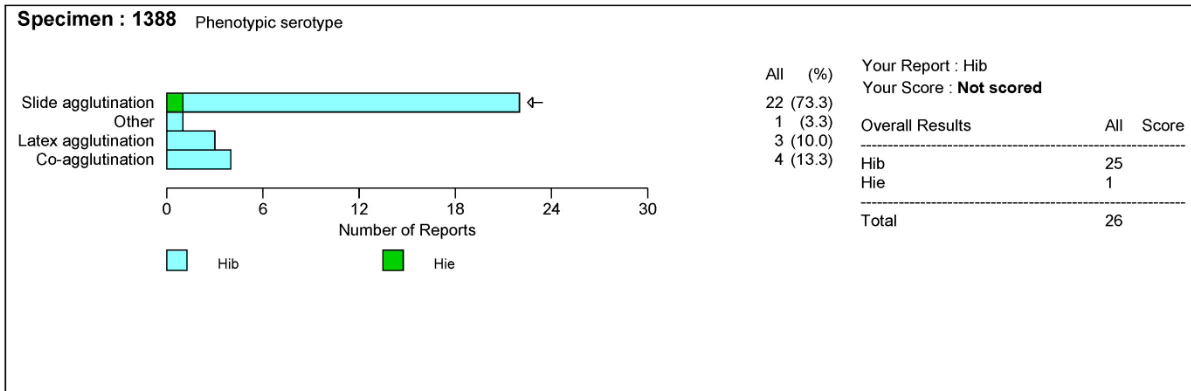
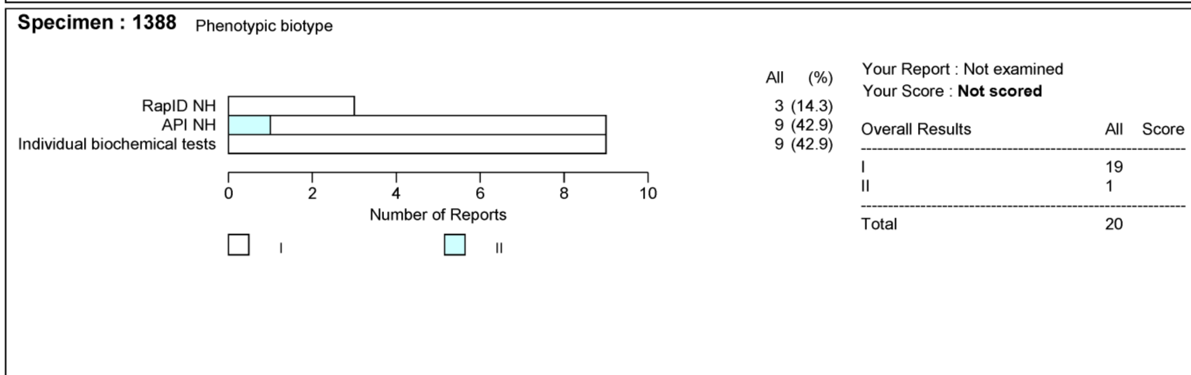
Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 7 of 30
Dispatch Date : 08-May-2012	







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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 8 of 30
Dispatch Date : 08-May-2012	

Specimen : 1388 Genotypic species ID

	All	(%)
ompP2 PCR ←	11	(78.6)
16S PCR	1	(7.1)
ompP6 PCR	2	(14.3)

Number of Reports (0, 4, 8, 12, 16, 20)

Legend: H. influenzae

Your Report : H. influenzae
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	15	
Total	15	

Specimen : 1388 Genotypic capsular type

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

Number of Reports (0, 4, 8, 12, 16, 20)

Legend: Hib; Hi non typable

Your Report : Hib
Your Score : **Not scored**

Overall Results	All	Score
Hib	18	
Hi non typable	1	
Total	19	

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 9 of 30
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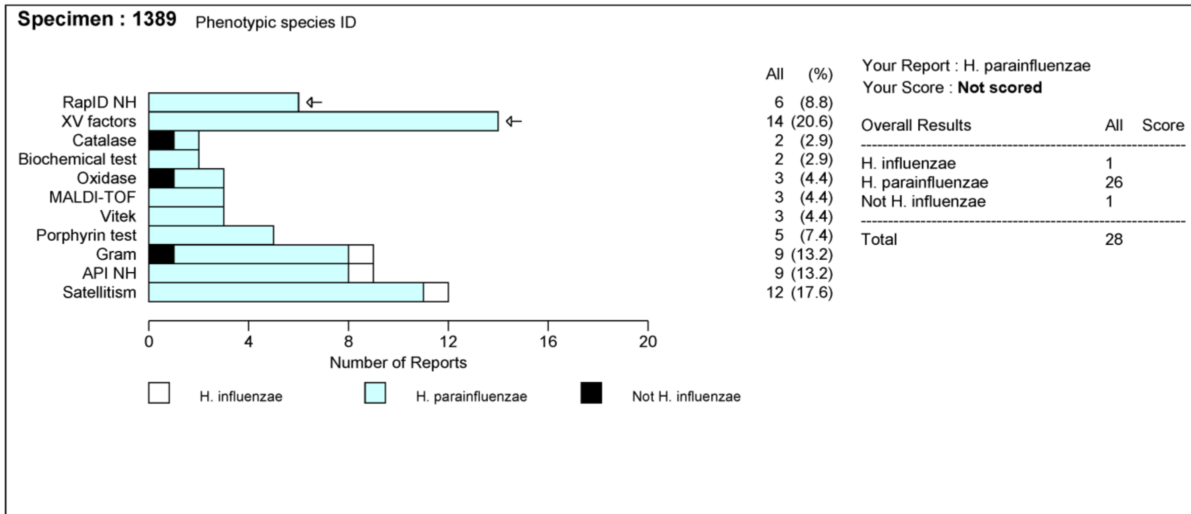

