TECHNICAL REPORT

External quality assurance scheme for *Streptococcus pneumoniae*

2010

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External quality assurance scheme for *Streptococcus pneumoniae* 2010

As part of the IBD-Labnet surveillance network

This report was commissioned by ECDC, coordinated by Dr Lucia Pastore-Celentano and produced by Prof Dominique A. Caugant (Oslo, Norway), Dr Ari van der Ende (Amsterdam, The Netherlands) and Dr Mary Slack (London, UK) on behalf of the IBD-Labnet consortium (referring to Specific Contract ECD. 1726).

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Abbreviations

BSAC	British Society for Antimicrobial Chemotherapy
CC	Clonal complex
CLIND	Clindamycin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
CSF	Cerebrospinal fluid
ECDC	European Centre for Disease Prevention and Control
ERY	Erythromycin
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU-IBIS Network	European Invasive Bacterial Infections Surveillance Network
I	Intermediate
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence type
MLST CC	Multilocus sequence type and clonal complex
NT	Non-typable
PEN	Penicillin
PCR	Polymerase chain reaction
R	Resistant
RIF	Rifampicin
S	Susceptible
SRGA	Swedish Reference Group for Antibiotics
ST	Sequence type

Executive summary

Streptococcus pneumoniae is the causative agent of a wide spectrum of diseases ranging from upper respiratory tract infections to severe invasive disease. *S. pneumoniae* is the most frequently isolated respiratory pathogen in community-acquired pneumonia and the most commonly observed infection due to it is otitis media. Invasive pneumococcal disease (IPD), defined as the isolation of *S. pneumoniae* or detection of *S. pneumoniae* nucleic acid or antigen from a normally sterile fluid, may feature as meningitis, bacteraemic pneumonia, occult bacteraemia, septic shock and, less frequently, arthritis and peritonitis [1].

Essentially all strains of *S. pneumoniae* have a polysaccharide capsule, which is the basis for serotyping. Currently, 91 distinct serotypes have been identified. Overall, 20 serotypes account for more than 80% of IPD.

Prevention of the invasive pneumococcal disease can be achieved by vaccination. The heptavalent pneumococcal conjugate vaccine (PCV7) targets seven out of these serotypes.

The universal implementation of the vaccine in the USA has led to a dramatic decrease of IPD caused by vaccine serotypes and a decrement of overall incidence. This decline in the incidence was observed mainly among those vaccinated but also in non-vaccinated groups (herd immunity). However, several post-licensure studies have revealed significant increase in non-vaccine serotypes. Post-vaccine surveillance faces important challenges: monitoring the vaccine impact on the target group, to assess its effect in non-vaccinated groups (herd immunity), detect any serotype replacement and estimate the impact of introducing new vaccines (i.e. PCV13, PCV10 recently introduced in Europe) [2,3].

Invasive pneumococcal disease surveillance varies significantly across Europe. Published data on surveillance systems demonstrate variations in reporting methods, case definitions, laboratory methods and medical practices, especially blood-culturing in febrile children [4].

Among the activities that ECDC is developing in relation to IPD surveillance, it has been considered crucial to strengthen laboratory surveillance for invasive pneumococcal disease. Therefore, ECDC has supported an external quality assurance (EQA) exercise on *S. pneumoniae* with the aim to improve laboratory capacity for detection, characterisation, molecular typing and antimicrobial susceptibility testing of *S. pneumoniae.*

In April 2010, a collection of six strains of *S. pneumoniae* and one other microorganism (which could be misidentified as *S. pneumoniae*) was sent to 27 participating laboratories within the IBD-Labnet surveillance network, for quality assurance testing. The participants were requested to test the submitted samples according to their routine standard methods, namely species identification, serogrouping and serotyping, multilocus sequence type (MLST) to derive the sequence type (ST) and clonal complex (CC). The laboratories were also asked to carry out antimicrobial susceptibility testing on the isolates using their regular procedures and interpreting the results as susceptible, resistant or intermediate.

The EQA exercise has shown that the European pneumococcal reference laboratories vary in the level to which they characterise the strains referred to them, ranging from simple speciation to full serotyping and sequence typing.

Overall, out of 182 results submitted, there were 24 errors in the serotyping results (13.1%). The errors were at the species level in six cases (40.0%) where either *S. pneumoniae* was identified as *Gemella sp.* or *S. mitis,* or the *S. mitis* isolate was identified as a pneumococcus. In five cases (33.3%) the errors were at serogroup or serotype level outside the correct serogroup. In four cases (26.6%) a wrong serotype within a correct serogroup was stated.

The EQA distribution scheme has identified some problem areas that need to be addressed. The serotyping results that were reported identified some problems and also the sequence typing. These difficulties are, most probably, due to varying reagents and methods that make difficult the interpretation.

Fifty percent (13/26) of the laboratories did not perform molecular typing by MLST, most likely due to limited financial resources. One third of the laboratories reporting ST did not assign a CC, most likely because CC are not automatically assigned in the pneumococcal MLST database at http://spneumoniae.mlst.net/.

The antimicrobial susceptibility testing indicated that the majority of laboratories have relatively little difficulty in identifying susceptible strains, but problems arose when strains are of reduced susceptibility to antimicrobials. An additional, unforeseen problem was that of interpretation of MIC results for beta-lactam antimicrobials according to the source of the isolate when using Clinical and Laboratory Standards Institute (CLSI) guidelines. All of the strains included in this EQA distribution were non-meningitis blood culture isolates and in retrospect the organisers should have stated this when the strains were distributed.

Penicillin minimum inhibitory concentration (MIC) interpretation is the major problem at present and is related to the guideline chosen. Specifically, there was considerable variation in the interpretation of the susceptibility of one of the samples.

In conclusion, some laboratories would benefit from training in phenotypic and molecular characterisation of *S. pneumoniae*.

The establishment of a regular EQA for the European pneumococcal reference laboratories is recommendable to ensure improved quality of surveillance and epidemiological reports.

It would also be advisable to harmonise performance and interpretation criteria of antimicrobial susceptibility testing, according to only one guideline for consistency, preferably the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methods. This will facilitate comparison of results from different laboratories.

The completion and submitting of results electronically would allow a much easier analysis of the results.

Introduction

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

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

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

Streptococcus pneumoniae is the most common cause of community-acquired pneumonia, meningitis and bacteraemia in children and adults. Invasive pneumococcal disease (IPD) primarily affects young children (mostly younger than three years), older adults (> 65 years), and individuals with comorbidities and impaired immune system.

In essence, all strains of *S. pneumoniae* have a polysaccharide capsule, which is the basis for serotyping. Presently, 91 distinct capsular types have been identified. Overall, nearly 20 serotypes account for more than 80% of IPD in all age groups. There is some evidence of an existing association between serotype and enhanced severity of disease. Serotype prevalence varies among geographic regions and may change over time in response to selective pressure or clonal spread. Furthermore, capsular switching may occur by providing evolutive advantages to the microorganism and allowing survival of specific clones.

At the moment there is no EU enhanced surveillance for *S. pneumoniae* in place. ECDC has funded a project for establishing an inventory of current surveillance systems for invasive pneumococcal disease in Europe. This study pointed out that surveillance systems differ significantly in terms of reporting methods, case definitions, laboratory methods and medical practices concerning blood-culturing.

