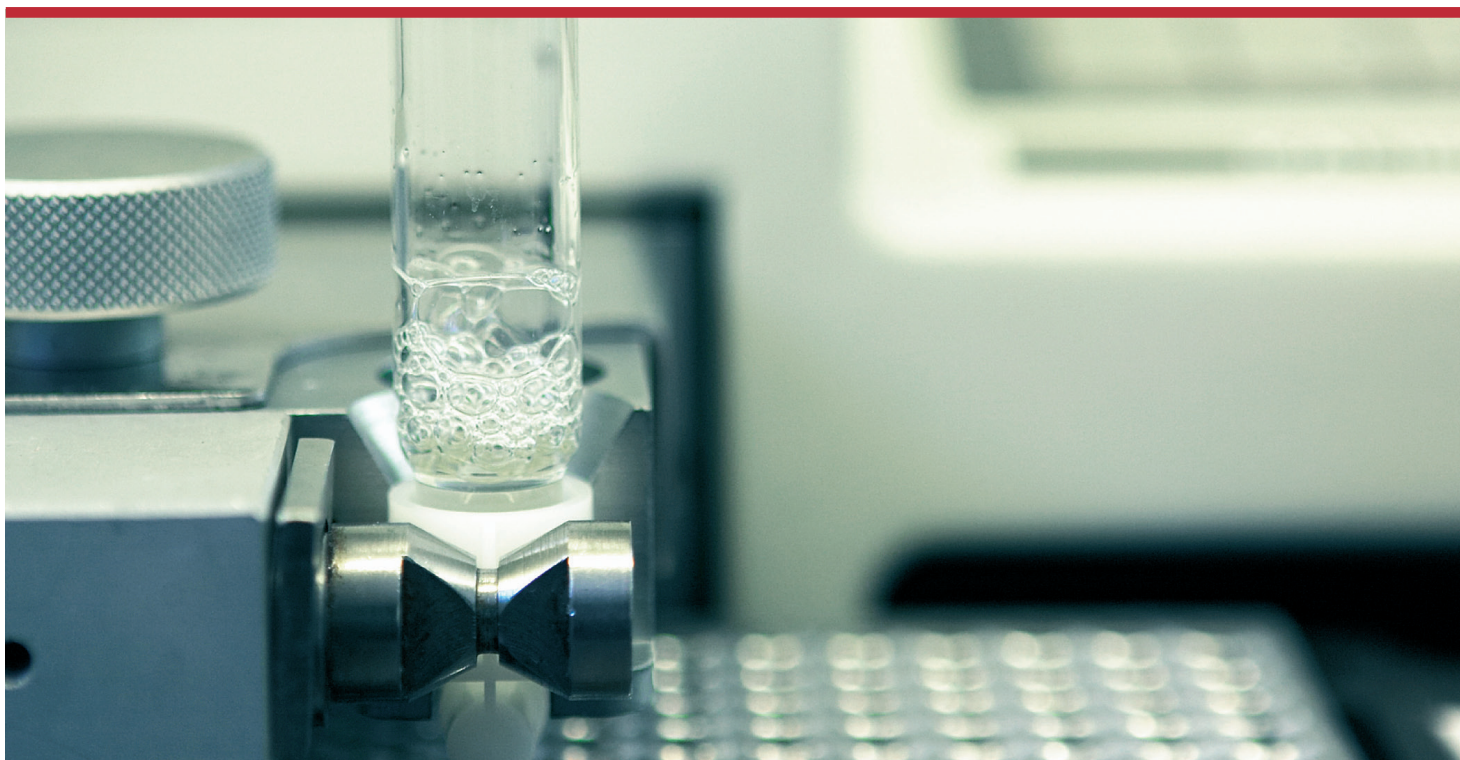


The 14th EURL-AR Proficiency Test - enterococci, staphylococci and *E. coli* 2013



Lina Cavaco
Susanne Karlsmose
Rene S. Hendriksen
Frank M. Aarestrup



DTU Food
National Food Institute

The 14TH EURL-AR Proficiency Test
Enterococci, Staphylococci and *Escherichia coli* - 2013

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National Food Institute
Technical University of Denmark
Kemitorvet, Building 204
DK-2800 Lyngby



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1. Introduction

This report describes the results from the fourteenth proficiency test conducted by the National Food Institute as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). This proficiency test focuses on antimicrobial susceptibility testing (AST) of enterococci, staphylococci and *Escherichia coli*. It is the seventh External Quality System Assurance System (EQAS) conducted for these microorganisms.

This EQAS aims to: i) monitor the quality of AST results produced by National Reference Laboratories (NRL-AR), ii) identify laboratories which may need assistance to improve their performance in AST, and iii) determine possible topics for further research or elaboration.

In reading this report, the following important considerations should be taken into account:

1) Expected results were generated by performing Minimum Inhibitory Concentration (MIC) determinations for all test strains in two different occasions at the Technical University of Denmark, National Food Institute (DTU-FOOD). These results were then verified by the United States Food and Drug Administration (FDA), Centre for Veterinary Medicine. Finally, a fourth MIC determination was performed at DTU-FOOD after preparation of the agar stab culture for shipment to participants to confirm that the vials contained the correct strains with the expected MIC values.

2) Evaluation is based on interpretations of AST values determined by the participants. This is in agreement with the method used by MS to report AST data to the European Food Safety Authority (EFSA), and complies with “the main objective of this EQAS to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported to EFSA by the different NRLs”, as stated in the protocol.

3) The EURL-AR network agreed on setting the accepted deviation level for laboratory performance to 5%.

Evaluation of a result as “deviating from the expected interpretation” should be carefully analyzed in a self-evaluation procedure performed by the participant. Since methods used for MIC determination have limitations, it is not considered a mistake to obtain a one-fold dilution difference in the MIC of a specific antimicrobial when testing the same strains. However, if the expected MIC is close to the breakpoint value for categorizing the strain as susceptible or resistant, a one-fold dilution difference, which is acceptable, may result in two different interpretations, i.e. the same strain will be categorized as susceptible and resistant, which will be evaluated as correct in one case and incorrect in the other if the evaluation is based on interpretation of MIC values. Since this report evaluates the interpretations of AST values, some participants may find their results classified as wrong even though the actual MIC they reported is only one-fold dilution different from the expected MIC. In these cases, the participants should be confident about the good quality of their performance of AST. In the organization of the EQAS we try to avoid these situations by choosing test strains with MIC values distant from the breakpoints for resistance, which is not always feasible for all strains and all antimicrobials. Therefore, the EURL-AR network unanimously established in 2008 that if there are less than 75% correct results for a specific strain/antimicrobial combination, the reasons for this situation must be further examined and, on selected occasions explained in details case by case, these results may subsequently be subtracted from the evaluation report.

This report is approved in its final version by a technical advisory group composed by competent representatives from all NRLs who meet once a year at the EURL-AR workshop.

All conclusions presented in this report are publically available. However, participating laboratories are identified by codes and each code is known only by the corresponding laboratory. The full list of laboratory codes is confidential information known only by relevant

representatives of the EURL-AR and the EU Commission.

The EURL-AR is accredited by DANAK as provider of proficiency testing (accreditation no. 516); working with zoonotic pathogens and indicator organisms as bacterial isolates (identification, serotyping and antimicrobial susceptibility testing).

2. Materials and Methods

2.1 Participants in EQAS 2013

A pre-notification to announce the EQAS 2013 on AST of enterococci, staphylococci and *E. coli* was sent by e-mail on the 16th April 2013 to the 41 NRLs in the network (App. 1) including seven additional laboratories (one from each of the following countries: Iceland, Norway, Serbia, Spain, Switzerland, The Netherlands and Turkey). These were invited to take part in

the EQAS 2013 on the basis of their participation in previous EQAS iterations and/or affiliation to the EU network. Participants represented all EU countries (except for Luxembourg) and Norway, Switzerland and Iceland (App. 2).

In total, this report includes AST results of enterococci strains submitted by 29 laboratories, and AST results of staphylococci

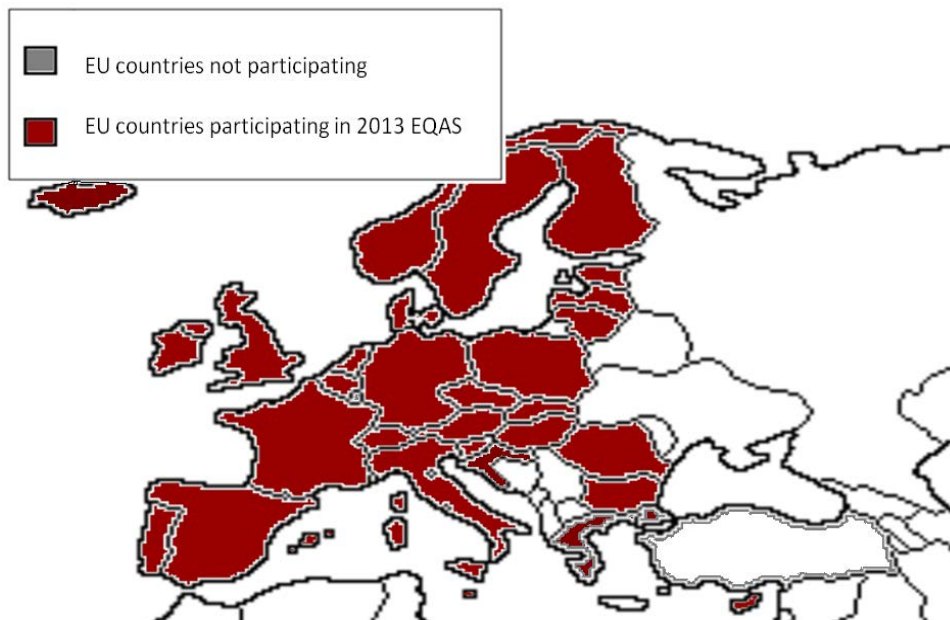


Figure 1 Participating countries in susceptibility testing of Enterococci, staphylococci and/or *E. coli*



strains submitted by 34 laboratories and *E. coli* strains submitted by 34 laboratories (Figure 1).

2.2 Strains

Bacterial strains included in this EQAS (eight enterococci, eight staphylococci and eight *E. coli*) were selected among the DTU-Food strain collection on the basis of antimicrobial resistance profiles and MIC values. For quality assurance purposes, one strain per each bacterial species tested has been included in all EQAS iterations performed to date, which represents an internal control.

AST of the EQAS strains was performed at DTU-Food by MIC determination using the Sensititre panels from Trek Diagnostic Systems. The MIC values obtained (App. 3) were used as reference values for this EQAS trial after verification performed by the U.S. FDA. Results from the following antimicrobials were however not verified by FDA: daptomycin, tigecycline, teicoplanin and ampicillin for enterococci. Ceftazidime, meropenem, colistin, cefotaxime, cefotaxime+clavulanic acid, ceftazidime, ceftazidime+clavulanic acid, imipenem, imipenem+EDTA, florfenicol and trimethoprim for *E. coli* and furthermore, florfenicol, sulfamethoxazol and trimethoprim for Staphylococci. After comparison and verification of the MIC values obtained at DTU-Food and FDA, the strains were inoculated in agar as stab cultures and dispatched to the participating laboratories.

Reference strains *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were provided to new participating laboratories with instructions to store and maintain them for quality assurance purposes and future EQAS trials.

2.3 Antimicrobials

The panels of antimicrobials recommended for

AST in this trial are listed in Table 1.