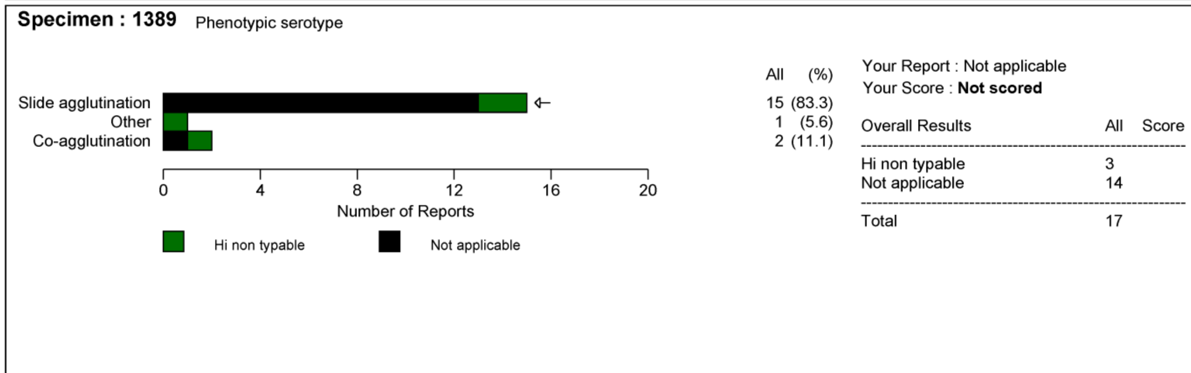

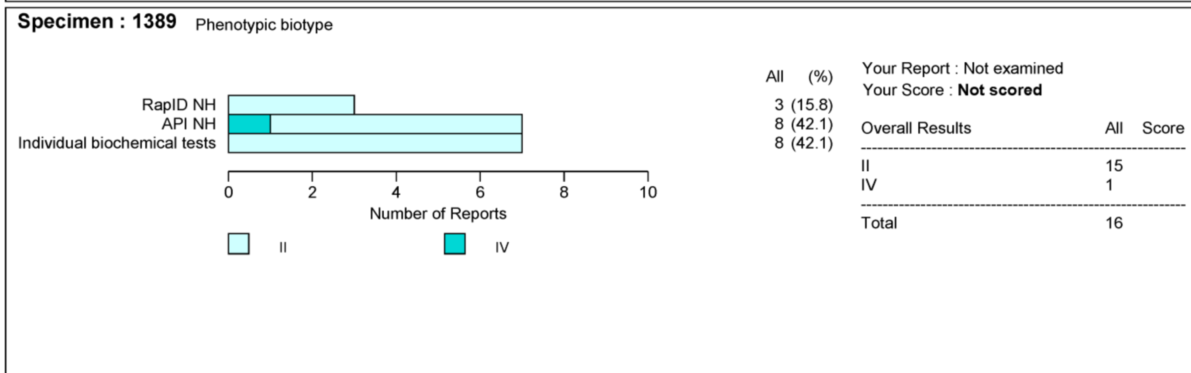

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 10 of 30
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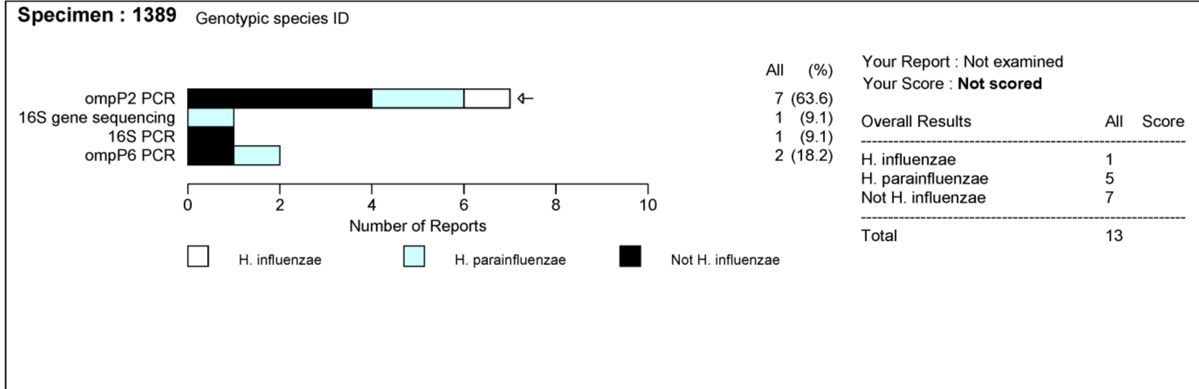
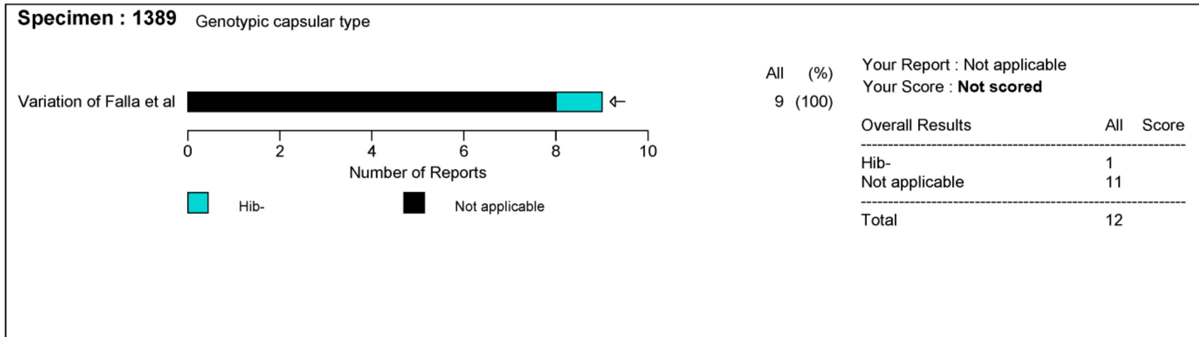
Specimen : 1389 Genotypic species ID



Your Report : Not examined
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	1	