Since the introduction of the heptavalent pneumococcal vaccine (PCV7) in the EU and the recent availability of two new vaccines (10-valent and 13-valent), implementing an IPD European surveillance is more urgent than ever. The objectives of this surveillance aim to monitor the impact of the vaccines in the European countries, to compare the impact of different vaccine schedules adopted by the Member States and to detect and study changes in serotype distribution and any possible replacement due to vaccine pressure. The conclusions of the analysis of EU surveillance data might be crucial to advise the Member States on optimal vaccine schedules.

Laboratory diagnostics and molecular epidemiology of *S. pneumoniae* (due to the importance of serotypes) are paramount in the surveillance of this pathogen. Therefore, ECDC has sponsored this EQA exercise to strengthen *S. pneumoniae* laboratory surveillance by identifying gaps, evaluating existing systems, promoting benchmarking and determining training needs for capacity building in order to establish and harmonise the most appropriate methods and establish the basis for future network activities. Moreover, ECDC promoted the performance of this EQA scheme to ensure high quality results to be reported as part of the laboratory surveillance.

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

1 Material and methods

The objectives of this exercise were:

- To design an EQA scheme utilising a small panel of material comprising viable *Streptococcus pneumoniae* and one other microorganism isolates for phenotypic characterisation and sequence typing by MLST and clonal complex (where possible), as well as antimicrobial susceptibility testing, to all EU/EEA countries with suitable reference facilities.
- To improve quality, assisting in the standardisation of techniques, evaluating systems and detecting training needs and thereby facilitating the consistency of epidemiological data for submission to ECDC as part of the IPD laboratory surveillance.

1.1 Study design

In April 2010 a collection of six strains of *S. pneumoniae* and one other organism which could be misidentified as *S. pneumoniae,* was sent to 27 participating reference laboratories in the IBD-Labnet surveillance network for quality assurance testing (see Annex 1). The strains were from the collections of the three coordinators (Prof Caugant, Dr Slack and Dr van der Ende), providing a selection of clinically relevant isolates. The strains were assembled at the Norwegian Institute of Public Health (NIPH) in Oslo, where they were confirmed for the parameters to be evaluated. Thirty copies of each isolate were lyophilised at NIPH and one copy set was opened and re-tested for confirmation of the material before its distribution to the laboratories participating in the EQA.

It was requested that all seven strains be tested using standard laboratory protocols for the methods normally used by the laboratory to characterise submitted isolates of *S. pneumoniae*, namely species identification, serogrouping and serotyping, MLST to derive the sequence type (ST) and clonal complex (CC).

It was also requested that antimicrobial susceptibility testing (penicillin, erythromycin, clindamicin and ceftriaxone) be carried out using normal laboratory procedures. For the antimicrobial susceptibility testing, the participants could either carry out disc susceptibility testing or MIC determination, but in both cases the laboratory was asked to provide an interpretation of the results, namely whether the strain was susceptible (S), resistant (R) or of intermediate susceptibility (I). An electronic template was provided for recording of results and the deadline for returning the results was 2 June 2010. The strains were sent as lyophilised cultures and shipped by courier.

On receipt the strains were viable. The strains were processed as requested and the results were returned to Prof Caugant by 26 laboratories.

Laboratories received feedback on their performance through distribution of the consensus results obtained by all respondents by e-mail on 28 June 2010 (see Annex 1). Further presentation and discussion of the results were made by Prof Caugant at the second annual meeting of the IBD-Labnet in Würzburg on 1 July 2010. Individual feedback was also provided to a number of laboratories that had specific questions and a new set of lyophilised strains was sent to one laboratory that had experienced some mix-up of samples.

1.2 Participants

The list of the participating reference laboratories is attached as Annex 1.

2 Results

2.1 Strain identification and characterisation

The characterisations requested and intended results of the participating reference laboratories were:

Table 1 Intended results of pneumococcal EQA distribution

EQA number	Strain identification	Serogroup/serotype	MLST ST	CC	Antimicrobial susceptibility
1001	*S. pneumoniae*	19A	276	230	PEN I; ERY R; CLIND R; CRO S
1002	*S. pneumoniae*	6A	207	207	PEN S; ERY S; CLIND S; CRO S
1003	*S. pneumoniae*	NT/rough	448	448	PEN S; ERY S; CLIND S; CRO S
1004	*S. pneumoniae*	6C	1692	395	PEN S; ERY S; CLIND S; CRO S
1005	*S. mitis*				PEN R; ERY R; CLIND S; CRO I
1006	*S. pneumoniae*	6B	176	176	PEN S; ERY S; CLIND S; CRO S
1007	*S. pneumoniae*	14	9	9	PEN S; ERY R; CLIND S; CRO S

The strains sent comprised of six *S. pneumoniae* and one *Streptococcus mitis.*

Serotyping was considered to be correct if the serogroup and/or serotype reported were in agreement with the intended result. A serogroup result was correct if the factor antisera required for serotyping were not available in the particular laboratory. A summary of the identification and serogrouping/serotyping results obtained by the countries is given in Tables 2 and 3. Four countries did not serogroup/serotype the isolates of *S. pneumoniae.* A summary of the molecular characterisation results obtained by the countries is given in Table 4.

Thirteen laboratories reported the serotype of the isolates of *S. pneumoniae* and nine laboratories identified the CC (detailed results in Annex 3).

Table 2 Results of strain identification

EQA number	Strain identification	Number of laboratories reporting consensus (%)	Non-consensus results reported
1001	*S. pneumoniae*	26/26 (100%)	
1002	*S. pneumoniae*	26/26(100%)	
1003	*S. pneumoniae*	23/26 (88%)	*S. mitis* (1); *Gemella spp.* (1); negative (1)
1004	*S. pneumoniae*	26/26 (100%)	
1005	*S. mitis*	17/26 (65%)	Not *S. pneumoniae* (3), *Streptococcus species* (1), negative (1), *S. pneumoniae* (2), mixed *S. pneumoniae* and other organism (2)
1006	*S. pneumoniae*	26/26 (100%)	
1007	*S. pneumoniae*	26/26 (100%)	

Table 3 Results of strain serogrouping/serotyping

EQA number	Serogrouping/ serotyping	Number of laboratories reporting consensus (%)	Non-consensus results reported
1001	19A	19/22(86%)	19 (1), 18 (1), 18C (1)
1002	6A	16/22 (73%)	6 (3), 6A/6C (1), 6B (1), 6C (1)
1003	NT/rough	18/20 (90%)	17A (1), 19A (1)
1004	6C	13/22 (59%)	6A (4), 6 (1), 6B (1), 6C/D (1), 17 (1), NT (1)
1005	NA	NA	NA
1006	6B	19/22 (86%)	6 (3)
1007	14	21/22 (95%)	NT (1)

NA= Not applicable

Table 4 MLST results

EQA number	ST (n=13)	Number of consensus responses (%)	Other results	CC (n=9)	Other results
1001	276	13 (100%)		230	
1002	207	13 (100%)		207	Not assigned (1)
1003	448	13 (100%)		448	
1004	1692	13 (100%)		395	327(1)
1005	NA	NA	176 (1), 276 (1)		
1006	176	12 (92%)	170 (1)	176	176/138 (1); not assigned (1)
1007	9	12 (92%)	3898	9	

Table 5 Overview of the number of participating laboratories per method

Method	Participants
Strain identification	26
Serogrouping/Serotyping	22*
Sequence typing (MLST)	13
Clonal Complex (CC)	9

**Only 20 participants for sample 1003.*

2.2 Antimicrobial susceptibility testing

All of the laboratories determined the antimicrobial susceptibility of the isolates. The laboratories were asked to determine the susceptibility of the isolates to four antimicrobial agents (penicillin, erythromycin, clindamycin and ceftriaxone) and to give their interpretation of the results of MIC determinations and/or disc diffusion testing. Differences in methodologies for antimicrobial susceptibility testing make it difficult to compare results from different centres. As an illustration of the problem, the breakpoints (in mg/L) for *S. pneumoniae* in the CLSI, BSAC and EUCAST schemes are shown in Annex 2.