The antimicrobials tested were changed in relation to previous trials by adjusting to the new EFSA recommendations; however, since the work at EU level on the panel of antimicrobials which would become part of the EU regulation was ongoing at the time, the choice of the antimicrobial panel did not correspond to the final panel set in the new legislation. The antimicrobials included in the EQAS will again be revised for the next trial, to adjust to the final panel to be used in the EU monitoring starting in 2014.

Guidelines for performing AST were set according to the Clinical and Laboratory Standards Institute (CLSI) document – M7-A9 (2012) “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Ninth Edition”.

MIC results were interpreted by using EUCAST epidemiological cut-off values (www.eucast.org), as recommended by EFSA and described in the protocol (App. 4). Results of ESBL detection tests were interpreted according to the recommendations reported in the EUCAST expert rules.

2.4 Distribution

Protocols and all relevant information were uploaded on the EURL-AR website (<http://www.eurl-ar.eu>), thereby EQAS participants could access necessary information at any time. In June 2013, bacterial strains in agar stab cultures were dispatched in double pack containers (class UN 6.2) to the participating laboratories according to the International Air Transport Association (IATA) regulations as UN3373, biological substances category B.

Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2013 component

Enterococci	Staphylococci	<i>Escherichia coli</i>
Ampicillin , AMP	Cefoxitin, FOX	Ampicillin, AMP
Chloramphenicol, CHL	Chloramphenicol, CHL	Cefotaxime, CTX
Ciprofloxacin , CIP	Ciprofloxacin, CIP	Ceftazidime, CAZ
Daptomycin, DAP	Clindamycin, CLN	Chloramphenicol, CHL
Erythromycin, ERY	Erythromycin, ERY	Ciprofloxacin, CIP
Gentamicin, GEN	Florfenicol, FFN	Colistin, COL
Linezolid, LZD	Gentamicin, GEN	Florfenicol, FFN
Quin.-Dalf. (Synercid), SYN	Linezolid, LZD	Gentamicin, GEN
Teicoplanin, TEI	Mupirocin, MUP	Meropenem, MER
Tetracycline, TET	Penicillin, PEN	Nalidixic acid, NAL
Tigecycline, TGC	Quin.-Dalf. (Synercid), SYN	Sulfamethoxazole, SMX
Vancomycin, VAN	Sulfamethoxazole, SMX	Tetracycline, TET
	Tetracycline, TET	Trimethoprim, TMP
	Trimethoprim, TMP	
	Vancomycin, VAN	

2.5 Procedure

Participants were instructed to keep the agar stab cultures refrigerated until performance of AST according to the information posted on the EURL-AR website (App. 4b, 4c, 4d and 4e). In addition, instructions for interpretation of AST results were provided. For interpretation of MIC determination results, cut-off values were reported in the protocol (App. 4b: Tables 1, 2 and 3). For interpretation of disk-diffusion (DD) method results, participants were advised to use interpretive breakpoints as in their routine methods. In both cases, the EQAS test strains should have been categorized as resistant or susceptible, and the EURL-AR recommended interpreting intermediate results as susceptible.

The EURL-AR is aware that there are two different types of interpretative criteria of results, clinical breakpoints and epidemiological cut-off values. The terms 'susceptible', 'intermediate' and 'resistant' should be reserved for classifications made in relation to the therapeutic application of antimicrobial agents.

When reporting data using epidemiological cut-off values, bacteria should be reported as 'wild-type' or 'non-wild-type' (Schwarz et al., 2010). Due to the different methods of AST used by the participants and also to simplify the interpretation of results, throughout this report, we will still maintain the terms susceptible and resistant, even in cases where we are referring to wild-type and non-wild-type strains.

All participating laboratories were invited to enter the obtained results into an electronic record sheet at the EURL-AR web-based database through a secured individual login and password. Alternatively, it was offered the possibility to fill-in a record sheet (provided with the protocol) and to send it to the EURL-AR by fax, mail or email.

The record sheet contained also space for reporting the results (zone diameters in millimeters or MIC values in µg/ml) obtained for the reference strains. These results were compared to the quality control ranges reported by CLSI in documents M31-A3 (2008) / M100-S23 (2013) (App. 5).



The database was finally closed and evaluations were made available to participants on the 10th September 2013.

After this date, the participants were invited to login again to retrieve a database-generated individual report which contained an evaluation

of the submitted results including possible deviations from the expected interpretations. Finally, participants were encouraged to complete an evaluation form available at the EURL-AR database with the aim to improve future EQAS trials

3. Results

The participants were asked to report results, including MIC values or inhibition zone diameters obtained by DD together with the categorisation as resistant or susceptible. Only the categorisation was evaluated, whereas the MIC values and disk diffusion inhibition zones were used as supplementary information.

As mentioned in the introduction, the EURL-AR network established that data should be examined and possibly subtracted from the general analysis if there are less than 75% correct results for a strain/antimicrobial combination in the ring trial. In this respect, we have noticed in the raw data analysis at database closing that four antimicrobial / strain combinations were causing 25% or more deviations and these were excluded from the analysis before the evaluation was opened to the participants. This was the case for ENT 7.2/ampicillin (61%), ENT 7.2/Quinupristin-dalfopristin (75%), ENT 7.3/ampicillin (71%) and ENT 7.3/quinupristin-dalfopristin (71%). Similarly, the results for Staphylococci for two combinations were excluded from the raw data analysis due to problems observed in relation to breakpoints. This was the case of strain ST 7.4/ciprofloxacin (50%) and strain ST 7.6/quinupristin+ dalfopristin (43%) which had low percentages of correct results as described above and were therefore excluded from the evaluation before the database evaluation was opened. The cause for these deviations was that the expected values were lying just one step from the breakpoint. For this reason, these tests were not considered representative of the

capacity of the laboratories for performing AST and were therefore not interpreted in the database when the evaluation was performed. Similarly, the results for the combination EURL ENT 7.8/daptomycin were found to lead to a deviation level above 25% also because the expected value lay/was close to the breakpoint. Therefore, as the results were evaluated for the report, further analysis was carried out to decide if they should be omitted as well.

In the EQAS 2013, the overall percentages of deviations from the expected results were 1.5%, 2.2% and 0.9% for enterococci, staphylococci and *E. coli*, respectively (Figure 2). These percentages were lower for enterococci, and *E. coli* trials when compared to the ones observed in 2012. The internal control strains (ENT-7.7, ST-7.4 and EC-7.4) followed the same decreasing pattern for the enterococci strain with 2.7 % deviation, while for *E. coli* the deviations were 0.5% as in 2012 and for staphylococci the deviation percent increased to 2.9% (Figure 2). Of note, these percentages do not include specific combinations strain/antimicrobial for which we observed less than 75% reported results in agreement with the expected results (detailed explanation is provided in the paragraphs below).

3.1 Methods

In the data analysis, results were grouped according to the methods used by the participants as follows. The agar dilution method and MIC determination were evaluated together as they are both quantitative methods

Table 2. Total number of antimicrobial susceptibility tests (AST) performed for each EQAS 2013 strain and percentage (%) of correct results

Strain*	No. AST	No correct	% correct	Strain*	No. AST	No correct	% correct	Strain*	No. AST	No correct	% correct
ENT-7.1	231	229	99,1%	ST-7.1	409	398	97,3%	EC 7.1	404	402	99,5%
ENT-7.2	220	215	97,7%	ST-7.2	408	392	96,1%	EC 7.2	406	401	98,8%
ENT-7.3	220	218	99,1%	ST-7.3	408	403	98,8%	EC 7.3	407	405	99,5%
ENT-7.4	261	258	98,9%	ST-7.4	376	365	97,1%	EC 7.4	406	404	99,5%
ENT-7.5	236	232	98,3%	ST-7.5	410	402	98,0%	EC 7.5	405	400	98,8%
ENT-7.6	234	232	99,1%	ST-7.6	386	379	98,2%	EC 7.6	404	398	98,5%
ENT-7.7	261	254	97,3%	ST-7.7	408	401	98,3%	EC 7.7	404	402	99,5%
ENT-7.8	255	255	100%	ST-7.8	407	402	98,8%	EC 7.8	407	402	98,8%

*ENT, enterococci; ST, staphylococci; EC, *Escherichia coli*.

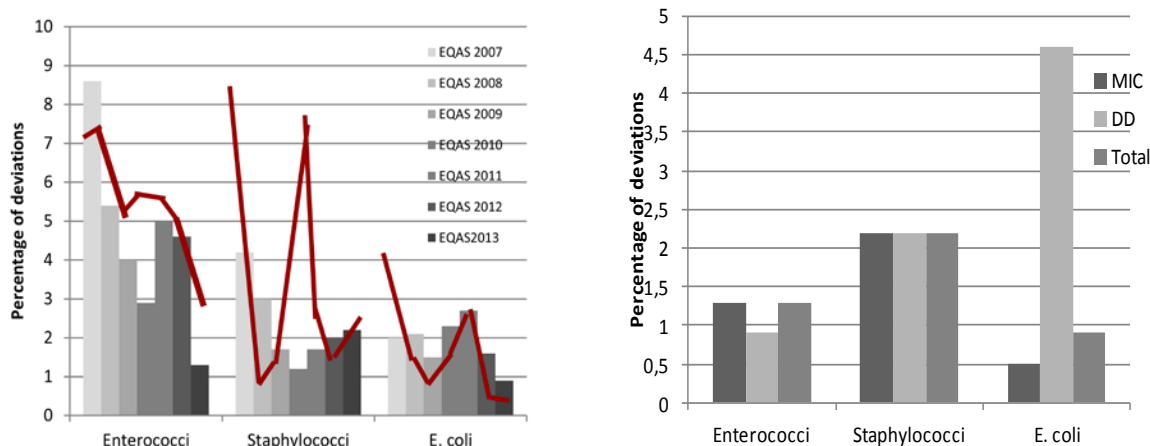


Figure 2 Overview of the percentages of deviations from expected results obtained in different EQAS iterations for the three bacterial species tested. The internal control strain is represented by a red line.

Figure 3 EQAS 2013: Percentage of deviations from the expected interpretation subdivided by tested species and antimicrobial susceptibility test method used.

giving results corresponding to the MIC of the bacterial strain tested. The Rosco and DD methods were evaluated together since they are based on the same principle of antimicrobial diffusion in the agar.

In the EQAS 2013, 26, 27 and 31 participants performed AST by MIC determination for enterococci, staphylococci and *E. coli*, respectively, and three, seven and three

participants performed AST by agar diffusion techniques for enterococci, staphylococci and *E. coli*, respectively.

3.2 Deviations overall

The list of deviations is illustrated in Appendixes 8a, 8b and 8c. Figure 2 shows the overall deviation levels.

The percentages of deviations were less



affected by the method used, than in previous years. Only for *E. coli* the results show a higher percentage of deviations performing AST by DD as compared to MIC determinations. For the enterococci the deviation percent obtained with DD was actually lower and for staphylococci it was similar (Figure 3),

Overall, the percentage of results in agreement with the expected values ranged from a minimum of 96.1% (strain ST 7.2) to a maximum of 99.5% (strains EC 7.1, EC 7.3 and EC 7.7), as shown in Table 2. The *E. coli* trial resulted in the highest percentages of results in agreement with the expected values.

Detailed analyses of the results obtained for each species are reported in the following paragraphs.

3.2.1 Enterococci

Analysis of results from the Enterococci trial showed that one additional antimicrobial combination had more than 25% deviation due to expected results being very close to the breakpoint. This was the case of the combination daptomycin and strain EURL ENT 7.8 for which only 11 laboratories uploaded results and three of these reported results just one step above the expected result but already above the resistance breakpoint. These results were subtracted from the calculations in this report as they do not reflect the capacity of the laboratories to perform AST.