H. parainfluenzae	5	
Not H. influenzae	7	
Total	13	

Specimen : 1389 Genotypic capsular type



Your Report : Not applicable
Your Score : **Not scored**

Overall Results	All	Score
Hib-	1	
Not applicable	11	
Total	12	

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 11 of 30
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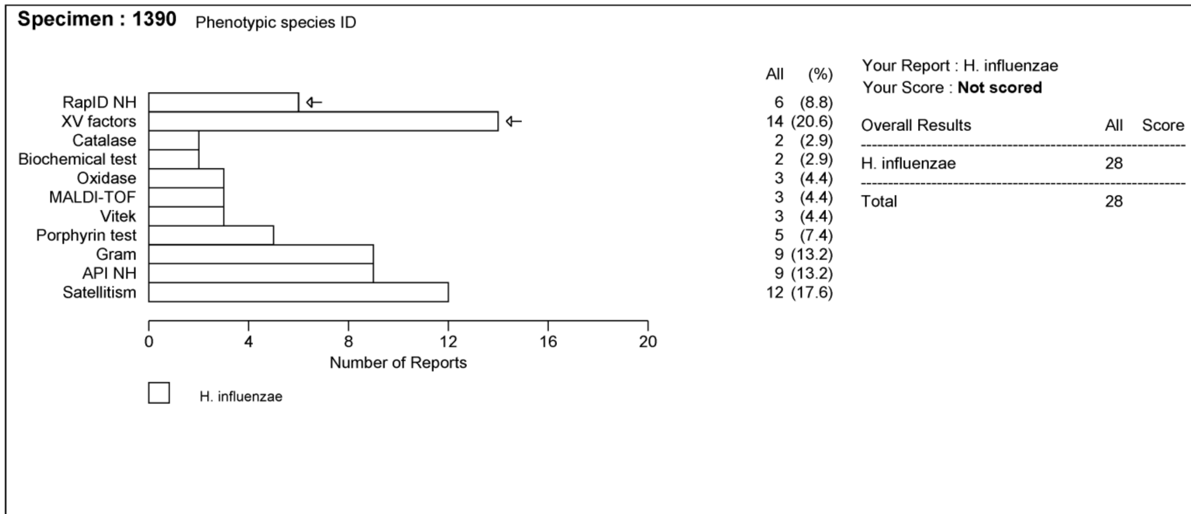