2.2.1 Penicillin

Table 6 Penicillin susceptibility testing

EQA number	MIC (mg/L)	Zone in mm	Consensus result	Number of consensus responses (%)	Other results
1001	0.38–4	0–25	I	8 (33%)	S (3), R (5), Rm/S (5), Rm/I/S (2), Im/S (1)
1002	0.004–0.03	24–47	S	25 (100%)	
1003	0.004–0.016	23–42	S	25 (100%)	
1004	0.008–0.032	25–44	S	23 (100%)	
1005	2–>32	0–22	R	13 (65%)	I (3), Rm/I (1), Rm/S (2), S (1)
1006	0.004–0.03	20–43	S	25 (100%)	
1007	0.008–0.03	24–41	S	25 (100%)	

Note: S = susceptible; I = intermediate; R = resistant; Rm/S= resistant if meningitis isolate but susceptible if non-meningitis isolate; Rm/I = resistant if meningitis isolate but intermediate if non-meningitis isolate; Rm/I/S = resistant if meningitis isolate, intermediate if parenteral non-meningitis isolate and susceptible for oral penicillin V; Im/S = intermediate if meningitis isolate but susceptible if non-meningitis parenteral isolate.

This highlights the problem of interpreting the significance of the penicillin MIC, as the laboratories that currently use CLSI guidelines needed to know whether the isolate was from CSF or blood culture. As shown in Annex 2, CLSI guidelines specify that for meningitis isolates a MIC of ≤ 0.06 mg/L is required to signify sensitivity, whereas for non-meningitis parenteral isolates a MIC of ≤ 2.0 mg/L signifies sensitivity. The interpretation of penicillin MIC results thus varies considerably.

2.2.2 Erythromycin

Table 7 Erythromycin susceptibility testing

EQA number	MIC (mg/L)	Zone in mm	Consensus result	Number of consensus responses (%)	Other results
1001	>1–>256	0–15	R	24 (96%)	S (1)
1002	0.016–0.19	19–35	S	25 (100%)	
1003	0.03–0.19	22–35	S	22 (100%)	
1004	0.015–0.19	23–37	S	25 (100%)	
1005	1.5–8	6–26	R	13 (68%)	I (3), S (3)
1006	<0.06–0.125	20–31	S	24 (96%)	R (1)
1007	>1–>128	6–15	R	25 (100%)	

2.2.3 Clindamycin

Table 8 Clindamycin susceptibility testing

EQA number	MIC (mg/L)	Zone in mm	Consensus result	Number of consensus responses (%)	Other results
1001	0.125–>256	6–20	R	20 (91%)	I (1), S (1)
1002	0.012–0.25	20–35	S	22 (100%)	
1003	<0.012–0.125	20–37	S	21 (100%)	
1004	<0.012–0.19	20–30	S	22 (100%)	
1005	<0.012–0.125	19–35	S	17 (94%)	R (1)
1006	<0.012–0.19	19–28	S	21 (95%)	I (1)
1007	<0.012–0.19	20–31	S	21 (95%)	I (1)

2.2.4 Ceftriaxone

Table 9 Ceftriaxone susceptibility testing

EQA number	MIC (mg/L)	Zone in mm	Consensus result	Number of consensus responses(%)	Other results
1001	0.25–32	24–30	S	12 (52%)	I (4), Rm/I (3), Im/S (2), R (2)
1002	0.008–0.125	32–40	S	23 (100%)	
1003	0.004–0.047	36–40	S	21 (100%)	
1004	0.008–0.125	30–40	S	23 (100%)	
1005	0.031–4	23–28	I	14 (64%)	S (4), Rm/I (2), R (2)
1006	0.008–0.064	30–40	S	23 (100%)	
1007	0.008–0.064	30–40	S	23 (100%)	

2.3 Comments on individual strains

2.3.1 Strain 1001

This strain was S. *pneumoniae,* serotype 19A, ST 276, CC 230. The strain was intermediate in its susceptibility to penicillin, resistant to erythromycin and clindamycin, and susceptible to ceftriaxone.

There was full agreement on the identification of the strain as *S. pneumoniae.* Four laboratories did not serogroup or serotype the strain. Of 22 laboratories, 19 correctly identified the strain as serotype 19A, one laboratory, which did not perform serotyping, stated that it was serogroup 19.

One laboratory identified it as serogroup 18 and one identified it as serotype 18C. All 13 laboratories that reported STs identified it correctly as ST 276 and all nine laboratories that assigned the ST to a clonal complex assigned it correctly to CC 230.

The antimicrobial susceptibilities proved problematic. Penicillin MICs, ranging from 0.38–4.0 mg/L and disc zone diameters of 0–25mm were reported. The consensus report (eight laboratories) was that the strain was of intermediate susceptibility. Some laboratories who used CLSI guidelines gave various interpretations, dependent on the source of the isolate (meningeal, non-meningeal).

Erythromycin susceptibility testing proved less problematic with 24/25 laboratories agreeing that the isolate was resistant to erythromycin. However, one laboratory stated that the isolate had an erythromycin MIC of 16 mg/L yet called it susceptible to erythromycin, which is incorrect. Twenty laboratories identified the strain as clindamycin resistant (one laboratory stated that it was of intermediate susceptibility and one laboratory stated that it was susceptible to this antimicrobial). The MICs for ceftriaxone reported ranged from 0.25–32 mg/L. The consensus report (12 laboratories) was that the strain was susceptible to ceftriaxone. As with penicillin, some laboratories gave different interpretations dependent on the source of the isolate.

2.3.2 Strain 1002

This strain was *S. pneumoniae,* serotype 6A, ST 207, CC 207. This strain was susceptible to all four antimicrobials.

The strain was correctly identified as *S. pneumoniae* by 26 laboratories. Sixteen laboratories identified the strain correctly as serotype 6A, three laboratories (that only performed serogrouping) called the isolate serogroup 6. One laboratory stated that it was 6A/6C, one identified it as 6B and one identified it as 6C. The ST and CC were correctly identified by all of the laboratories reporting these parameters.

There were no problems with the antimicrobial susceptibility testing, with all laboratories reporting the strain as susceptible to all four agents.