In general, 99% of the results were interpreted correctly. Figure 3 shows the total percentage

of deviations assigned to AST by MIC testing or DD. The percentage of deviation for the labs performing DD was lower in general (the DD column represents only three laboratories).

Results deviating from expected interpretation subdivided by strain showed that percentage of deviations from expected results ranged from 0.0% (ENT 7.8) to 2.7% (ENT 7.7) (Figure 4). Laboratories performing AST by DD reported very good results for most of the strains ranging from percentage of deviation of 0% (for ENT-7.3, 7.4, 7.5, 7.6, 7.7 and 7.8) to 4% (for ENT-7.2), as shown in Figure 4. Out of 29 laboratories participating in the enterococci trial, only three performed AST by DD. Analysis of the results according to the tested antimicrobials showed that the highest percentages of deviation from expected interpretations were obtained in testing susceptibility to gentamicin (3.9%) and ampicillin (3.5%) (Figure 5). An overview of obtained and expected results is reported in Appendix 7a.

Enterococci identification (ID)

For the first time in this EQAS, the participants were requested to identify the Enterococci species. The exercise went very well and only three deviations were obtained in 224 tests performed. One participant did not upload data for Enterococci ID (Lab #40). The registered deviations were obtained by three participants who failed to ID one strain each (Labs #11, #26 and 45)

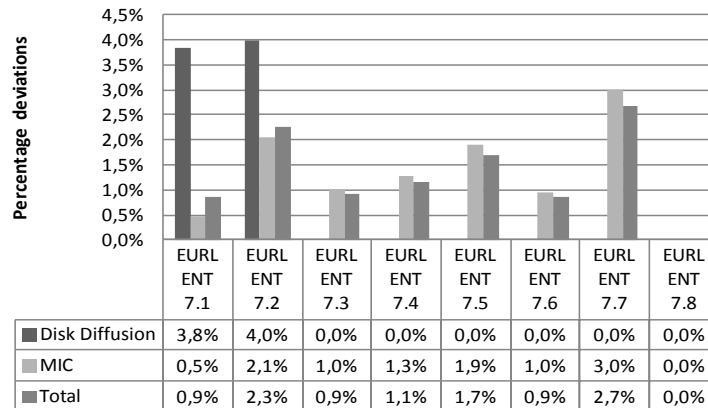


Figure 4 Enterococci trial: results deviating from the expected interpretation subdivided by tested strain and antimicrobial susceptibility test method used

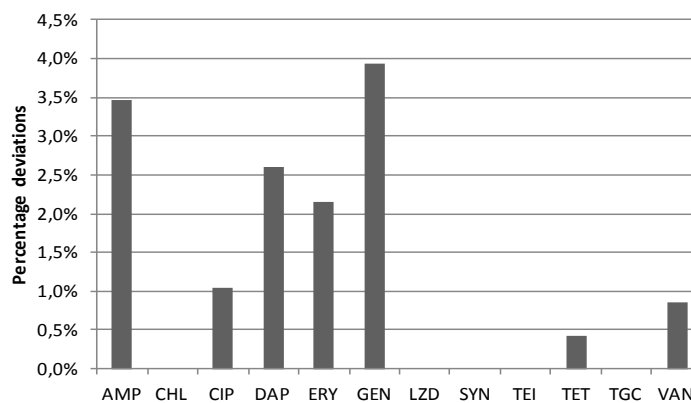


Figure 5. Enterococci trial: results deviating from the expected interpretation according to tested antimicrobials.

3.2.2 Staphylococci

Analysis of results from the Staphylococci trial showed that 97.8% of the results were interpreted correctly. Figure 3 shows the total percentage of deviations assigned to AST by MIC testing or DD. This shows that the percentage of deviation of the labs performing DD was similar between methods in general (the DD column represents seven laboratories).

Analysis of results deviating from expected interpretation subdivided by strain showed that percentage of deviations from expected results ranged from 1.2% to 2.9% (Figure 6). The highest percentage (2.9%) of disagreement with

the expected results was obtained for ST-7.4 (Figure 6). The lowest percentage of disagreement with the expected results was 1.2% for strain ST 7.3 and 7.8 (Figure 6). Laboratories performing AST by DD obtained results deviating from the expected categories in percentages comparable to the ones obtained by MIC determination, as shown in Figure 7. Out of 34 laboratories participating in the staphylococci trial, six performed AST by DD and one performed diffusion using Rosco tablets (for analysis purposes summed in the DD).

Analysis of the results according to the tested

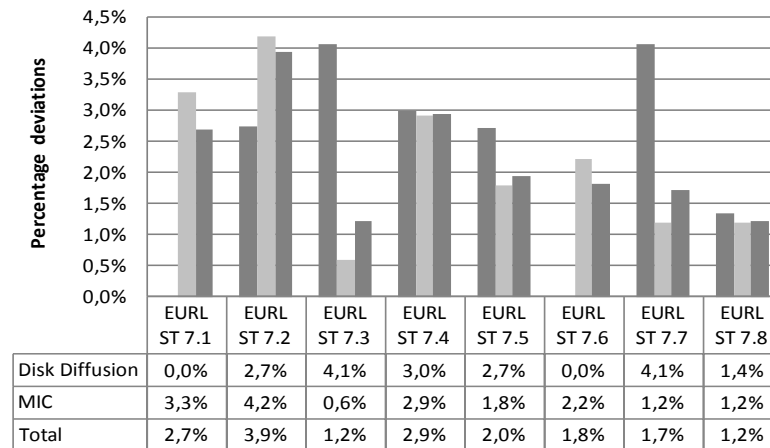


Figure 6 Staphylococci trial: results deviating from the expected interpretation subdivided by tested strain and antimicrobial susceptibility test method used

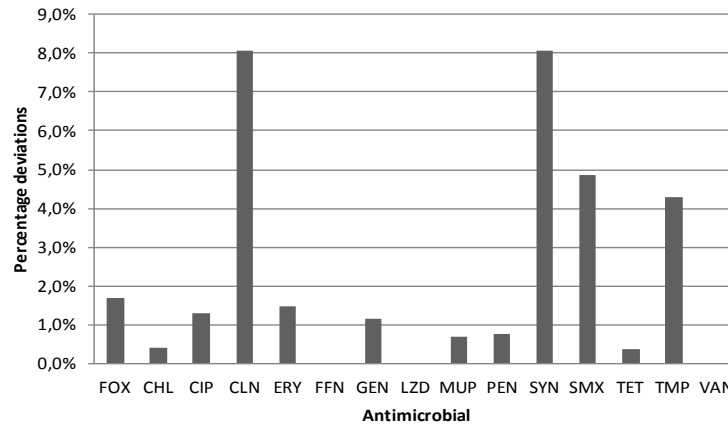


Figure 7. Staphylococci trial: results deviating from the expected interpretation according to tested antimicrobial.

antimicrobials showed that the highest percentages of deviation from expected interpretations were obtained in testing susceptibility to clindamycin and quinupristin-dalfopristin (both 8.1% deviations), followed by sulfamethoxazole (4.9%) and trimethoprim (4.3%) (Figure 7).

An overview of obtained and expected results is reported in Appendix 7b.

Methicillin-resistant *S. aureus*

Strains ST 7.1, 7.3, 7.4, 7.5, 7.7 and 7.8 were methicillin-resistant. Among 34 participants

testing staphylococci strains, one (#45) did not report results concerning methicillin resistance.

One participant (lab #39) failed in detecting methicillin resistance in strain ST 7.1 and two laboratories found either ST 7.2 (lab #6) or ST 7.6 (lab #30) as false methicillin positive.

All remaining results were correct.

3.2.3 *Escherichia coli*

Analysis of results from the *E. coli* trial showed that 99.1% of the results were interpreted correctly. Figure 3 shows the total percentage of deviations assigned to AST by MIC testing or DD. The percentage of deviation of the labs

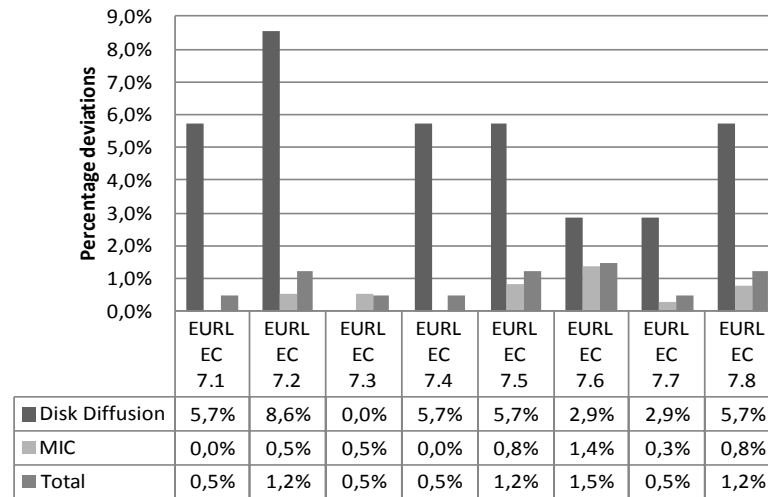


Figure 8 *E. coli* trial: results deviating from the expected interpretation subdivided by tested strain and antimicrobial susceptibility test method used

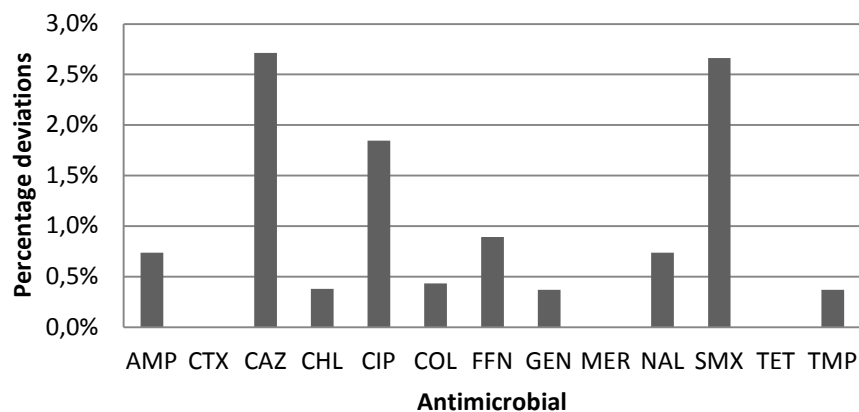


Figure 9 *E. coli* trial: results deviating from the expected interpretation according to tested antimicrobials.

performing DD was higher (4.6%) in relation to the MIC testing results that showed only 0.5% deviations (the DD column represents three laboratories).

Analysis of results deviating from expected interpretation subdivided by strain showed that percentage of deviations from all expected results ranged from 0.5% to 1.5% (Figure 8). The highest percentage (1.5%) of disagreement with expected results was obtained for EC 7.6 (Figure 8). Laboratories performing AST by DD obtained results deviating from the expected

categories in percentages higher than the ones obtained by MIC determination, as shown in Figure 8. The results obtained by DD varied from 0.0%- 8.6% (Figure 8). Out of 34 laboratories participating in the *E. coli* trial, three performed AST by DD.

An overview of obtained and expected results is reported in Appendix 7c.

Analysis of the results according to the tested antimicrobials showed that the highest percentages of deviation from expected

interpretations were obtained in testing susceptibility to ceftazidime and sulfamethoxazole (2.7%), (Figure 9). Ciprofloxacin caused the third highest deviation percentage (1.8%) while tests of susceptibility to the remaining antimicrobials resulted in less than 1.0% results deviating from the expected (Figure 9). No deviations were observed for cefotaxime, tetracycline and meropenem susceptibility testing (Figure 9).