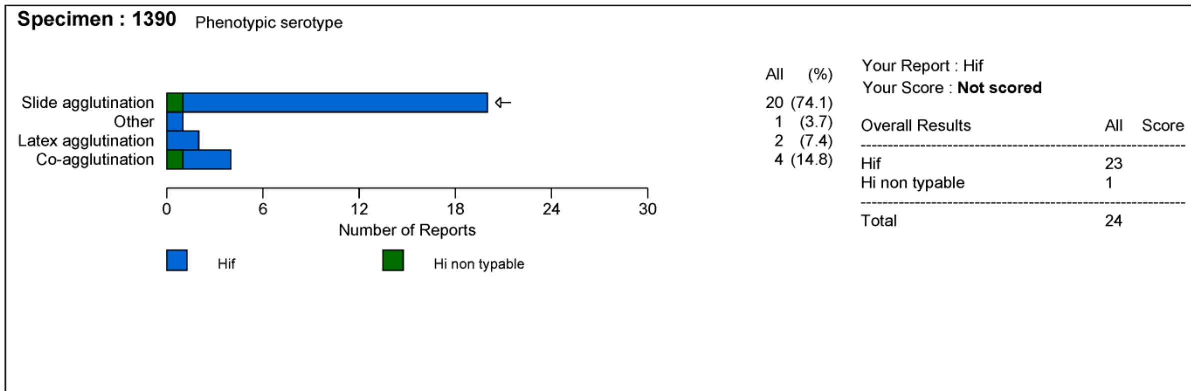

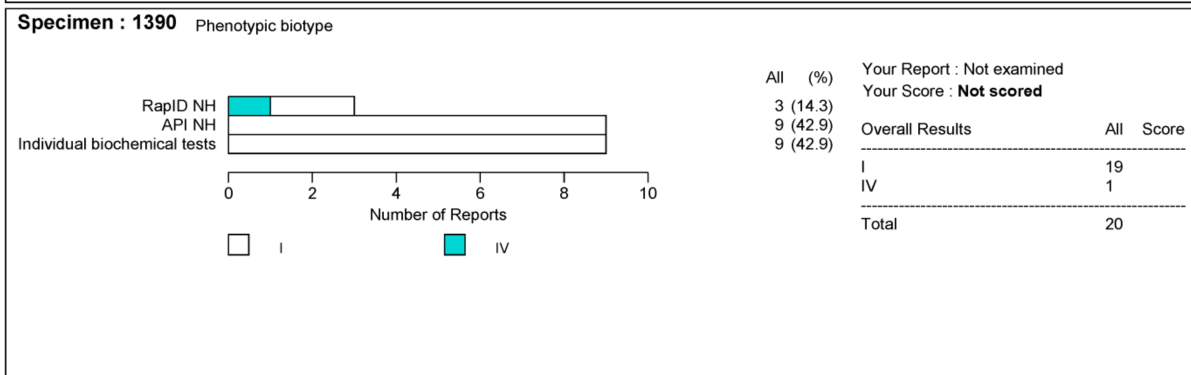

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Haemophilus influenzae	Laboratory : **NM10**
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Specimen : 1390 Genotypic species ID

	All	(%)
ompP2 PCR	11	(78.6)
16S PCR	1	(7.1)
ompP6 PCR	2	(14.3)

Your Report : H. influenzae
Your Score : **Not scored**

Overall Results	All	Score
H. influenzae	15	
Total	15	

Specimen : 1390 Genotypic capsular type

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

Your Report : Hif
Your Score : **Not scored**

Overall Results	All	Score
Hif	18	
Hif-	1	
Total	19	

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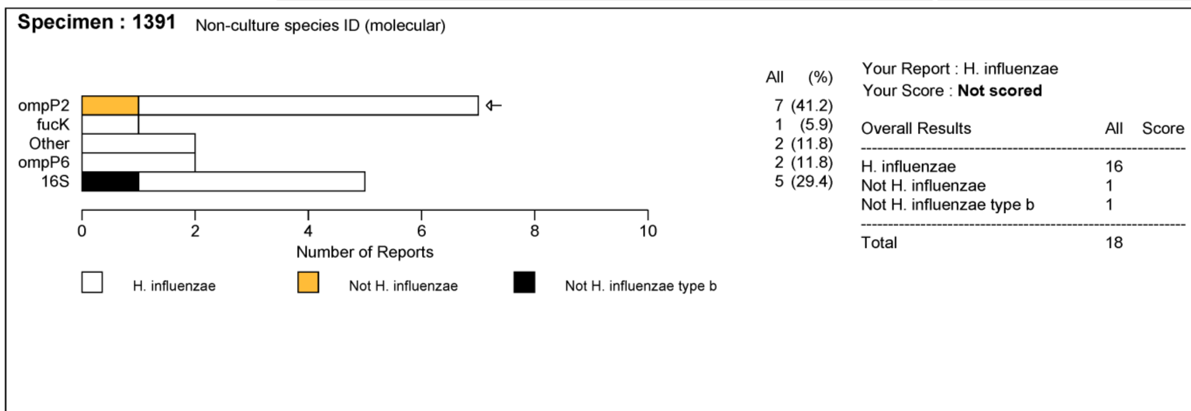