2.3.3 Strain 1003

This strain was a non-typable/rough *S. pneumoniae*. Of 26 laboratories, 23 correctly identified the strain as *S. pneumoniae,* one identified it as *S. mitis,* one as *Gemella* sp., and one stated that it was negative. Eighteen laboratories correctly identified it as a non-typable/rough strain, one stated that it was serotype 17A and one stated that it was serotype 19A. All laboratories reporting ST and CC correctly assigned the strain to ST 448, CC 448. All laboratories were in agreement that the isolate was susceptible to all four antimicrobial agents.

2.3.4 Strain 1004

This was a strain of *S. pneumoniae* serotype 6C. All laboratories correctly identified the strain as *S. pneumoniae,* but only 13 stated that it was serotype 6C. Four stated that it was serotype 6A, one serogroup 6, one serotype 6B, one serotype 6C/6D, one serotype 17 and one stated that it was non-typable. As noted in the questionnaire, not all laboratories are testing for serotype 6C and this strain would be identified as serotype 6A by laboratories not yet testing for 6C. All laboratories reporting ST assigned the strain to ST 1692 and eight out of nine assigned this ST to CC 395 (one laboratory reported CC327). All laboratories were in agreement that the strain was susceptible to all four antimicrobial agents.

2.3.5 Strain 1005

This was a strain of *S. mitis.* Seventeen laboratories correctly speciated the strain. The discordant results were: not *S. pneumoniae* (1), *Streptococcus* sp. (1), negative (1), *S. pneumoniae* (2), mixed *S. pneumoniae* + other organism (2). Two laboratories incorrectly ascribed a pneumococcal ST to this organism.

Of 20 laboratories, 13 described the strain as resistant to penicillin. Again, there were varying interpretations of the results depending on the source of the isolate. Of 19 laboratories, 13 described the strain as erythromycin resistant, three said it was of intermediate susceptibility and three called it susceptible. Of 18 laboratories reporting results for clindamycin, 17 described it as susceptible and one laboratory called it clindamycin resistant. Fourteen of 18 laboratories described it as of intermediate susceptibility to ceftriaxone, four called it susceptible and two called it resistant.

2.3.6 Strain 1006

This was a strain of *S. pneumoniae* serotype 6B. All laboratories correctly speciated the strain and 19 of 22 laboratories identified it as serotype 6B. Three laboratories labelled it as serogroup 6.

Twelve of 13 laboratories designated the strain as ST 176 (one laboratory called it ST 170). Eight laboratories assigned the ST to CC 176 (one designated it CC 176/138). There was almost complete agreement on the antimicrobial susceptibilities. All laboratories called the strain susceptible to penicillin, 24 of 25 stated that it was susceptible to erythromycin (one stated it was resistant), all but one laboratory described it as clindamycin susceptible and all found it to be susceptible to ceftriaxone.

2.3.7 Strain 1007

This was a strain of *S. pneumoniae* serotype 14. All laboratories correctly speciated this strain and all but one identified it as serotype 14 (one laboratory found it to be non-typable). It was ST 9 and CC 9. There was almost complete agreement with the antimicrobial susceptibility testing.

The strain was penicillin susceptible, erythromycin resistant, clindamycin susceptible (one laboratory described it of intermediate susceptibility to clindamycin) and susceptible to ceftriaxone.

2.4 Overall comments

The laboratory EQA scheme has shown that the European pneumococcal reference laboratories vary in the level to which they characterise strains referred to them, ranging from simple speciation to full serotyping and sequence typing.

Overall out of 182 results submitted, there were 24 errors in the serotyping results (13.2%). The errors were at the species level in six cases (40.0%), where either *S. pneumoniae* was identified as *Gemella sp.* or *S. mitis,* or the *S. mitis* isolate was identified as a pneumococcus. In five cases (33.3%), the errors were at serogroup or serotype level outside the correct serogroup. In four cases (26.6%) a wrong serotype within a correct serogroup was stated (note that only results where the laboratory stated that they had the required serotyping antisera are included here).

The EQA distribution scheme has identified some problem areas that need to be addressed. Some laboratories lack the necessary reagents to fully serotype isolates and this renders surveillance of invasive pneumococcal disease

difficult. Comprehensive data on serotype distribution is essential in order to establish the baseline epidemiology of invasive pneumococcal infections and also to study the impact of the use of pneumococcal vaccines.

The serotyping results that were reported identified some problems. These may be due to the varying reagents in use or problems in interpretation. The variety of the methods and reagents employed by the different laboratories has been mapped by a questionnaire. Detailed analyses of the relationships between methodology and obtained results were not attempted in this exercise, but might provide valuable information. Similarly, there were a few problems with sequence typing. Both of these issues could be addressed by training in the use of these techniques.

Fifty percent (13/26) of the laboratories did not perform molecular typing by MLST, most likely due to limited financial resources. One third of the laboratories reporting ST did not assign a CC, most likely because CC are not automatically assigned in the pneumococcal MLST database at http://spneumoniae.mlst.net/.

The antimicrobial susceptibility testing indicated that the majority of laboratories have relatively little difficulty in identifying susceptible strains, but problems arose when strains are of reduced susceptibility to antimicrobials. An additional, unforeseen problem was that of interpretation of MIC results for beta-lactam antimicrobials according to the source of the isolate when using CLSI guidelines. All of the strains included in this EQA distribution were non-meningitis blood-culture isolates and, in retrospect, the organisers should have stated this when the strains were distributed. Penicillin MIC interpretation is the major problem at present and is related to the guideline chosen. Specifically, there was considerable variation in the interpretation of the susceptibility of sample 1001.

Some laboratories returned their results electronically and in clearly legible form. Others faxed the results. In some cases the results were handwritten and in a few cases deciphering the writing was quite challenging.

3 Conclusions

Some laboratories would benefit from practical training in phenotypic and molecular characterisation of isolates of *S. pneumoniae.* This could take the form of either a laboratory training workshop or an exchange, in which individuals from laboratories requiring training could visit other laboratories to acquire the necessary skills.

In future, an electronic form completed online would make analysis of the results much easier.

The establishment of a regular EQA scheme for the European pneumococcal reference laboratories is required in order to ensure improved quality of epidemiological reports. Where possible, an EQA scheme should be frequent with a small number of samples per distribution. Involvement of a reputable and independent provider is advisable.

For European surveillance, it would be preferable to collect the MIC values that have been derived by one standard method and then interpret the results according to one guideline for consistency. The pneumococcal questionnaire results indicated that the CLSI guidelines are the most commonly used, but European laboratories should be adopting the EUCAST methods of antimicrobial susceptibility testing (www.EUCAST.org) in the near future. This will facilitate comparison of results from different laboratories.

A validation process requiring regular submission of a sample of isolates to a supranational European reference laboratory for verification of serotype and MIC would theoretically be of great value in ensuring high quality of epidemiological data throughout Europe.

References

[1] Lynch JP, Zhanel GG. Streptococcus pneumoniae: Epidemiology, Risk Factors, and Strategies for Prevention. Semin Resp Crit Care M. 2009;30(2):189-209.

[2] Hanquet G, Kissling E, Fenoll A, George R, Lepoutre A, Lernout T, et al. Pneumococcal serotypes in children in 4 European countries. Emerg Infect Dis. 2010;16(9):1428-39.