An overview of obtained and expected results is reported in Appendix 7c.

Beta-lactamase-producing *E. coli*

Confirmation of beta-lactamase production is a mandatory component of this EQAS.

According to the protocol, which was based on the EFSA recommendations the confirmatory test for ESBL production requires use of both cefotaxime (CTX) and ceftazidime (CAZ) alone and in combination with a β -lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC CTX : CTX/CL or CAZ : CAZ/CL ratio ≥ 8) or ii) a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production. Resistance to cefepime gives further indication of ESBL production.

Confirmatory test for carbapenemase production requires the testing of meropenem (MER).

Detection of AmpC-type beta-lactamase producing bacteria can be performed by testing the isolates for susceptibility to ceftaxitin (FOX). Resistance to FOX could indicate the presence

of an AmpC-type beta-lactamase, that may be verified by PCR and sequencing.

The classification of the phenotypic results should be based on the most recent EFSA recommendations (EFSA 2012), indicating as:

- Presumptive ESBL: strains with positive synergy test, susceptible to ceftaxitin and resistant to cefepime
- Presumptive ESBL+pAmpC: -strains with positive or negative synergy test, resistant to ceftaxitin and resistant to cefepime
- Presumptive pAmpC phenotype: - strains with negative synergy test
- Presumptive carbapenemase phenotype: -strain resistant to meropenem
- Unusual phenotype: any other combinations

In this EQAS all laboratories have uploaded results at least for the strains harbouring resistance to the cephalosporins tested.

In this trial, EC 7.2 and EC 7.4 were extended-spectrum beta-lactamase (ESBL) producers and EC 7.3 and EC 7.8 were AmpC-producers. For the strain 7.2 as the cefepime resistance was difficult to detect, the result "unusual phenotype" was accepted as correct as well as the classification as presumptive ESBL.

Deviations from expected results were obtained as follows:

Two participants (Lab #6 and #22) did not identify EC 7.4 as ESBL producing strain but they classified it as presumptive ESBL+pAmpC.

This can be explained as Lab #6 has found this strain resistant to ceftaxitin. For Lab #22 the reason might be related to the interpretation as



the results uploaded would indicate it as an ESBL phenotype. Regarding the AmpC strains, strain EC 7.3 was misclassified as ESBL+pAmpC by six laboratories which either considered it as ESBL+pAmpC (Lab #6, #22, #40, #45 and #58) or as an unusual phenotype (#37). Most of these labs seemed to have misclassified the phenotype, whereas lab #22 found synergy to ceftazidime and Lab #58 found resistance to cefepime in addition to the AmpC phenotype. Regarding strain EC 7.8, the results were misclassified by seven laboratories three of which considered it as an unusual phenotype (Lab #19, #37 and #45). These labs had different reasons for the misclassification. Lab#19 reported synergy for ceftazidime, whereas Lab #37 did not find the isolate resistant to ceftazidime and Lab #45 reported results consistent to an AmpC profile but considered the profile as unusual. Three participants considered strain EC 7.8 as harboring both ESBL and pAmpC (Labs #6, #39 and #58). However, two of them reported results that represented an AmpC phenotype whereas Lab#58 reported this strain to be additionally resistant to cefepime. One participant considered strain EC 7.8 as an ESBL based on the determination of synergy for cefotaxime with clavulanic acid, however this laboratory did not provide results for ceftazidime or cefepime (Lab #41) (please refer to protocol, App.4b).

3.3 Deviations by participating laboratory

Figures 10, 11 and 12 illustrate the percentage of deviations for each participant laboratory.

Two out of 29 participants obtained a percentage of deviations from expected results higher than 5% for enterococci (Figure 10), three out of 34 participants had above 5% deviation in the staphylococci trial (Figure 11) and three out of 34 participants had above 5%

deviation in the *E. coli* trial. These results will be in focus in the next sections.

3.3.1 Enterococci

Participant #11 obtained the largest number of deviations (7.4%). This percentage of deviations was due to four deviations for strain ENT 7.5. After communicating with this laboratory it was found that the participant had mixed up this strain with the *Enterococcus faecium* control strain BM 4147 why the four deviations could be explained. Re-testing the actual ENT 7.5 strain allowed to obtain the expected results.

The second highest level of deviations was 7.1% obtained by Lab #26. All the deviations were caused by MICs for gentamicin much higher than those expected, leading to misinterpretation of susceptible strains as resistant.

For further information please consult the overview in the Appendixes (App. 8a).

In summary, 27 of the 29 participants in the enterococci trial achieved the acceptance level by having less than 5% of results deviating from the expected values (Figure 10). Among the two participants who did not meet the acceptance level, none was considered an outlier (Figure 10).

3.3.2 Staphylococci

Analysis of laboratory performance of AST showed that three out of 34 participants obtained a percentage of deviations from expected results higher than 5.0% (Figure 11). One out of seven participants performing AST by DD obtained more than 5.0% deviations from expected results (Figure 11).

Participant #39 and #6 were considered outliers due to the percentages of deviations obtained. The participant with highest level of deviation

(Lab #39) had 14.3% deviations corresponding to nine deviations. These deviations were regarding the testing of strains towards ciprofloxacin, clindamycin and erythromycin (all of them are deviations towards reporting higher MIC results than expected). After conversations with this laboratory the results for six of these deviations might have to do with wrong reading of the MIC for clindamycin, regarding ciprofloxacin and erythromycin further investigations are needed.

Participant #6 obtained 10.9% due to 12 deviations from expected results. These deviations are regarding the testing of strains towards several antimicrobials (many of them are deviations where higher MIC results than expected were obtained and two maybe related to the interpretation or breakpoint. The participant suspected that the problem has been the calibration of the autoinoculator and

new calibration has been solicited and it was reported to the EURL-AR that the results were improved.

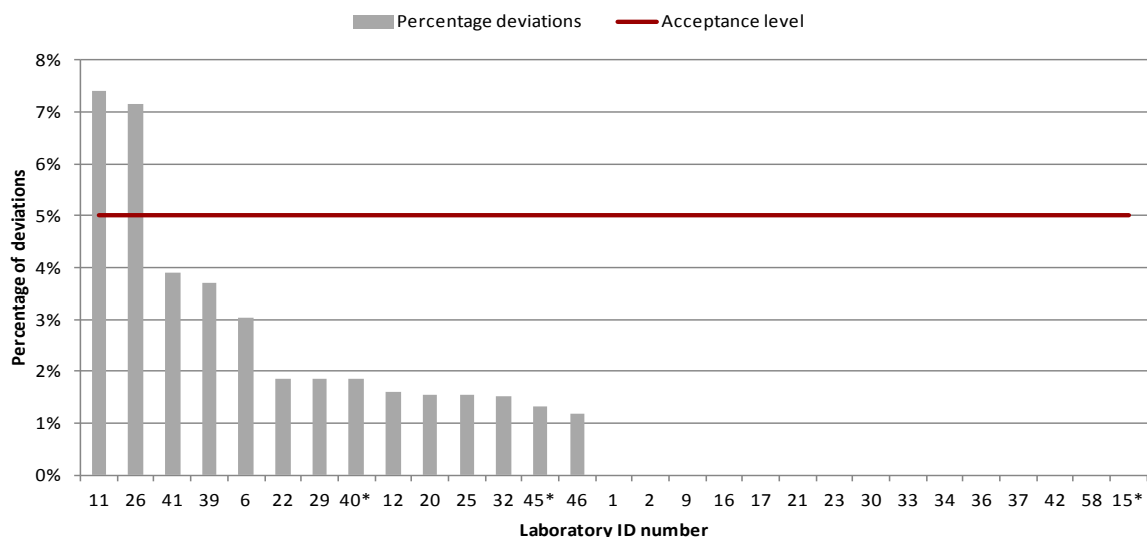
In summary, 31 of 34 participants in the staphylococci trial achieved the acceptance level by having less than 5.0% of results deviating from the expected values (Figure 11).

Deviations from expected results obtained by each participant in the staphylococci trial are reported in Appendix 8b.

3.3.3 *Escherichia coli*

Analysis of laboratory performance of AST showed that two out of 34 participants obtained a percentage of deviations from expected results higher than 5% (Figure 12).

Participant # 45 obtained eight deviations from

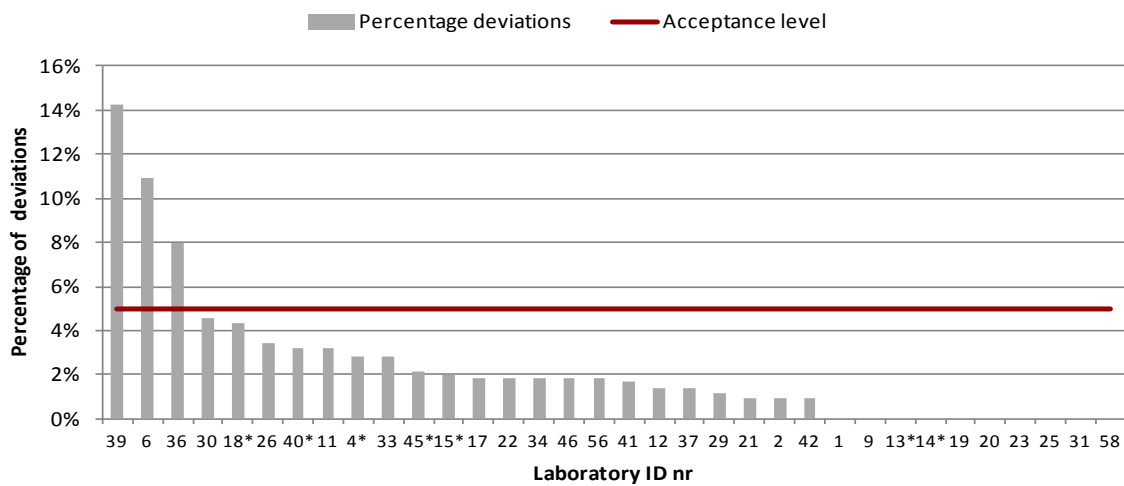


*Laboratories performing AST by disk diffusion

Figure 10. Percentage of deviations from expected results obtained by each laboratory in the enterococci trial. The laboratories were ranked by decreasing percentage of deviations from expected results in antimicrobial susceptibility testing.