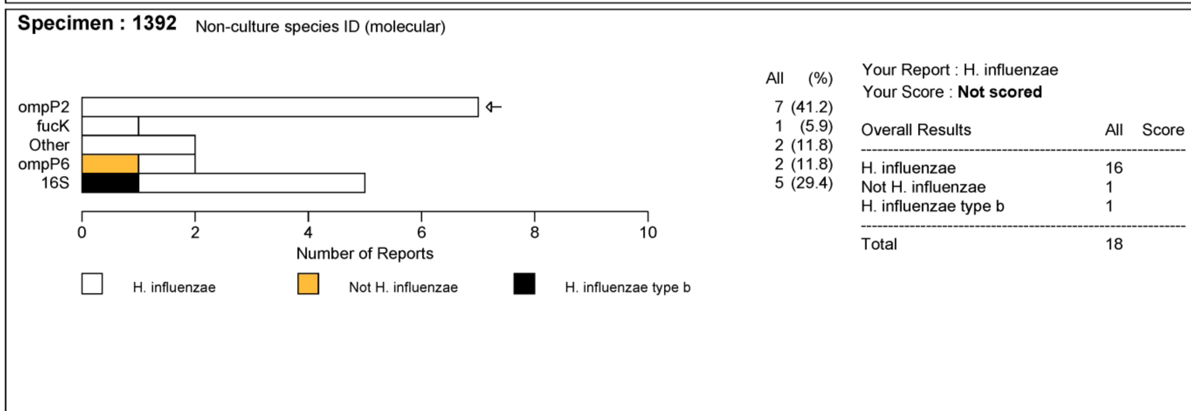

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Haemophilus influenzae	Laboratory : **NM10**
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Specimen : 1386 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S All	M/I	R	% of Laboratories with correct result All
Ampicillin	susceptible	23	0	0	100
Co-amoxiclav	susceptible	17	0	0	100
Beta-lactamase	negative	27	0	0	100
Chloramphenicol	susceptible	18	0	0	100
Ciprofloxacin	susceptible	22	0	0	100
Ceftriaxone	susceptible	18	0	0	100
Cefotaxime	susceptible	20	0	0	100

Specimen : 1387 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S All	M/I	R	% of Laboratories with correct result All
Ampicillin	resistant	3	6	14	60.9
Co-amoxiclav	susceptible	11	0	6	64.7
Beta-lactamase	negative	26	0	0	100
Chloramphenicol	susceptible	18	0	0	100
Ciprofloxacin	susceptible	21	0	0	100
Ceftriaxone	susceptible	19	0	0	100
Cefotaxime	susceptible	19	1	0	95.0

Specimen : 1388 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S All	M/I	R	% of Laboratories with correct result All
Ampicillin	resistant	0	0	23	100
Co-amoxiclav	susceptible	17	0	0	100
Beta-lactamase	positive	0	0	28	100
Chloramphenicol	resistant	0	0	17	100
Ciprofloxacin	susceptible	22	1	0	95.7
Ceftriaxone	susceptible	19	0	0	100
Cefotaxime	susceptible	20	0	0	100

Specimen : 1389 H. parainfluenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S All	M/I	R	% of Laboratories with correct result All
Ampicillin	susceptible	21	0	1	95.5
Co-amoxiclav	susceptible	15	0	1	93.8
Beta-lactamase	negative	25	0	0	100
Chloramphenicol	susceptible	18	0	0	100
Ciprofloxacin	susceptible	21	0	0	100
Ceftriaxone	susceptible	17	0	0	100
Cefotaxime	susceptible	19	0	0	100

Specimen : 1390 H. influenzae

Antimicrobial agent	Correct result	No. of laboratories reporting as S All	M/I	R	% of Laboratories with correct result All
Ampicillin	susceptible	22	0	1	95.7
Co-amoxiclav	susceptible	16	0	0	100
Beta-lactamase	negative	26	0	1	96.3
Chloramphenicol	susceptible	17	0	1	94.4
Ciprofloxacin	susceptible	22	0	0	100
Ceftriaxone	susceptible	18	0	0	100
Cefotaxime	susceptible	20	0	0	100

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Haemophilus influenzae	Laboratory : **NM10**
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Dispatch Date : 08-May-2012	

Specimen : 1386

Ampicillin - specimen 1386

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.032	1
gradient MIC	0.047	1
gradient MIC	0.064	4
gradient MIC	0.094	2
gradient MIC	0.12	2
gradient MIC	0.125	2
gradient MIC	0.19	1
gradient MIC	0.25	2
gradient MIC	0.5	1
MIC microdilution	0.125	1
Other	0.125	1

Ampicillin

score	S	I	R	% concordance
EUCAST	11	0	0	100
CLSI	10	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	23	0	0	100.0
UK	1	0	0	100.0

Beta-lactamase - specimen 1386

Intended result : negative

Your guideline : **EUCAST**

MIC method	MIC	Count

Beta-lactamase

score	S	R	% concordance
EUCAST	15	0	100
CLSI	10	0	100
NWGA	1	0	100
SFM	1	0	100
All	27	0	100.0
UK	1	0	100.0

Cefotaxime - specimen 1386

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.016	6
gradient MIC	<0.03	1
gradient MIC	0.004	2
gradient MIC	0.008	2
gradient MIC	0.01	1
gradient MIC	0.016	1
gradient MIC	0.38	1
MIC microdilution	0.004	1

Cefotaxime

score	S	I	R	% concordance
EUCAST	10	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	20	0	0	100.0
UK	1	0	0	100.0