[3] Lynch JP, Zhanel GG. Streptococcus pneumoniae: epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines. Curr Opin Pulm Med 2010;16:217-25

[4] Hanquet G, Perrocheau A, Kissling E, Bruhl DL, Tarragó D, Stuart J, et al. Surveillance of invasive pneumococcal disease in 30 EU countries: Towards a European system? Vaccine. 2010;28(23):3920-8.

Annex 1: Participating reference laboratories

Country	Contact person	Institution
Austria	Dr Sigrid Heuberger	National Reference Centre for Meningococci, Pneumococci and *Haemophilus influenzae* Austrian Agency for Health and Food Safety Beethovenstraße 6 A-8010 Graz, Austria
Belgium	Dr Jan Verhaegen	National Reference Laboratory UH Gasthuisberg Laboratory of microbiology Herestraat 49 B-3000 Leuven, Belgium
Bulgaria	Dr Antoaneta Decheva	National Reference Laboratory for Streptococci and Diphteria National Center of Infectious and Parasitic Diseases 26, Yanko Sarazov blvd Sofia 1504, Bulgaria
Cyprus	Dr Despo Pieridou Bagkatzouni	Microbiology Department Nicosia General Hospital 215, Palaios Dromos Lefkosias-Lemesou 2029 Strovolos, Nicosia, Cyprus
Czech Republic	Dr Jitka Motlová	National Reference Laboratory for Streptococci and Enterococci National Institute of Public Health Šrobárova 48 100 42 Prague 10, Czech Republic
Denmark	Dr Lotte Lambertsen	Statens Serum Institut 5 Artillerivej, building 37/133 2300 Copenhagen S, Denmark
England and Wales	Dr Androulla Efstratiou	Streptococcus and Diphteria Reference Unit Respiratory and Systemic Infection Laboratory Bacteriology Department, HPA Centre for Infections 61 Colindale Avenue London NW9 5EQ, United Kingdom
Estonia	Dr Rita Peetso	Laboratory of Communicable Diseases Health Protectorate Inspectorate Kotka 2 11315 Tallinn, Estonia
Finland	Dr Anni Virolainen-Julkunen	National Institute for Health and Welfare Mannerheimintie 166 Fi-00300 Helsinki, Finland
France	Dr Emmanuelle Varon	National Reference Centre for Pneumococci Laboratory of Microbiology, Georges Pompidou European Hospital (teaching hospital) 20 rue Leblanc F-75908 Paris Cedex 15, France
Germany	Dr Mark van der Linden	German National Reference Center for Streptococci Department of Medical Microbiology University Hospital RWTH Aaachen Pauwelsstrasse 30 52074 Aachen, Germany
Greece	Dr Georgina Tzanakaki	National Meningitis Reference Laboratory National School of Public Health 196 Alexandras Avenue 115 21 Athens, Greece
Iceland	Dr Karl G. Kristinson	Landspitali University Hospital Department Clonical Microbiology v/Barongstig 101, Reykjavik, Iceland
Ireland	Prof Hilary Humphreys	The Royal College of Surgeons in Ireland Department of Clinical Microbiology RCSI Education and Research Centre Smurfit Building Beaumont Hospital PO Box 9063, Dublin 9, Ireland

Country	Contact person	Institution
Italy	Dr Annalisa Pantosti	Unit of Respiratory and Systemic Bacterial Diseases Department of Infectious, Parasitic and Immunomediated Diseases Instituto Superiore di Sanità Viale Regina Elena 299 00161 Rome, Italy
Latvia	Dr Jelena Galajeva	Infectology Center of Latvia 3 Linezera Riga, LV 1006, Latvia
Lithuania	Dr Migle Janulaitiene	High Threat Pathogens Subdivision of Microbiology Department National Public Health Surveillance Laboratory Zolyno str. 36 10210 Vilnius, Lithuania
Luxembourg	Dr Jos Even	Laboratoire National de Santé 42 rue du Laboratoire L-1911 Luxembourg
Netherlands	Dr Arie van der Ende	Netherlands Reference Laboratory for Bacterial Meningitis Academic Medical Canter PO Box 22660 1100 DD Amsterdam, The Netherlands
Norway	Dr Martin Steinbakk	Division of Infectious Disease Control National Institute of Public Health Department of Bacteriology and Immunology PO Box 4404 Nydalen N-0403 Oslo, Norway
Poland	Dr Waleria Hryniewicz	Department of Epidemiology and Clinical Microbiology National Medicines Institute Chelmska 30/34 00-725 Warsaw, Poland
Portugal	Dr Manuela Caniça	National Laboratory of Antimicrobial Resistance Instituto Nacional de Saúde Dr Ricardo Jorge Avenida Padre Cruz 1649-016 Lisboa, Portugal
Romania	Dr Marina Pana	Cantacuzino Institute Bacterial Respiratory Infections 102 Splaiul Independentei, Sector 5 C.P.1-525, 050096 Bucharest, Romania
Scotland (UK)	Dr Mathew Diggle	Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory Stobhill Hospital, Microbiolgy Department 133 Balornock Road Glasgow G21 3UW, UK
Slovenia	Dr Metka Paragi	Department of medical Microbiology National Institute of Public Health Grablovičeva 44 1000 Ljubljana, Slovenia
Sweden	Prof Birgitta Henriques Normark	Swedish Institute for Infectious Disease Control Nobelsväg 18 SE 17182 Solna, Sweden
Switzerland	Prof Kathrin Mülemann	Institute for Infectious Diseases University of Bern Friedbühlstrasse 51 3010 Bern, Switzerland

Annex 2: Antimicrobial susceptibility testing breakpoints (mg/L) for *S. pneumoniae* from three different schemes

Antibiotic		Committee	MIC breakpoint (mg/L)			Disc content (µg)	Interpretation of zone diameter (mm)		
			S	I	R		S	I	R
Penicillin	Parenteral non-meningitis	CLSI	≤2	4	≥8	1 µg oxacillin	≥20		
	Parenteral meningitis	CLSI	≤0.06		≥0.12				
		BSAC	≤0.06	0.12–2	>2	1 µg oxacillin	≥20	11–19	≤10
	Pneumonia	EUCAST	*			1 µg oxacillin	≥20		<20
	Meningitis	EUCAST	≤0.06						
Erythromycin		CLSI	≤0.25	0.5	≥1	15 µg	≥21	16–20	≤15
		BSAC	≤0.25	0.5	>0.5	5 µg	≥22	20–21	≤19
		EUCAST	≤0.25		>0.5	15 µg	≥22		<19
Clindamycin		CLSI	≤0.25	0.5	≥1	2 µg	≥19	16–18	≤15
		BSAC	-	-	-	-	-	-	-
		EUCAST	≤0.5		>0.5	2 µg	≥19		<19
Ceftriaxone	Non-meningitis	CLSI	≤1	2	≥4	-	-	-	-
	Meningitis	CLSI	≤0.5	1	≥2	-	-	-	-
		BSAC	≤0.5	1-2	>2	30 µg	≥28	-	≤23
		EUCAST	≤0.5		>2		**		**

** In pneumonia, when a dose of 1.2g x 4 is used, isolates with MIC ≤ 0.5 mg/L should be regarded as susceptible to benzylpenicillin; when a dose of 2.4g x 4 or 1.2g x 6 is used, isolates with MIC ≤ 1 mg/L should be regarded as susceptible; when a dose of 2.4g x 6 is used then isolates with MIC ≤ 2.0 mg/L should be regarded as susceptible. In meningitis only isolates with MIC ≤ 0.06 mg/L (susceptible by oxacillin disc screen) should be regarded as susceptible, otherwise report resistant.*