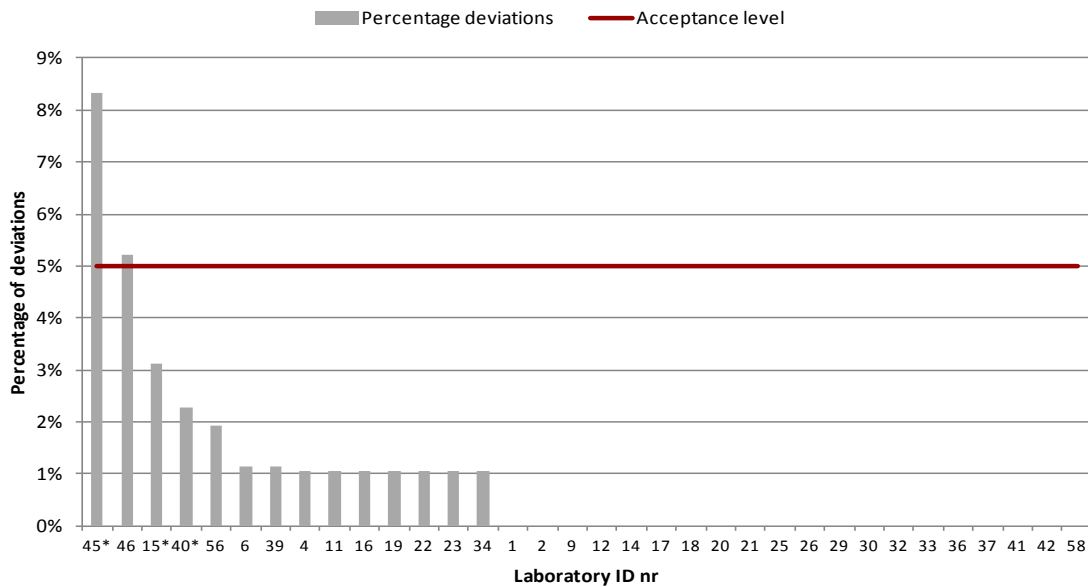
expected results accounting for a total deviation of 8.3%. These deviations are regarding the testing of strains towards several antimicrobials with disk diffusion and they may be related to the interpretation or breakpoint or test results whereas participant #46 had 5.2% deviations by

testing ampicillin and ciprofloxacin by agar dilution and sulfamethoxazole and trimethoprim by E-test. (App. 8c). In summary, 32 of 34 participants in the *E. coli* trial achieved the acceptance level by having less than 5% of results deviating from the expected values.



*Laboratories performing AST by disk diffusion

Figure 11 Percentage of deviations from expected results obtained by each laboratory in the Staphylococci trial. The laboratories were ranked by decreasing percentage of deviations from expected results in antimicrobial susceptibility testing



*Laboratories performing AST by disk diffusion

Figure 12. Percentage of deviations from expected results obtained by each laboratory in the *Escherichia coli* trial. The laboratories were ranked by decreasing percentage of deviations from expected results in antimicrobial susceptibility testing

3.4 Deviations from expected results for the reference strains

The results for antimicrobial susceptibility testing of the reference strains have been evaluated according to the CLSI-established quality control (QC) ranges (App. 5).

Table 3. Antimicrobial susceptibility testing of *Enterococcus faecalis* ATCC 29212 by MIC determination: deviations from expected values.

Antimicrobial	Proportion outside of range	Below QC range	Above QC range
Ampicillin	1/21 (5%)	-	>2 steps
Chloramphenicol	0/26 (0%)	-	-
Ciprofloxacin	1/19 (5%)	-	2 steps
Daptomycin	0/9 (0%)	-	-
Erythromycin	0/26 (0%)	-	-
Gentamicin	0/24 (0%)	-	-
Linezolid	1/23 (4%)	1 step	-
Quinu-dalfo-pristin	1/16 (6%)	-	1 step
Teicoplanin	1/3 (33%)	>5 steps	-
Tetracycline	0/26 (0%)	-	-
Tigecycline	1/10 (10%)	-	1 step
Vancomycin	1/25 (4%)	-	3 steps

Table 4. Antimicrobial susceptibility testing of *Staphylococcus aureus* ATCC 29213 by MIC determination: deviations from expected values.

Antimicrobial	Proportion outside of range	Below QC range	Above QC range
Cefoxitin	0/21 (0%)	-	-
Chloramphenicol	0/25 (0%)	-	-
Ciprofloxacin	0/25 (0%)	-	-
Clindamycin	1/21 (5%)	-	5 steps
Erythromycin	0/26 (0%)	-	-
Florfenicol	0/6 (0%)	-	-
Gentamicin	0/25 (0%)	-	-
Linezolid	0/15 (0%)	-	-
Penicillin	1/26 (4%)	1 step	-
Quinu-dalfo-pristin	0/15 (0%)	-	-
Sulfisoxazole	0/12 (0%)	-	-
Tetracycline	0/26 (0%)	-	-
Trimethoprim	0/25 (0%)	-	-
Vancomycin	0/15 (0%)	-	-

3.4.1 *Enterococcus faecalis* ATCC 29212

26 participants performed AST of *E. faecalis* ATCC 29212 by MIC determination. Seven results were found outside of range. In summary, out of 228 tests performed 221 were correct (Table 3).

As CLSI has not published a QC range for *E. faecalis* ATCC 29212 using DD, the two laboratories (#40 and #45) that have entered data for the reference strain performing this method for AST could not be evaluated. One participant performing AST of enterococci by DD (#15) did not provide data for the Enterococci reference strain.

3.4.2a *Staphylococcus aureus* ATCC 25923

Six participants performed AST of *S. aureus* ATCC 25923 by DD. One result outside of the QC range was obtained for cefoxitin, penicillin, sulfisoxazole, vancomycin and tetracycline susceptibility tests. In summary, out of 49 tests performed by DD overall, 44 were correct. One participant (lab # 4) performed AST of *S. aureus* ATCC 25923 by Rosco method. This participant (lab #4) obtained one results outside the QC range for cefoxitin.

3.4.2b *Staphylococcus aureus* ATCC 29213

Twenty-six participants performed AST of *S. aureus* ATCC 29213 by MIC determination (Table 5) and one additional laboratory #46 did perform MIC testing but did not upload data for this reference strain. In this EQAS, two deviations were obtained, one for clindamycin and one for penicillin. In summary, out of 283 tests performed, 281 were correct.



Table 5. Antimicrobial susceptibility testing of *Escherichia coli* ATCC 25922 by MIC: deviations from expected values.

Antimicrobial	Proportion outside of range	Below QC range	Above QC range
Ampicillin	0/30 (0%)		
Cefotaxime	0/30 (0%)		
Ceftazidime	0/28 (0%)		
Chloramphenicol	0/29		
Ciprofloxacin	4/30 (13%)		1 step
Colistin	0/24 (0%)		
Florfenicol	0/24 (0%)		
Gentamicin	0/30 (0%)		
Meropenem	0/8 (0%)		
Nalidixic acid	0/30 (0%)		
Sulfisoxazole	0/21 (0%)		
Tetracycline	0/30 (0%)		
Trimethoprim	0/30(0%)		

3.4.3 *Escherichia coli* ATCC 25922

Three participants performed AST of *E. coli* ATCC 25922 by DD. Only one deviation was observed by DD for ampicillin. In summary, out of 29 tests performed overall, 28 were correct. Thirty participants performed AST of *E. coli* ATCC 25922 by MIC determination and one participant (Lab #46) performed MIC determination but did not upload reference strain data even though this is a compulsory part of the EQAS. Four deviations were observed which were all for ciprofloxacin (Table 5). In summary, out of 344 tests performed, 340 were correct. For further information please consult App 6a, 6b and 6c.

4. Discussion

4.1 General overview

In the overall analysis of the results, it was observed that the levels of deviations from the expected results were comparable to last year for AST of staphylococci and *E. coli*, while there was a decrease in deviations from the expected results for AST of enterococci (Figure 2). The percentage of deviations from the expected results for AST of the internal control strains followed a trend towards a decrease for enterococci, maintained the same level for *E. coli* and increased for the staphylococci internal control strain (Figure 2).

It is important to consider that the number of EQAS participants changes from year to year, which implies that comparisons among different EQAS iterations are difficult to interpret. Furthermore, results from three laboratories from EU-affiliated countries non-MS were included in this report.

The EURL-AR has emphasized the need for harmonization of AST methodology among NRLs, and has recommended MIC determination on several occasions. In this

EQAS trial, the number of participants performing MIC determination is comparable to the high numbers observed last year and the new EU regulation will be based on MIC testing alone.

4.2 Enterococci

The percentages of results deviating from the expected interpretations varied from 0.0% to 2.7% among the different test strains (Figure 4). These percentages of deviation are quite lower than the 2012 trial and most of the deviations were due to deviations occurring in testing MIC even though strains ENT 7.1 and 7.2 showed some deviations testing by DD (Figure 4).

Even though CLSI does not provide a QC range for the testing of the reference strain by DD, the results of DD were found quite good this time as DD was performed by laboratories experienced in this method.

The number of participants submitting more than 5% results deviating from the expected interpretation was two and none were considered outliers (Figure 10), which is less



than last year. The participants have been contacted by the EURL-AR to identify possible causes of this unsatisfactory performance and to improve the quality of results. Furthermore the level of deviation was in general lower than in the 2012 iteration.

The number of participants performing AST with 100% agreement with the expected results was 15 (52%), which is a higher level than last year.

AST of the quality control strain *E. faecalis* ATCC 29212 was very good for the 26 participants that tested this strain by MIC determination (Table 3). In summary, out of 228 tests performed overall, 221 (97%) were within range.

4.3 Staphylococci

The percentages of results deviating from the expected interpretations ranged from 1.2% to 3.9% among the different test strains (Figure 6). The percentages of deviations from expected results generated by participants performing MIC and DD were this time similar in general. However, some higher deviation levels were observed for DD when testing particular strains (Figure 6).

Identification of methicillin-resistant strains was in generally satisfactory, which demonstrated that laboratories within the EURL-AR network correctly identify MRSA. However, a few issues are prevailing. One participant (Lab #45) did still not report results for methicillin resistance and one lab (Lab #39) failed in detecting methicillin resistance in strain ST 7.1 because they have not set up the recommended testing method. Furthermore, two laboratories found either ST 7.2 (Lab #6) or ST 7.6 (Lab #30) as false methicillin positive.

Three participants (Lab #9, #6 and #36) submitted results with more than 5% deviations from the expected interpretation (Figure 11), which is similar to last year. Two outliers were observed (Lab #39 and #6) with percentages of deviation up to 14.3% and 10.9%, respectively.

The EURL-AR has contacted the three participants to identify possible causes of this unsatisfactory performance and to improve the quality of results.

The number of participants performing AST with 100% agreement with the expected results was lower than in the past year and consisted of 10 participants (29%).

AST of the quality control strain *S. aureus* ATCC 25923 (for DD) resulted in 90% correct tests (Table 4), and AST of the quality control strain *S. aureus* ATCC 29213 (for MIC determination) resulted in 99% correct tests (Table 5). Overall, this performance was quite satisfactory.

4.4 Escherichia coli

The percentages of results deviating from the expected interpretations varied from 0.5% to 1.5% among the different test strains (Figure 8). These percentages of deviations from expected results were lower than in the previous year and mainly generated by participants performing AST by DD (Figure 8).

Susceptibility tests to ceftazidime and sulfamethoxazole resulted in the highest percentages (2.7%) of results deviating from the expected interpretations (Figure 9). For ceftazidime, the incorrect classification was mostly represented by resistant strains reported as susceptible by testing using DD, one susceptible strain interpreted as resistant using DD and one deviation due to one step lower MIC testing that influenced the interpretation (App. 8c). For sulfamethoxazole all of the deviations were related to considering resistant strains expected to be susceptible, which might be related to the end-point reading of this drug (App. 8c).

The number of participants submitting more than 5% results deviating from the expected interpretation was two, which is lower than last year when three participants performed outside the acceptance level (Figure 12). The



Laboratory obtaining highest deviation levels at 8.3% (Lab #45) performed testing *E. coli* by DD whereas the second highest obtaining 5.2% deviations (Lab #46) did MIC testing (Figure 15). The two laboratories reporting deviation levels above the threshold have been contacted by the EURL-AR to identify possible causes of this unsatisfactory performance and to improve the quality of results.

The number of participants performing AST with 100% agreement with the expected results was 20 (59%).

Detection of beta-lactamases of the ESBL and AmpC-type should be further improved especially concerning the classification of the profiles found, especially taking in account the possibility of mixed profiles as it is included in the EFSA classification included in the new EC regulation (EU Decision 2013/652/EC). Therefore we still consider there is some need for improvements for correct performance and

interpretation of ESBL and AmpC confirmatory tests as well as detection of carbapenemases.