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Ceftriaxone - specimen 1386
Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<=0.002	1
gradient MIC	<=0.25	1
gradient MIC	<0.016	5
gradient MIC	<0.03	1
gradient MIC	0.002	1
gradient MIC	0.003	3
Other	0.016	1

Ceftriaxone

score	S	I	R	% concordance
EUCAST	7	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	18	0	0	100.0
UK	1	0	0	100.0

Chloramphenicol - specimen 1386
Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.094	1
gradient MIC	0.25	2
gradient MIC	0.38	1
gradient MIC	0.5	2
gradient MIC	0.50	2
gradient MIC	0.75	1
gradient MIC	1	2
Other	0.5	1

Chloramphenicol

score	S	I	R	% concordance
EUCAST	9	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
All	18	0	0	100.0
UK	1	0	0	100.0

Ciprofloxacin - specimen 1386
Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<=0.002	1
gradient MIC	<0.002	1
gradient MIC	<0.03	1
gradient MIC	0.002	2
gradient MIC	0.004	4
gradient MIC	0.008	3
gradient MIC	0.01	1
MIC microdilution	0.006	1
Other	0.008	1

Ciprofloxacin

score	S	I	R	% concordance
EUCAST	11	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	22	0	0	100.0
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
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Dispatch Date : 08-May-2012	

Co-amoxiclav - specimen 1386

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.125	2
gradient MIC	0.19	1
gradient MIC	0.25	5
gradient MIC	0.4	1
gradient MIC	0.5	2
MIC microdilution	0.38	1
Other	0.25	1

Co-amoxiclav

score	S	I	R	% concordance
EUCAST	7	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	17	0	0	100.0
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
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Dispatch Date : 08-May-2012	

Specimen : 1387

Ampicillin - specimen 1387

Intended result : resistant

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	1.5	3
gradient MIC	2	9
gradient MIC	2.0	1
gradient MIC	3	1
gradient MIC	4.0	1
gradient MIC	8	1
MIC microdilution	2	1
Other	4	1

Ampicillin

score	S	I	R	% concordance
EUCAST	0	0	11	100
CLSI	2	6	2	20.0
NWGA	1	0	0	0.0
SFM	0	0	1	100
All	3	6	14	60.9
UK	0	0	1	100.0

Beta-lactamase - specimen 1387

Intended result : negative

Your guideline : **EUCAST**

MIC method	MIC	Count

Beta-lactamase

score	S	R	% concordance
EUCAST	15	0	100
CLSI	9	0	100
NWGA	1	0	100
SFM	1	0	100
All	26	0	100.0
UK	1	0	100.0

Cefotaxime - specimen 1387

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.047	1
gradient MIC	0.06	1
gradient MIC	0.064	1
gradient MIC	0.094	4
gradient MIC	0.12	1
gradient MIC	0.125	2
gradient MIC	0.25	3
gradient MIC	8	1
MIC microdilution	0.12	1

Cefotaxime

score	S	I	R	% concordance
EUCAST	10	0	0	100
CLSI	7	1	0	87.5
NWGA	1	0	0	100
SFM	1	0	0	100
All	19	1	0	95.0
UK	1	0	0	100.0

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Distribution : **3213**	Page 19 of 30
Dispatch Date : 08-May-2012	

Ceftriaxone - specimen 1387

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.023	1
gradient MIC	0.03	1
gradient MIC	0.032	8
gradient MIC	0.047	1
gradient MIC	0.064	1
gradient MIC	0.5	1
Other	0.032	1

Ceftriaxone

score	S	I	R	% concordance
EUCAST	8	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	19	0	0	100.0
UK	1	0	0	100.0

Chloramphenicol - specimen 1387

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.38	2
gradient MIC	0.75	2
gradient MIC	1	3
gradient MIC	1.0	3
gradient MIC	2	1
MIC microdilution	<=1	1
Other	0.5	1

Chloramphenicol

score	S	I	R	% concordance
EUCAST	9	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
All	18	0	0	100.0
UK	1	0	0	100.0

Ciprofloxacin - specimen 1387

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.03	1
gradient MIC	0.015	1
gradient MIC	0.016	5
gradient MIC	0.023	4
gradient MIC	0.03	1
gradient MIC	0.064	1
MIC microdilution	<=0.06	1
Other	0.023	1

Ciprofloxacin

score	S	I	R	% concordance
EUCAST	11	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
All	21	0	0	100.0
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 20 of 30
Dispatch Date : 08-May-2012	

Co-amoxiclav - specimen 1387

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	1.5	1
gradient MIC	12	1
gradient MIC	2	3
gradient MIC	3	1
gradient MIC	4	4
gradient MIC	8	1
MIC microdilution	2	1
Other	2	1

Co-amoxiclav

score	S	I	R	% concordance
EUCAST	3	0	4	42.9
CLSI	8	0	0	100
NWGA	0	0	1	0.0
SFM	0	0	1	0.0
All	11	0	6	64.7
UK	0	0	1	0.0