*** Screen for beta-lactam resistance with 1µg oxacillin disc. Isolates categorised as susceptible may be reported as susceptible to ceftriaxone.*

Annex 3: Identification and molecular typing results

EQA number	Intended result	Laboratory number						
		1	2	3	4	5	6	7
Serotyping								
1001	**19A**	19A	18C	19A	19	19A	19A	19A
1002	**6A**	6A	6C	6A	6	6A	6A	6A
1003	**NT/rough**	NT	19A	Rough	NT	NT	NT	NT
1004	**6C**	6C	6A	6C	NT	6C	6C	6C
1005	***S. mitis***	Probably not *S. pneumoniae*	-	*S. mitis* gr.	*S. oralis*	*S. mitis/ oralis*	*S. mitis*	Not *S. pneumoniae*
1006	**6B**	6B	6B	6B	6	6B	6B	6B
1007	**14**	14	14	14	14	14	14	14
ST								
1001	**276**	276		276		276	276	
1002	**207**	207		207		207	207	
1003	**448**	448		448		448	448	
1004	**1692**	1692		1692		1692	1692	
1005		-		-		-	-	
1006	**176**	176		176		176	176	
1007	**9**	9		9		9	9	
Clonal complex								
1001	**230**	230				230	230	
1002	**207**	207				207	207	
1003	**448**	448				448	448	
1004	**395**	395				395	395	
1005	**-**	-				-	-	
1006	**176**	176/138				176	176	
1007	**9**	9				9	9	

For laboratories that do not serotype the serogroup result is scored as correct or incorrect.

For strain 1004: for laboratories that stated they do not have a serotype 6C antisera, 6A regarded as correct answer.

Discrepant results in red.

EQA number	Intended result	Laboratory number						
		8	9	10	11	12	13	14
Serotyping								
1001	**19A**	*S. pneumoniae*	19A	19A	19A	*S. pneumoniae*	19A	19A
1002	**6A**	*S. pneumoniae*	6A	6A	6B	*S. pneumoniae*	6A	6A
1003	**NT/rough**	*S. pneumoniae*	NT	NT	Rough	*S. pneumoniae*	NT	NT
1004	**6C**	*S. pneumoniae*	6C	6C	6C	*S. pneumoniae*	6C	6C
1005	***S. mitis***	*S. pneumoniae*	Not *S. pneumoniae*	*S. mitis*	19A (mixed)	*S. mitis*	*S. mitis*	*S. mitis*
1006	**6B**	*S. pneumoniae*	6B	6B	6B	*S. pneumoniae*	6B	6B
1007	**14**	*S. pneumoniae*	14	14	14	*S. pneumoniae*	14	14
ST								
1001	**276**		276	276	276		276	276
1002	**207**		207	207	207		207	207
1003	**448**		448	448	448		448	448
1004	**1692**		1692	1692	1692		1692	1692
1005			-	-	276		-	-
1006	**176**		176	176	176		176	176
1007	**9**		9	9	9		9	9
Clonal complex								
1001	**230**		230		230		230	230
1002	**207**		207		207		207	207
1003	**448**		448		448		448	448
1004	**395**		395		395		395	395
1005	**-**		-		230		-	-
1006	**176**		176		176		176	176
1007	**9**		9		9		9	9

For laboratories that do not serotype the serogroup result is scored as correct or incorrect.

For strain 1004: for laboratories that stated they do not have a serotype 6C antisera, 6A regarded as correct answer.

Discrepant results in red.

EQA number	Intended result	Laboratory number						
		15	16	17	18	19	20	21
Serotyping								
1001	**19A**	*S. pneumoniae*	18	19A	19A	19A	19A	19A
1002	**6A**	*S. pneumoniae*	6	6A	6A	6A	6A/6C	6A
1003	**NT/rough**	*S. pneumoniae*	*S. mitis*	17A	NT	Rough	NT	NT
1004	**6C**	*S. pneumoniae*	17	6A	6C	6C	6B	6C
1005	***S. mitis***	*S. viridans*	*S. mitis/ oralis*	*S. mitis*	*S. mitis*	*S. mitis gr.*	*Streptococcus* sp.	*S. mitis*
1006	**6B**	*S. pneumoniae*	6	6B	6B	6B	6B	6B
1007	**14**	*S. pneumoniae*	NT	14	14	14	14	14
ST								
1001	**276**				276			276
1002	**207**				207			207
1003	**448**				448			448
1004	**1692**				1692			1692
1005					-			-
1006	**176**				176			176
1007	**9**				9			9
Clonal complex								
1001	**230**							
1002	**207**							
1003	**448**							
1004	**395**							
1005	**-**							
1006	**176**							
1007	**9**							

For laboratories that do not serotype the serogroup result is scored as correct or incorrect.

For strain 1004: for laboratories that stated they do not have a serotype 6C antisera, 6A regarded as correct answer.

Discrepant results in red.

EQA number	Intended result	Laboratory number				
		22	23	24	25	26
Serotyping						
1001	**19A**	*S. pneumoniae*	19A	19A	19A	19A
1002	**6A**	*S. pneumoniae*	6	6A	6A	6A
1003	**NT/rough**	Negative	NT	NT	NT	*Gemella* sp.
1004	**6C**	*S. pneumoniae*	6	6C/D	6A	6A
1005	***S. mitis***	*S. mitis/oralis*	NT	6B + other	*S. mitis*	*S. mitis*
1006	**6B**	*S. pneumoniae*	6	6B	6B	6B
1007	**14**	*S. pneumoniae*	14	14	14	14
ST						
1001	**276**			276	276	
1002	**207**			207	207	
1003	**448**			448	448	
1004	**1692**			1692	1692	
1005				176	-	
1006	**176**			170	176	
1007	**9**			3898	9	
Clonal complex						
1001	**230**			230	230	
1002	**207**			207	-	
1003	**448**			448	448	
1004	**395**			395	327	
1005	**-**			176	-	
1006	**176**			176	-	
1007	**9**			9	9	

For laboratories that do not serotype the serogroup result is scored as correct or incorrect.

For strain 1004: for laboratories that stated they do not have a serotype 6C antisera, 6A regarded as correct answer.

Discrepant results in red.