AST of the quality control strain *E. coli* ATCC 25922 resulted in 97% and 99% correct tests by DD and MIC determination, respectively (Table 5). Overall, this performance was quite satisfactory. However, as for previous years the majority of deviations was observed for testing ciprofloxacin and this results must be improved in future trials since ciprofloxacin is among the critically important antimicrobials as defined by the WHO. The participant with highest deviations for testing *E. coli* with DD reported data for the reference strain with only one deviation for testing ampicillin, however, the testing for this antimicrobial drug is not found deviating for the test strains. The participant with the second highest deviation level (Lab # 46) performed MIC determination but did not upload data for the testing of the reference strain.

5. Conclusions

The number of laboratories not performing AST within the acceptable level (i.e. > 5% results deviating from the expected values) was three for the Staphylococci trial and two for the *E. coli* and enterococci trials. In the enterococci and *E. coli* trial none of them was considered as outliers, whereas for the staphylococci trial, two laboratories were considered outliers due to their higher deviation levels. Since one of the tasks of the EURL-AR is to give specific recommendations targeting individual difficulties in performing acceptable AST, laboratories outside the acceptable level have been contacted to assess individually the causes of inadequate AST performance and provide guideline to improve the methods used. These individual contacts should be taken as an opportunity to improve knowledge on AST.

One participant did not provide data on methicillin resistance and false negative and

false positive results were reported in this trial, therefore the EURL-AR will also follow up on any needs regarding the implementation of the correct detection and confirmation methods.

Additional improvements are needed to correctly identify *E. coli* producing beta-lactamases of the ESBL and AmpC-type as this is a priority area within the EURL-AR activities. We strongly encourage participants having problems in identifying these strains to perform a re-test of the test strains as a training exercise and to contact the EURL-AR in case any discussion is needed.

Finally, the EURL-AR is open to suggestions to improve future EQAS trials and invites the entire network to contribute with ideas for training courses and specific focus areas to expand the network's knowledge in antimicrobial resistance.



6. References

Schwarz S, Silley P, Simjee S, Woodford N, van DE, Johnson AP & Gaastra W. (2010) Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. *J Antimicrob Chemother* 65: 601-604.

EFSA, Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted

through food. *EFSA Journal* 2012;10(6):2742 [64 pp.].

European Commission, 2013/652/EU: Commission Implementing Decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria



M00-06-001/01.12.2011

Appendix 1. Pre notification EURL-AR EQAS 2013

EQAS 2013 FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *E. COLI*, STAPHYLOCOCCI AND ENTEROCOCCI, AND IDENTIFICATION AND TYPING OF MRSA

The EURL-AR announces the launch of another EQAS, thus providing the opportunity for proficiency testing which is considered an essential tool for the generation of reliable laboratory results of consistently good quality.

This EQAS consists of antimicrobial susceptibility testing of eight *E. coli* isolates, eight staphylococci and eight enterococci isolates. The EQAS on identification and typing of MRSA strains will consist on the confirmation of identification and methicillin resistance by multiplex PCR for the eight Staphylococcus isolates and two isolates provided additionally for the MRSA trial. Additionally, *spa* typing of the strains confirmed as MRSA is offered as an optional module.

Additionally, quality control (QC) strains *E. coli* ATCC 25922 (CCM 3954), *E. faecalis* ATCC 29212 (CCM 4224), *S. aureus* ATCC 25923 (CCM 3953) (for disk diffusion) and *S. aureus* ATCC 29213 (CCM 4223) (for MIC) will be distributed to new participants.

This EQAS is specifically for NRL's on antimicrobial resistance and additional designated laboratories performing the selective isolation and identification of MRSA from pig farms. These laboratories do not need to sign up to participate but are automatically regarded as participants. You may contact the EQAS-coordinator, if you wish to inform of changes. Participation is free of charge for all above-mentioned designated laboratories.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

The content of the parcel is "UN3373, Biological Substance Category B": eight *E. coli*, ten staphylococci, eight enterococci and for new participants also the QC strains mentioned above. Please provide the EQAS coordinator with documents or other information that can simplify customs procedures (e.g. specific text that should be written on the pro-forma invoice). To avoid delays, we kindly ask you to send this information already at this stage.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

Shipment of isolates and protocol: The isolates will be shipped in June 2013. The protocol for this proficiency test will be available for download from the website (www.eurl-ar.eu).

Submission of results: Results must be submitted to the National Food Institute no later than the 6th of September 2013 via the password-protected website. Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.

EQAS report: A report summarising and comparing results from all participants will be issued. In the report, laboratories will be presented coded, which ensures full anonymity. The EURL-AR and the EU Commission, only, will have access to un-coded results. The report will be publicly available.

Next EQAS: The next EURL-AR EQAS that we will have is on antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* which will be carried out in October 2013

Please contact me if you have comments or questions regarding the EQAS.

Sincerely,

Lina Cavaco - EURL-AR

Appendix 2- List of participants

Institute	Country	E coli	Ent	Staph
Austrian Agency for Health and Food Safety	Austria	x	x	x
Institute of Public Health	Belgium	x		x
Veterinary and Agrochemical Research Centre	Belgium			x
Nacional Diagnostic and Research Veterinary Institute	Bulgaria	x	x	x
Croatian Veterinary Institut	Croatia	x		x
Veterinary Services	Cyprus	x	x	x
State Veterinary Institute Praha	Czech Republic	x	x	x
SVI Olomouc	Czech Republic			x
National Food Institute	Denmark	x	x	x
Estonian Veterinary and Food Laboratory	Estonia	x	x	x
Finnish Food Safety Authority EVIRA	Finland	x	x	x
Agence nationale de sécurité sanitaire ANSES- Maisons-Alfort	France			x
Agence nationale de sécurité sanitaire ANSES - Ploufragan	France	x		x
Agence nationale de sécurité sanitaire ANSES - Lyon	France	x	x	x
Agence nationale de sécurité sanitaire ANSES - Fougères	France	x	x	
Federal Institute for Risk Assessment	Germany	x	x	x
Veterinary Laboratory of Chalkis	Greece	x		x
Central Agricultural Office Veterinary Diagnostic Directorate	Hungary	x		x
University of Iceland	Iceland	x	x	x
Central Veterinary Research Laboratory	Ireland	x	x	x
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy	x	x	x
Institute of Food Safety, Animal Health and Environment "BIOR"	Latvia	x	x	x
National Food and Veterinary Risk Assessment Institute	Lithuania	x	x	x
Public Health Laboratory	Malta	x	x	x
Food and Consumer Product Safety Authority (VWA)	Netherlands	x	x	x
Central Veterinary Institute of Wageningen UR	Netherlands	x	x	x
Faculty of Veterinary Medicine,	Netherlands			x
Veterinærinstituttet	Norway	x	x	x
National Veterinary Research Institute	Poland	x	x	x
Laboratorio Nacional de Investigação Veterinaria	Portugal	x	x	x
Institute for Hygiene and Veterinary Public Health	Romania	x	x	x
Institute for Diagnosis and Animal Health	Romania	x	x	x
State Veterinary and Food Institute (SVFI)	Slovakia	x	x	x
National Veterinary Institute	Slovenia	x	x	x
Laboratorio Central de Sanidad, Animal de Santa Fe	Spain			x
Laboratorio Central de Sanidad, Animal de Algete	Spain	x	x	
VISAVET Health Surveillance Center, Complutense University	Spain	x	x	x
Agencia Espanola de Seguridad Alimentria y Nutricion	Spain			
National Veterinary Institute, SVA	Sweden	x	x	x
Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland	x	x	x
Public Health England - Colindale	UK	x	x	x
The Veterinary Laboratory Agency	United Kingdom	x	x	x

NRL's	
non- NRL enrolled for EQAS	
not EU-member state	

Appendix 3a- Expected results for the enterococci trial (MIC- values and interpretations)

Strain nr	Species	DAP	TIG	TEI	Amp	CHL	CIP	ERY	GEN	LZD	Q-D	TET	VAN
EURL ENT 7.1	<i>E. faecalis</i>	2	0,06	<=0,25	<=2	8	2	2	<=16	2	16	<=1	2
EURL ENT 7.2	<i>E. faecium</i>	4	0,06	<=0,25	8	8	1	>32	<=16	2	4	<=1	<=1
EURL ENT 7.3	<i>E. faecium</i>	1	0,06	>32	4	8	1	>32	<=16	2	8	>32	>32
EURL ENT 7.4	<i>E. faecium</i>	4	0,03	0,5	<=2	8	8	2	<=16	2	1	<=1	2
EURL ENT 7.5	<i>E. faecalis</i>	2	0,06	<=0,25	<=2	8	1	>32	>1024	2	16	>32	1
EURL ENT 7.6	<i>E. faecalis</i>	1	0,12	<=0,25	<=2	>64	1	>32	512	2	16	>32	1
EURL ENT 7.7	<i>E. faecium</i>	0,25	0,06	>32	4	8	<=0,5	1	<=16	2	4	>32	>32
EURL ENT 7.8	<i>E. faecium</i>	4	0,03	<=0,25	>32	8	64	>32	>1024	2	2	<=1	2

Strain nr	Species	DAP	TIG	TEI	Amp	CHL	CIP	ERY	GEN	LZD	Q-D	TET	VAN
EURL ENT 7.1	<i>E. faecalis</i>	S	NA	S	S	S	S	S	S	S	NA	S	S
EURL ENT 7.2	<i>E. faecium</i>	S	S	S	R	S	S	R	S	S	S	S	S
EURL ENT 7.3	<i>E. faecium</i>	S	S	R	S	S	S	R	S	S	R	R	R
EURL ENT 7.4	<i>E. faecium</i>	S	S	S	S	S	R	S	S	S	S	S	S
EURL ENT 7.5	<i>E. faecalis</i>	S	NA	S	S	S	S	R	R	S	NA	R	S
EURL ENT 7.6	<i>E. faecalis</i>	S	NA	S	S	R	S	R	R	S	NA	R	S
EURL ENT 7.7	<i>E. faecium</i>	S	S	R	S	S	S	S	S	S	S	R	R
EURL ENT 7.8	<i>E. faecium</i>	S	S	S	R	S	R	R	R	S	S	S	S

Resistant

Not applicable

Abbreviations: DAP- daptomycin, TIG- tigecycline, TEI- teicoplanin, AMP-ampicillin, CHL-chloramphenicol, CIP- ciprofloxacin, ERY- erythromycin, GEN- gentamicin, LZD- linezolid, Q-D- quinupristin-dalfopristin, TET- tetracycline, VAN- vancomycin

Appendix 3b - Expected results for the staphylococci trial (MIC-values and interpretations)

Strain nr	Species	VAN	Q-D	LZN	MUP	CLN	CHL	CIP	ERY	FFN	FOX	GEN	PEN	SMX	TET	TMP	methicillin R
EURL ST 7.1	<i>S. aureus</i>	1	0,5	4	0,06	0,12	8	0,25	0,5	4	8	<=0,25	0,5	<=32	<=0,5	1	yes
EURL ST 7.2	<i>S. aureus</i>	2	1	2	0,06	0,12	8	0,5	0,5	4	4	<=0,25	8	<=32	<=0,5	2	no
EURL ST 7.3	<i>S. aureus</i>	1	2	2	0,12	8	8	0,25	0,5	4	16	0,5	>16	<=32	>32	>32	yes
EURL ST 7.4	<i>S. aureus</i>	0,5	0,5	2	0,06	0,12	8	2	<=0,25	4	8	>16	>16	256	32	<=0,5	yes
EURL ST 7.5	<i>S. aureus</i>	1	0,5	2	0,06	0,12	8	0,25	0,5	4	16	0,25	8	<=32	>32	1	yes
EURL ST 7.6	<i>S. aureus</i>	1	2	2	0,12	>256	16	0,5	>16	8	4	>16	16	<=32	>32	>32	no
EURL ST 7.7	<i>S. aureus</i>	1	2	2	0,12	8	8	0,25	0,5	4	8	<=0,25	>16	<=32	>32	>32	yes
EURL ST 7.8	<i>S. aureus</i>	1	1	1	0,12	0,12	8	0,25	<=0,25	2	8	<=0,25	8	<=32	>32	1	yes