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Haemophilus influenzae	Laboratory : **NM10**
Distribution : **3213**	Page 21 of 30
Dispatch Date : 08-May-2012	

Specimen : 1388

Ampicillin - specimen 1388

Intended result : resistant

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	>256	3
gradient MIC	1.5	1
gradient MIC	12	2
gradient MIC	16	1
gradient MIC	2	1
gradient MIC	4	2
gradient MIC	6	1
gradient MIC	6.0	1
gradient MIC	8	3
MIC microdilution	12	1
Other	6	1

Ampicillin

score	S	I	R	% concordance
EUCAST	0	0	11	100
CLSI	0	0	10	100
NWGA	0	0	1	100
SFM	0	0	1	100
All	0	0	23	100.0
UK	0	0	1	100.0

Beta-lactamase - specimen 1388

Intended result : positive

Your guideline : **EUCAST**

MIC method	MIC	Count

Beta-lactamase

score	S	R	% concordance
EUCAST	0	15	100
CLSI	0	11	100
NWGA	0	1	100
SFM	0	1	100
All	0	28	100.0
UK	0	1	100.0

Cefotaxime - specimen 1388

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.016	3
gradient MIC	0.008	1
gradient MIC	0.012	1
gradient MIC	0.016	3
gradient MIC	0.023	1
gradient MIC	0.03	2
gradient MIC	0.032	2
gradient MIC	0.5	1
MIC microdilution	0.04	1

Cefotaxime

score	S	I	R	% concordance
EUCAST	10	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	20	0	0	100.0
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
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Ceftriaxone - specimen 1388

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<=0.25	1
gradient MIC	<0.016	5
gradient MIC	<0.03	1
gradient MIC	0.003	1
gradient MIC	0.004	3
gradient MIC	0.006	1
Other	<0.016	1

Ceftriaxone

score	S	I	R	% concordance
EUCAST	8	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	19	0	0	100.0
UK	1	0	0	100.0

Chloramphenicol - specimen 1388

Intended result : resistant

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	12	1
gradient MIC	16	3
gradient MIC	3	1
gradient MIC	32	1
gradient MIC	4	1
gradient MIC	6.0	2
gradient MIC	8	2
Other	12	1

Chloramphenicol

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

Ciprofloxacin - specimen 1388

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.03	1
gradient MIC	0.004	2
gradient MIC	0.006	3
gradient MIC	0.008	6
gradient MIC	0.016	1
MIC microdilution	0.008	1
Other	0.012	1

Ciprofloxacin

score	S	I	R	% concordance
EUCAST	11	0	0	100
CLSI	9	1	0	90.0
NWGA	1	0	0	100
SFM	1	0	0	100
All	22	1	0	95.7
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
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Co-amoxiclav - specimen 1388

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.38	2
gradient MIC	0.5	3
gradient MIC	0.50	1
gradient MIC	0.75	1
gradient MIC	1	3
gradient MIC	2	1
MIC microdilution	0.75	1
Other	0.094	1

Co-amoxiclav

	S	I	R	% concordance
score				
EUCAST	7	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	17	0	0	100.0
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
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Specimen : 1389

Ampicillin - specimen 1389

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.015	1
gradient MIC	0.094	1
gradient MIC	0.12	1
gradient MIC	0.125	1
gradient MIC	0.25	3
gradient MIC	0.38	1
gradient MIC	0.380	1
gradient MIC	0.4	1
gradient MIC	0.5	3
gradient MIC	0.75	1
gradient MIC	1	1
MIC microdilution	0.25	1
Other	0.38	1
Other	0.75	1

Ampicillin

score	S	I	R	% concordance
EUCAST	10	0	1	90.9
CLSI	10	0	0	100
NWGA	1	0	0	100
All	21	0	1	95.5
UK	1	0	0	100.0

Beta-lactamase - specimen 1389

Intended result : negative

Your guideline : **EUCAST**

MIC method	MIC	Count

Beta-lactamase

score	S	R	% concordance
EUCAST	13	0	100
CLSI	10	0	100
NWGA	1	0	100
SFM	1	0	100
All	25	0	100.0
UK	1	0	100.0

Cefotaxime - specimen 1389

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.002	1
gradient MIC	<0.016	4
gradient MIC	0.012	2
gradient MIC	0.016	1
gradient MIC	0.03	1
gradient MIC	0.032	3
gradient MIC	0.5	1
MIC microdilution	<=0.03	1
Other	0.016	1

Cefotaxime

score	S	I	R	% concordance
EUCAST	10	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
All	19	0	0	100.0
UK	1	0	0	100.0

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Ceftriaxone - specimen 1389

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<=0.25	1
gradient MIC	<0.016	5
gradient MIC	0.004	4
gradient MIC	0.006	2
Other	<0.016	1

Ceftriaxone

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

Chloramphenicol - specimen 1389

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.25	1
gradient MIC	0.38	2
gradient MIC	0.5	1
gradient MIC	0.75	3
gradient MIC	0.750	1
gradient MIC	1	3
MIC microdilution	<=1	1
Other	0.5	1