Annex 4: Results for antimicrobial susceptibility testing

MIC values mg/L in green
Zone size mm in blue

EQA number	Laboratory number													
	1		2		3		4		5		6		7	
Pencillin – MIC,mg/L														
1001	1.0	I	1	Im/S	2	I	0.75	Rm/S	0.75	Rm/S	0.5	I	0.75	I
1002	0.016	S	<0.016	S	0.016	S	0.016	S	0.016	S	0.016	S	0.016	S
1003	0.016	S	<0.016	S	0.008	S	0.016	S	0.008	S	0.016	S	0.008	S
1004	0.032	S	<0.016	S	0.016	S	0.016	S	0.023	S	0.032	S	0.016	S
1005	4.0	R	2	Rm/S	4	R			4	R	8	R	4	R
1006	0.016	S	<0.016	S	0.016	S	0.016	S	0.016	S	0.016	S	0.012	S
1007	0.032	S	<0.016	S	0.016	S	0.016	S	0.016	S	0.016	S	0.023	S
Penicillin – Disc zone size,mm														
1001					25	I							0	I
1002					47	S							28	S
1003					42	S							28	S
1004					44	S							26	S
1005					18	R							0	R
1006					43	S							26	S
1007					41	S							26	S
Erythromycin – MIC,mg/L														
1001			>256	R	>256	R			>256	R			>256	R
1002			0.032	S	0.125	S			0.064	S			0.094	S
1003			0.047	S	0.125	S			0.064	S			0.125	S
1004			0.047	S	0.125	S			0.064	S			0.094	S
1005			4	R	2	R			1.5	I			4	R
1006			0.064	S	0.125	S			0.125	S			0.125	S
1007			12	R	8	R			24	R			24	R
Erythromycin – Disc zone size,mm														
1001	11	R	15	R	6	R	6	R			10	R	0	R
1002	32	S	22	S	35	S	29	S			29	S	30	S
1003	32	S	22	S	35	S	32	S			31	S	30	S
1004	28	S	25	S	33	S	23	S			28	S	26	S
1005	21	I	15	R	21	R					18	R	17	R
1006	27	S	22	S	31	S	27	S			28	S	27	S
1007	11	R	10	R	10	R	10	R			11	R	10	R

EQA number	Laboratory number													
	1		2		3		4		5		6		7	
Clindamycin – MIC,mg/L														
1001			>256	R	>256	R			>256	R			>256	R
1002			0.064	S	0.125	S			0.064	S			0.125	S
1003			0.064	S	0.064	S			0.094	S			0.125	S
1004			0.047	S	0.125	S			0.047	S			0.094	S
1005			0.023	S	0.032	S			0.064	S			0.094	S
1006			0.094	S	0.125	S			0.023	S			0.125	S
1007			0.19	S	0.125	S			0.094	S			0.19	S
Clindamycin – Disc zone size,mm														
1001	16	R	17	R	16	R	6	R			20	R	15	R
1002	26	S	20	S	28	S	25	S			27	S	26	S
1003	31	S	20	S	34	S	31	S			31	S	28	S
1004	25	S	20	S	30	S	27	S			27	S	24	S
1005	31	S	20	S	34	S					31	S	24	S
1006	23	I	19	S	27	S	24	S			27	S	23	S
1007	24	I	20	S	26	S	25	S			26	S	23	S
Ceftriaxone – MIC,mg/L														
1001			1.50	Rm/I	0.5	S	0.50	S	0.75	Im/S	0.25	S	0.50	S
1002			0.023	S	0.016	S	0.016	S	0.032	S	0.016	S	0.016	S
1003			0.023	S	0.008	S	0.016	S	0.016	S	0.016	S	0.006	S
1004			0.032	S	0.016	S	0.016	S	0.023	S	0.016	S	0.012	S
1005			4	R	2	I			2	I	2	I	0.75	I
1006			0.047	S	0.016	S	0.016	S	0.023	S	0.016	S	0.008	S
1007			0.032	S	0.016	S	0.016	S	0.023	S	0.016	S	0.008	S
Ceftriaxone – Disc zone size,mm														
1001													27	S
1002													38	S
1003													40	S
1004													35	S
1005													23	I
1006													34	S
1007													38	S

EQA number	Laboratory number													
	8		9		10		11		12		13		14	
Pencillin – MIC,mg/L														
1001			0.75	R	1.5	Rm/I/S	2	Rm/S	1	I	1.5	Rm/S	2	R
1002			0.012	S	0.03	S	0.03	S	0.012	S	0.012	S	<0.015	S
1003			0.012	S	0.015	S	0.015	S	0.012	S	0.012	S	<0.015	S
1004			0.032	S	0.03	S	0.03	S	0.023	S	0.023	S	<0.015	S
1005					6	R	2	Rm/S	4	R			4	R
1006			0.016	S	0.03	S	0.03	S	0.023	S	0.008	S	<0.015	S
1007			0.023	S	0.023	S	0.03	S	0.016	S	0.016	S	<0.015	S
Penicillin – Disc zone size,mm														
1001	25/6ox	R	6	R					24	I			5	R
1002	35/20ox	S	24	S					40	S			25	S
1003	36/21ox	S	23	S					40	S			30	S
1004	38/18ox	S	25	S					40	S			25	S
1005	22/6ox	R							18	R			5	R
1006	35/6ox	R	20	S					40	S			25	S
1007	38/21ox	S	24	S					40	S			24	S
Erythromycin – MIC,mg/L														
1001			>256	R	>256	R			>256	R	>256	R	>256	R
1002			0.19	S	0.03	S			0.032	S	0.064	S	0.094	S
1003			0.19	S	0.03	S			0.064	S	0.125	S	0.094	S
1004			0.19	S	0.015	S			0.047	S	0.064	S	0.125	S
1005					1.5	R			2	R			8	R
1006			0.125	S	0.06	S			0.094	S	0.125	S	0.094	S
1007			24	R	12	R			12	R	24	R	32	R
Erythromycin – Disc zone size,mm														
1001	6	R	6	R			6	R	6	R			5	R
1002	32	S	28	S			30	S	32	S			30	S
1003	35	S	29	S			31	S	32	S			31	S
1004	37	S	28	S			30	S	29	S			27	S
1005	26	I					6	R	15	R			16	R
1006	20	R	27	S			30	S	29	S			27	S
1007	15	R	6	R			10	R	11	R			8	R

EQA number	Laboratory number													
	8		9		10		11		12		13		14	
Clindamycin – MIC,mg/L														
1001			>256	R					0.38	I	>256	R	>16	R
1002			0.19	S					0.094	S	0.125	S	<0.012	S
1003			0.094	S					0.064	S	0.064	S	<0.012	S
1004			0.19	S					0.094	S	0.094	S	<0.012	S
1005									0.032	S			<0.012	S
1006			0.19	S					0.125	S	0.125	S	<0.012	S
1007			0.125	S					0.19	S	0.19	S	<0.012	S
Clindamycin – Disc zone size,mm														
1001	15	R	15	R			6	R	16	I			5	R
1002	35	S	26	S			25	S	26	S			24	S
1003	37	S	28	S			28	S	30	S			28	S
1004	30	S	24	S			26	S	28	S			24	S
1005	35	S	24	S			6	R	27	S			31	S
1006	28	S	23	S			25	S	24	S			22	S
1007	31	S	23	S			24	S	25	S			24	S
Ceftriaxone – MIC,mg/L														
1001			0.50	S	0.50	S	3	R	0.50	S	1	Im/s		
1002			0.023	S	0.023	S	0.094	S	0.016	S	0.016	S		
1003			0.012	S	0.015	S	0.047	S	0.016	S	0.012	S		
1004			0.023	S	0.023	S	0.064	S	0.016	S	0.023	S		
1005					2	R/I	2	R/I	2	I				
1006			0.016	S	0.023	S	0.064	S	0.016	S	0.016	S		
1007			0.023	S	0.015	S	0.064	S	0.016	S	0.016	S		
Ceftriaxone – Disc zone size,mm														
1001	26	I							30	S				
1002	37	S							40	S				
1003	36	S							40	S				
1004	39	S							40	S				
1005	25	I							25	I				
1006	36	S							40	S				
1007	38	S							40	S				