Strain nr	Species	VAN	Q-D	LZN	MUP	CLN	CHL	CIP	ERY	FFN	FOX	GEN	PEN	SMX	TET	TMP	methicillin R
EURL ST 7.1	<i>S. aureus</i>	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S	yes
EURL ST 7.2	<i>S. aureus</i>	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	no
EURL ST 7.3	<i>S. aureus</i>	S	R	S	S	R	S	S	S	S	R	S	R	S	R	R	yes
EURL ST 7.4	<i>S. aureus</i>	S	S	S	S	S	S	R	S	S	R	R	R	R	R	S	yes
EURL ST 7.5	<i>S. aureus</i>	S	S	S	S	S	S	S	S	S	R	S	R	S	R	S	yes
EURL ST 7.6	<i>S. aureus</i>	S	R	S	S	R	S	S	R	S	S	R	R	S	R	R	no
EURL ST 7.7	<i>S. aureus</i>	S	R	S	S	R	S	S	S	S	R	S	R	S	R	R	yes
EURL ST 7.8	<i>S. aureus</i>	S	S	S	S	S	S	S	S	S	R	S	R	S	R	S	yes

Resistant

Abbreviations: VAN- vancomycin, Q-D- quinupristin-dalfopristin, LZD- linezolid, MUP- mupirocin, CLN-Clindamycin, CHL-chloramphenicol, CIP- ciprofloxacin, ERY- erythromycin, FFN- florfenicol, FOX- ceftiofloxime, GEN- gentamicin, PEN- penicillin, SMX- sulphamethoxazol, TET- tetracycline, TMP- trimethoprim, methicillin R- methicillin resistance confirmed.

Appendix 3c- Expected results for the *E. coli* trial (MIC- values and interpretations)

Strain nr	Species	MER	COL	AMP	CAZ	CHL	CIP	CTX	FFN	FOX	GEN	NAL	SMX	TET	TMP	Presumptive phenotype
EURL EC 7.1	<i>E. coli</i>	0,03	<=1	>32	0,125	4	0,12	<=0,12	2	2	<=0,5	>64	>1024	>32	>32	Not resistant
EURL EC 7.2	<i>E. coli</i>	0,03	<=1	>32	1	4	<=0,015	>4	4	4	0,5	4	>1024	>32	>32	ESBL /Unusual phenotype
EURL EC 7.3	<i>E. coli</i>	0,03	<=1	>32	8	4	<=0,015	4	4	64	0,5	2	<=16	<=2	<=1	pAmpC
EURL EC 7.4	<i>E. coli</i>	0,03	<=1	>32	2	4	<=0,015	>4	4	4	0,5	2	<=16	<=2	<=1	ESBL
EURL EC 7.5	<i>E. coli</i>	0,03	<=1	4	0,25	8	0,03	0,125	8	4	1	4	<=16	<=2	<=1	Not resistant
EURL EC 7.6	<i>E. coli</i>	0,03	<=1	>32	0,25	32	0,03	<=0,125	16	4	1	4	<=16	>32	<=1	Not resistant
EURL EC 7.7	<i>E. coli</i>	0,03	<=1	>32	0,125	>64	<=0,015	<=0,125	>64	2	16	2	>1024	<=2	>32	Not resistant
EURL EC 7.8	<i>E. coli</i>	0,03	<=1	>32	16	4	0,25	8	4	32	1	>64	<=16	<=2	<=1	pAmpC

Strain nr	Species	MER	COL	AMP	CAZ	CHL	CIP	CTX	FFN	FOX	GEN	NAL	SMX	TET	TMP	Presumptive phenotype
EURL EC 7.1	<i>E. coli</i>	S	S	R	S	S	R	S	S	S	S	R	R	R	R	Not resistant
EURL EC 7.2	<i>E. coli</i>	S	S	R	R	S	S	R	S	S	S	S	R	R	R	ESBL /Unusual phenotype
EURL EC 7.3	<i>E. coli</i>	S	S	R	R	S	S	R	S	R	S	S	S	S	S	pAmpC
EURL EC 7.4	<i>E. coli</i>	S	S	R	R	S	S	R	S	S	S	S	S	S	S	ESBL
EURL EC 7.5	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	Not resistant
EURL EC 7.6	<i>E. coli</i>	S	S	R	S	R	S	S	S	S	S	S	S	R	S	Not resistant
EURL EC 7.7	<i>E. coli</i>	S	S	R	S	R	S	S	R	S	R	S	R	S	R	Not resistant
EURL EC 7.8	<i>E. coli</i>	S	S	R	R	S	R	R	S	R	S	R	S	S	S	pAmpC

Resistant

Abbreviations: MER- meropenem, COL-colistin, AMP-ampicillin, CAZ-ceftazidime, CHL-chloramphenicol, CIP- ciprofloxacin, CTX- cefotaxime, FFN- florfenicol, FOX- ceftiofloxacin, GEN- gentamicin, NAL- nalidixic acid, SMX- sulphamethoxazol, TET- tetracycline, TMP- trimethoprim.



M00-06-001/01.12.2011

EURL-AR External Quality Assurance System (EQAS) 2013:-*Escherichia coli*, staphylococci, enterococci and MRSA

Id:

Name of participant

Address

Country

Lyngby, 17th June 2013

Dear XX

Please find enclosed the bacterial strains for the EURL-AR EQAS 2013. Upon arrival to your laboratory, the strains should be stored dark and at 4°C for stabs, and dark and cool for freeze-dried strains.

On the EURL-AR-website (www.eurl-ar.eu) the following documents relevant for the EURL-AR EQAS are available:

- Protocol for *E. coli*, staphylococci, enterococci and MRSA including test forms
- Instructions for Opening and Reviving Lyophilised Cultures
- Subculture and Maintenance of Quality Control Strains

We ask you to examine the eight *E. coli*, *enterococci* and *S. aureus* strains that we send to you by performing antimicrobial susceptibility testing and the eight *S. aureus* plus the two additional strains for MRSA. In the protocol you can find detailed description of the procedures to follow. Additionally, you can find a description of the procedure to enter your results into the interactive web database. For accessing the database, you need this username and password:

Your username: xxx

Your password: xxx

Please keep this document
Your username and password will not appear in other documents

Results should be entered in the database no later than **6th September 2013**. Please acknowledge receipt of this parcel immediately upon arrival (to licav@food.dtu.dk) and do not hesitate to contact me for further information.

Yours sincerely,

Lina Cavaco



PROTOCOL

For antimicrobial susceptibility testing of *Escherichia coli*, enterococci and staphylococci, and identification and typing of MRSA

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1. INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *E. coli*, enterococci and staphylococci, and identification and typing of MRSA is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). The EC/Ent/Staph EQAS 2013 will include AST of eight *E. coli*, eight enterococci and eight staphylococci strains and AST of reference strains *E. coli* ATCC 25922 (CCM 3954), *E. faecalis* ATCC 29212 (CCM 4224), *S. aureus* ATCC 25923 (CCM 3953) (for disk diffusion) and *S. aureus* ATCC 29213 (CCM 4223) (for MIC). The above-mentioned reference strains are included in the parcel only for new participants of the EQAS who did not receive them previously. The reference strains are original CERTIFIED cultures provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The reference strains will not be included in the years to come. Therefore, please take proper care of these strains. Handle and maintain them as suggested in the manual ‘Subculture and Maintenance of QC Strains’ available on the EURL-AR website (see www.eurl-ar.eu).

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The strains included in the identification and typing of MRSA are the eight staphylococci strains for AST together with two additional *Staphylococcus* strains. This component of the EQAS functions as a continuation of the previous MRSA EQAS to evaluate the proficiency of the laboratories on procedures for confirmatory testing and *spa* typing.

Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs it is placed with a competent subcontractor and the National Food Institute is responsible to the scheme participants for the subcontractor's work.

1. OBJECTIVES

This EQAS aims to support laboratories to assess and, if necessary, to improve the quality of results obtained by AST of pathogens of food- and animal-origin, with special regard to *E. coli*, enterococci and staphylococci. Further objectives are to evaluate and improve the comparability of surveillance data on antimicrobial susceptibility of *E. coli*, enterococci and staphylococci reported to EFSA by different laboratories, and to harmonise the breakpoints for antimicrobial susceptibility used within the EU. Additionally, with the MRSA confirmation and *spa* typing components included in this iteration, we intend to continue the harmonization and /or implementation process of MRSA monitoring at the NRL level.

2. OUTLINE OF THE EC/ENT/STAPH EQAS 2013

2.1. Shipping, receipt and storage of strains

In June 2013, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight *E. coli*, eight enterococci and ten staphylococci strains from the National Food Institute, Denmark (two of the staphylococci strains are to be included in the MRSA components, only). This parcel will also contain reference strains, but only for participants who did not receive them previously. All strains belong to UN3373, Biological substance, category B. Extended spectrum beta-lactamase (ESBL)-producing strains as well as methicillin resistant *Staphylococcus aureus* (MRSA) will be included in the selected material.

The reference strains are shipped lyophilised, while the test strains are stab cultures. On arrival, the stab cultures must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

2.2. Suggested procedure for reconstitution of the lyophilised reference strains

Please refer to the document 'Instructions for opening and reviving lyophilised cultures' reported on the EURL-AR-website (see www.eurl-ar.eu).

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2.3. Antimicrobial susceptibility testing

The strains should be tested for susceptibility to the antimicrobials listed in Tables 1, 2 and 3, using the method implemented in your laboratory for performing monitoring for EFSA and applying the interpretative criteria listed below.

Participants performing minimum inhibitory concentration (MIC) determination should use the values listed in Tables 1, 2 and 3 for interpretation of results. These values represent the epidemiological cut-off values developed by EUCAST (www.eucast.org), and allow categorisation of bacterial isolates into two categories: Resistant or susceptible. A categorisation as intermediate is not accepted, and **intermediate results should be interpreted as susceptible.**

Participants using disk diffusion are recommended to interpret the results according to the breakpoints used routinely. However, when testing *E. coli* by disk diffusion, the interpretation of ciprofloxacin results should be done according to the guidelines described by Cavaco and Aarestrup in J Clin Microbiol. 2009 Sep;47(9):2751-8. Strains must be categorised resistant and susceptible. Also in this case, a categorization as intermediate is not accepted, and **intermediate results should be interpreted as susceptible.**

E. coli

Table 1: Antimicrobials recommended for AST of *Escherichia coli* and interpretative criteria

Antimicrobials for <i>E. coli</i>	MIC ($\mu\text{g/mL}$) R is >
Ampicillin, AMP	8
Cefepime	0.125
Cefotaxime, CTX	0.25
Cefoxitin, FOX	8
Ceftazidime, CAZ	0.5
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.06
Colistin	2
Florfenicol, FFN	16
Gentamicin, GEN	2
Meropenem, MER	0.125
Nalidixic acid, NAL	16
Sulfonamides, SMX	64
Tetracycline, TET	8
Trimethoprim, TMP	2

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Plasmid-mediated quinolone resistance

When performing antimicrobial susceptibility testing of *E. coli*, the interpretative criteria listed in Table 1 for results obtained by MIC-determination detect plasmid mediated quinolone resistant test strains. When interpreting a disk diffusion result, reference should be made to the guidelines in described by Cavaco and Aarestrup in J Clin Microbiol. 2009 Sep;47(9):2751-8.