Chloramphenicol

score	S	I	R	% concordance
EUCAST	9	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
All	18	0	0	100.0
UK	1	0	0	100.0

Ciprofloxacin - specimen 1389

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.002	1
gradient MIC	0.008	2
gradient MIC	0.012	2
gradient MIC	0.016	5
gradient MIC	0.064	1
gradient MIC	0.25	1
MIC microdilution	<=0.06	1
Other	0.012	1

Ciprofloxacin

score	S	I	R	% concordance
EUCAST	11	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
All	21	0	0	100.0
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
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Co-amoxiclav - specimen 1389

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.023	1
gradient MIC	0.064	1
gradient MIC	0.094	1
gradient MIC	0.125	1
gradient MIC	0.25	1
gradient MIC	0.5	4
gradient MIC	1	1
MIC microdilution	<=0.25	1
Other	0.094	1

Co-amoxiclav

score	S	I	R	% concordance
EUCAST	6	0	1	85.7
CLSI	8	0	0	100
NWGA	1	0	0	100
All	15	0	1	93.8
UK	1	0	0	100.0

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Haemophilus influenzae	Laboratory : **NM10**
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Specimen : 1390

Ampicillin - specimen 1390

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.19	5
gradient MIC	0.190	1
gradient MIC	0.25	6
gradient MIC	0.5	3
gradient MIC	1	1
MIC microdilution	0.12	1
Other	0.25	1

Ampicillin

score	S	I	R	% concordance
EUCAST	11	0	0	100
CLSI	9	0	1	90.0
NWGA	1	0	0	100
SFM	1	0	0	100
All	22	0	1	95.7
UK	1	0	0	100.0

Beta-lactamase - specimen 1390

Intended result : negative

Your guideline : **EUCAST**

MIC method	MIC	Count

Beta-lactamase

score	S	R	% concordance
EUCAST	15	0	100
CLSI	9	1	90.0
NWGA	1	0	100
SFM	1	0	100
All	26	1	96.3
UK	1	0	100.0

Cefotaxime - specimen 1390

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.023	1
gradient MIC	0.016	1
gradient MIC	0.023	4
gradient MIC	0.03	2
gradient MIC	0.032	2
gradient MIC	0.06	1
gradient MIC	0.064	2
gradient MIC	0.75	1
MIC microdilution	<=0.03	1

Cefotaxime

score	S	I	R	% concordance
EUCAST	10	0	0	100
CLSI	8	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	20	0	0	100.0
UK	1	0	0	100.0

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Ceftriaxone - specimen 1390

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<=0.25	1
gradient MIC	<0.016	3
gradient MIC	<0.03	1
gradient MIC	0.006	1
gradient MIC	0.008	3
gradient MIC	0.012	1
gradient MIC	0.016	1
gradient MIC	0.25	1
Other	<0.016	1

Ceftriaxone

score	S	I	R	% concordance
EUCAST	7	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	18	0	0	100.0
UK	1	0	0	100.0

Chloramphenicol - specimen 1390

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.38	1
gradient MIC	0.5	1
gradient MIC	0.75	2
gradient MIC	1	4
gradient MIC	1.0	2
gradient MIC	1.5	1
MIC microdilution	<=1	1
Other	0.75	1

Chloramphenicol

score	S	I	R	% concordance
EUCAST	9	0	0	100
CLSI	7	0	1	87.5
NWGA	1	0	0	100
All	17	0	1	94.4
UK	1	0	0	100.0

Ciprofloxacin - specimen 1390

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	<0.03	1
gradient MIC	0.01	1
gradient MIC	0.015	1
gradient MIC	0.016	6
gradient MIC	0.023	3
gradient MIC	0.032	1
MIC microdilution	<=0.06	1
Other	0.032	1

Ciprofloxacin

score	S	I	R	% concordance
EUCAST	11	0	0	100
CLSI	9	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	22	0	0	100.0
UK	1	0	0	100.0

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Co-amoxiclav - specimen 1390

Intended result : susceptible

Your guideline : **EUCAST**

MIC method	MIC	Count
gradient MIC	0.25	3
gradient MIC	0.38	2
gradient MIC	0.5	2
gradient MIC	1	4
MIC microdilution	<=0.25	1
Other	0.75	1

Co-amoxiclav

score	S	I	R	% concordance
EUCAST	7	0	0	100
CLSI	7	0	0	100
NWGA	1	0	0	100
SFM	1	0	0	100
All	16	0	0	100.0
UK	1	0	0	100.0

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PART 3

Non-culture DNA extraction method	Non-culture detection method	Combination count
Manual / in-house	PCR & gel electrophoresis	1
Manual / commercial kit	PCR & gel electrophoresis	6
Manual / commercial kit	Real-time PCR platform	4
Manual / commercial kit	PCR & sequencing	3
Automated / commercial kit	Real-time PCR platform	4

Genotypic DNA extraction method	Count
Manual / in-house	8
Manual / commercial kit	7
Automated / commercial kit	4
Other	1

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