EQA number	Laboratory number													
	15		16		17		18		19		20		21	
Pencillin – MIC,mg/L														
1001	0.38	I	>2	R	1	Rm/I/S	1	I	2	R	2	Rm/S	1.5	
1002	0.006	S	<0.06	S	0.023	S	<0.06	S	0.023	S	<0.06	S		
1003	0.004	S	0.012	S	0.012	S	<0.06	S	<0.016	S	<0.06	S		
1004	0.012	S	<0.06	S	0.023	S	<0.06	S	0.023	S	<0.06	S		
1005			4	R	3	I	4	R	3	I	4	Rm/I	8	
1006	0.004	S	<0.06	S	0.023	S	<0.06	S	0.016	S	<0.06	S		
1007	0.012	S	<0.06	S	0.023	S	<0.06	S	0.023	S	<0.06	S		
Penicillin – Disc zone size,mm														
1001					22	Rm/I/S			18	R				
1002					34	S			34	S			>20	
1003					38	S			37	S			>20	
1004					30	S			35	S			>20	
1005	6	R			15	I			11	I				
1006					30	S			40	S			>20	
1007					30	S			36	S			>20	
Erythromycin – MIC,mg/L														
1001			>1	R	>256	R	>16	R	>256	R	>8	R		
1002			<0.06	S	0.094	S	<0.25	S	0.125	S	<0.125	S		
1003					0.094	S	<0.25	S	0.125	S	<0.125	S		
1004			<0.06	S	0.094	S	<0.25	S	0.125	S	<0.125	S		
1005					3	R	8	R	2	R	2	R		
1006			<0.06	S	0.094	S	<0.25	S	0.125	S	<0.125	S		
1007			>1	R	16	R	16	R	4	R	>8	R		
Erythromycin – Disc zone size,mm														
1001	6	R			6	R			6	R				
1002	28	S			28	S			22	S				
1003	31	S	28.5	S	29	S			28	S				
1004	29	S			26	S			25	S				
1005	21	S	17	I	15	R			19	R				
1006	28	S			24	S			27	S				
1007	6	R			6	R			8	R				

EQA number	Laboratory number													
	15		16		17		18		19		20		21	
Clindamycin – MIC,mg/L														
1001				-	>256	R	>8	R			8	R		
1002				S	0.125	S	<0.25	S			<0.125	S		
1003					0.047	S	<0.25	S			<0.125	S		
1004				S	0.094	S	<0.25	S			<0.125	S		
1005					0.032	S	<0.25	S			<0.125	S		
1006				S	0.125	S	<0.25	S			<0.125	S		
1007				-	0.125	S	<0.25	S			<0.125	S		
Clindamycin – Disc zone size,mm														
1001	6	R			6	R			6	R				
1002	24	S			24	S			20	S				
1003	28	S	27	S	27	S			27	S				
1004	25	S			22	S			22	S				
1005	30	S	30	S	19	S			25	S				
1006	26	S			25	S			23	S				
1007	24	S			27	S			23	S				
Ceftriaxone – MIC,mg/L														
1001	0.50	S	2	Rm/l	0.50	S	0.50	S	1	I	2	Rm/l	1	
1002	0.016	S	<0.06	S	0.016	S	0.008	S	0.016	S	<0.125	S		
1003	0.004	S			0.008	S	0.004	S	0.008	S	<0.125	S		
1004	0.008	S	<0.06	S	0.012	S	0.008	S	0.012	S	<0.125	S		
1005					1.5	I	1	I	2	I	4	R	2	
1006	0.008	S	<0.06	S	0.012	S	0.008	S	0.016	S	<0.125	S		
1007	0.023	S	<0.06	S	0.016	S	0.008	S	0.016	S	<0.125	S		
Ceftriaxone – Disc zone size,mm														
1001					24	S			25	I				
1002					33	S			32	S				
1003			39	S	38	S			38	S				
1004					30	S			30	S				
1005	28	S	27.5	S	25	I			23	I				
1006					30	S			35	S				
1007					30	S			37	S				

EQA number	Laboratory number									
	22		23		24		25		26	
Pencillin – MIC,mg/L										
1001	0.75	S	3	S	4	R	1	I	1	S
1002	<0.016	S	0.032	S	0.031	S	0.004	S	0.012	S
1003			0.016	S	0.016	S	0.004	S	0.008	
1004	<0.016	S	0.032	S	0.031	S	0.008	S	0.023	S
1005			>32	R	0.016	S	2	I	3	
1006	<0.016	S	0.012	S	0.016	S	0.004	S	0.016	S
1007	<0.016	S	0.008	S	0.031	S	0.008	S	<0.016	S
Penicillin – Disc zone size,mm										
1001	20	S							6	S
1002	38	S							24	S
1003									26	
1004	26	S							25	S
1005									6	
1006	24	S							24	S
1007	27	S							24	S
Erythromycin – MIC,mg/L										
1001	>256	R	16	S	>128	R	>4	R	>256	R
1002	<0.016	S	0.125	S	0.125	S	0.125	S	0.064	S
1003			0.125	S	0.125	S	0.125	S	0.094	
1004	0.032	S	0.125	S	0.125	S	0.125	S	0.064	S
1005			6	S	0.125	S	4	R	1.5	
1006	0.047	S	0.125	S	0.125	S	0.125	S	0.094	S
1007	3	R	64	R	>128	R	>4	R	8	R
Erythromycin – Disc zone size,mm										
1001	6	R					6	R	8	R
1002	34	S					25	S	19	S
1003							29	S	32	
1004	29	S					25	S	29	S
1005							15	R	19	
1006	30	S					26	S	26	S
1007	10	R					6	R	8	R

EQA number	Laboratory number									
	22		23		24		25		26	
Clindamycin – MIC,mg/L										
1001	0.125	S			>128	R	>4	R	>256	R
1002	0.023	S			0.125	S	0.25	S	0.25	S
1003					0.063	S	0.063	S	0.064	
1004	0.047	S			0.063	S	0.125	S	0.12	S
1005					0.125	S	0.032	S	0.032	
1006	0.047	S			0.125	S	0.125	S	0.12	S
1007	0.016	S			0.125	S	0.125	S	0.094	S
Clindamycin – Disc zone size,mm										
1001	20	S					6	R		
1002	30	S					23	S		
1003							28	S		
1004	25	S					23	S		
1005							23	S		
1006	27	S					22	S		
1007	28	S					22	S		
Ceftriaxone – MIC,mg/L										
1001	0.75	S	32	R	1	I	1	I	0.75	S
1002	<0.016	S	0.125	S	0.031	S	0.016	S	0.016	S
1003			0.032	S	0.016	S	0.008	S	0.006	
1004	<0.016	S	0.125	S	0.031	S	0.016	S	0.012	S
1005			2	S	0.031	S	2	I	2	
1006	0.016	S	0.023	S	0.016	S	0.008	S	0.012	S
1007	<0.016	S	0.012	S	0.016	S	0.016	S	0.012	S
Ceftriaxone – Disc zone size,mm										
1001										
1002										
1003										
1004										
1005										
1006										
1007										