Beta-lactam resistance

Confirmatory tests for ESBL production are mandatory on all strains resistant to cefotaxime (CTX), ceftazidime (CAZ) or meropenem.

Confirmatory test for ESBL production requires use of both cefotaxime (CTX) and ceftazidime (CAZ) alone and in combination with a β -lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC CTX : CTX/CL or CAZ : CAZ/CL ratio ≥ 8) or ii) a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production. Resistance to cefepime gives further indication of ESBL production.

Confirmatory test for carbapenemase production requires the testing of meropenem (MER).

Detection of AmpC-type beta-lactamases can be performed by testing the bacterium for susceptibility to cefoxitin (FOX). Resistance to FOX could indicate the presence of an AmpC-type beta-lactamase, that may be verified by PCR and sequencing.

The classification of the phenotypic results should be based on the most recent EFSA recommendations (EFSA 2012), indicating as:

- Presumptive ESBL: strains with positive synergy test, susceptible to cefoxitin and resistant to cefepime
- Presumptive ESBL+pAmpC: -strains with positive or negative synergy test, resistant to cefoxitin and resistant to cefepime
- Presumptive pAmpC phenotype: -strains with negative synergy test.
- Presumptive carbapenemase phenotype: -strain resistant to meropenem
- Unusual phenotype: any other combinations

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The EURL-AR aims to harmonise with the new EU monitoring and the EUCAST expert rules. Accordingly, MIC values and relative interpretation of cefotaxime, ceftazidime and meropenem used for detection of beta-lactamase-producing strains in this EQAS should be reported as found.

Enterococci

Table 2: Antimicrobials recommended for AST of *Enterococcus* spp. and interpretative criteria

Antimicrobials for enterococci	MIC ($\mu\text{g/mL}$)	MIC ($\mu\text{g/mL}$)
	R is > <i>E. faecium</i>	R is > <i>E. faecalis</i>
Ampicillin, AMP	4	4
Chloramphenicol, CHL	32	32
Ciprofloxacin, CIP	4	4
Daptomycin, DAP	4	4
Erythromycin, ERY	4	4
Gentamicin, GEN	32	32
Linezolid, LZD	4	4
Quinupristin-dalfopristin (Synercid), SYN	4*	Not applicable
Teicoplanin	2	2
Tetracycline, TET	4	4
Tigecycline, TGC	0,25	Not applicable
Vancomycin, VAN	4	4

*DANMAP 2009 (www.danmap.org)

Identification of the *Enterococcus* spp.

In 2013, species identification of the Enterococci must be performed by the NRLs using in-house methods or adopting the protocol available on the EURL-AR website under: <http://eurl-ar.eu/233-protocols.htm>.

Staphylococci

Eight of the staphylococci strains sent should be tested both in the AST component and in the MRSA components (EURL ST-7.1 to ST-7.8) whereas the two additional strains (EURL ST-7.9 and 7.10) are intended for the MRSA EQAS components only.

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013Table 3: Antimicrobials recommended for AST of *Staphylococcus aureus* and interpretative criteria

Antimicrobials for <i>S. aureus</i>	MIC ($\mu\text{g/mL}$) R is >
Cefoxitin, FOX	4
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Clindamycin, CLI	0.25
Erythromycin, ERY	1
Florfenicol, FFN	8
Gentamicin, GEN	2
Linezolid, LNZ	4
Mupirocin, MUP	1
Penicillin, PEN	0.125*
Quinupristin-dalfopristin (Synercid), SYN	1
Sulfonamides, SMX	128
Tetracycline, TET	1
Trimethoprim, TMP	2
Vancomycin	2

*CLSI M100 Table 2C

Identification and typing of MRSA

All ten staphylococci (ST 7.1 to 7.10) are to be used in the MRSA identification and typing components of the proficiency test. Some test strains may be methicillin-resistant. **Confirmation of *mecA* and/or *mecC* presence is mandatory** in this EQAS. For this purpose, you are welcome to use the method you prefer, and upload the result as 'positive' or 'negative'. According to CLSI recommendations (M100, Table 2C), all MRSA should be regarded as resistant to all β -lactam antibiotics.

The ten staphylococci strains are either positive or negative for methicillin resistance and or, represent other methicillin susceptible or resistant *Staphylococcus* species.

As part of the identification and typing of MRSA, handle the strains as follows:

1. Take up the strains from the agar sticks and plate onto a blood agar plate (you may use additional selective media, but it is not compulsory).
2. Incubate 24-48 h at 37 °C.
3. Observe the colony morphology of the isolates on the blood agar plate (colour, appearance, haemolysis). Check for purity.

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4. At this stage, the isolates should either be processed immediately or stored under appropriate conditions (-80°C) for later identification and characterisation.

Identification of MRSA

Presumptive MRSA isolates should be confirmed as *Staphylococcus aureus* isolates carrying the *mecA* gene or the *mecC* gene (previously known by *mecA_{LGA251}*) by PCR. There is no need to perform other screening methods (such as screening with either oxacillin or cefoxitin), thus, the presence of the *mecA* or *mecC* gene can be directly confirmed by PCR amplification. The species identification is simultaneously confirmed by using the EURL-AR recommended multiplex PCR protocol (<http://eurl-ar.eu/233-protocols.htm>) including the amplification of the *spa* gene (specific for *Staphylococcus aureus* species and may be sequenced for *spa* typing), the *mecA*-gene and the *mecC* gene (both encoding methicillin resistance) and the *pvl* gene (encoding the Panton Valentine Leukocidin).

Spa typing

Spa typing of the MRSA isolates may be performed additionally if the laboratory has the capacity to perform and analyse the *spa*-typing data. In case you decide to include *spa* types in the submitted data, these will be evaluated on the accuracy of the *spa* typing.

3. REPORTING OF RESULTS AND EVALUATION

3.1. AST of E. coli, enterococci and staphylococci

Please write your results in the test forms, and enter your results into the interactive web database. In addition, we kindly ask you to report in the database the tested MIC range and/or antimicrobial disk content. Finally, if you did **not** use the cut-off values recommended in the protocol for interpretation of AST results, please report the breakpoints used in the database.

3.2. MRSA identification and typing

Fill in your results in the enclosed MRSA EQAS test forms. Please enter your results into the interactive web database. Please read the detailed description below before entering the web database. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print a submission report of your results.

3.3. General recommendations for data upload

We recommend reading carefully the description reported in paragraph 5 before entering your results in the web database. **Results must be submitted no later than September, 6th 2013.** After the deadline when all participants have uploaded results, you will be able to login to the database

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once again, and to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as ‘correct’, while results deviating from the expected interpretation are categorised as ‘incorrect’.

If you experience difficulties in entering your results, please return the completed test forms by e-mail, fax or mail to the National Food Institute, Denmark.

All results will be summarized in a report which will be publicly available. The data in the report will be presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the complete list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions will be public.

If you have questions, please do not hesitate to contact us:

Lina Cavaco
National Food Institute
Technical University of Denmark
Kemitorvet, Building 204 st room 51,
DK-2800 Kgs. Lyngby
Denmark
Tel: +45 3588 6269
Fax: +45 3588 6341
E-mail: licav@food.dtu.dk

4. HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read carefully this paragraph before entering the web page.

Remember that you need by your side the completed test forms and the breakpoint values you used.

Enter the EURL-AR EQAS 2013 start web page (<http://thor.dfvf.dk/crl>), write your username and password in lower-cases and press enter. Your username and password are the same used in the previous EQAS's arranged by The National Food Institute, Denmark. Do not hesitate to contact us if you experience problems with the login.

You can browse back and forth by using the back and forward keys and by clicking on the EURL logo.

4.1. AST of *E. coli*, enterococci and staphylococci

Click on either “*E. coli* test results”, “enterococci test results” or “staphylococci test results” based on the results you are going to upload. The description reported below is based on *Salmonella* test

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result entry, but it is the exact same procedure for entering *E. coli*, enterococci and staphylococci test results.

Click on "Start of Data Entry - Methods and Breakpoints for Salm."

In the next page, you can navigate among fields with the Tab-key and the mouse.

Complete the fields related to the method used for antimicrobial susceptibility testing of *Salmonella* and the brand of discs, tablets, MIC trays, etc.

Fill in the fields related to either antimicrobial disk content or tested MIC range. If you used disk diffusion, please upload the breakpoints used for interpretation of results.

Click on "save and go to next page"

In the data entry pages, enter the obtained values and the interpretation (R, resistant or S, susceptible) for each *E. coli*, enterococcus and staphylococcus strain.

For *E. coli* strains, remember to report also the results for the ESBL detection tests.

For *S. aureus* strains, remember to report also the results for presence/absence of methicillin resistance.

If you did not test for susceptibility to a given antimicrobial, please leave the field empty.

Click on "save and go to next page"

When uploading data on the reference strains, please enter the zone diameters in mm and MIC values in $\mu\text{g/ml}$. Remember to use the operator keys to show symbols like "equal to", etc... If you do not use CLSI guidelines for AST of the reference strains, please add a comment on the method used.

Click on "save and go to next page"

This page is a menu that allows you to review the input pages and approve your input.

Browse through the pages and make corrections if necessary. Remember to save a page if you make corrections. If you save a page without changes, you will see an error screen. In this case, click on "back" to get back to the page and "go to next page" to continue.

Please complete the evaluation form.

Before approving your input, please be sure that you have filled in all the relevant fields because **YOU CAN ONLY APPROVE ONCE!** The approval blocks your data entry in the interactive database.

4.2. EQAS on identification and typing of MRSA

Click on "[MRSA tests](#)" to start entering your data regarding the MRSA EQAS.

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Please read carefully the instructions on the webpage and start by answering to the questionnaire on the work performed in your laboratory relative to MRSA by clicking on “[General MRSA questionnaire](#)”.

Please choose the options that more correctly describe your work on MRSA and before you leave this page, click on “[Save page](#)” which will take you back to the previous menu. Then, fill in the methods used in a second page by clicking on “[Methods for MRSA test samples](#).”

In the next page you navigate to fields with the Tab-key and mouse.

Fill in what kind of method you have used for the confirmation of MRSA in this EQAS.

Click on “[Save and go to the next page](#)”

In the data entry pages for each strain, you enter the obtained results for each of the MRSA EQAS strains (EURL ST 7.1...7.10).

If you wish to participate in the *spa* typing trial, you will have the option to include the *spa*-typing results.

If you have performed the *spa* typing, choose the *spa* type from the list. If you did not perform *spa* typing, leave the field blank. In case the isolate is not a methicillin resistant *Staphylococcus aureus*, choose “not applicable (N/A)”. Click on “[save and go to next page](#)” to navigate to the next sample results, until you finish uploading all your data.

From the last result sheet you get into the general menu, from where you can review the input pages: Browse through the pages and make corrections if necessary. Remember to save a page if you make any corrections. If you save a page without changes, you will see an error screen, and you just have to click on “[back](#)” to get back to the page and “[go to next page](#)” to continue.

At the end, approve your input. Be sure that you have filled in all the results before approval, as

YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database, but allows you to see and print the submitted results.

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External Quality Assurance System (EQAS) 2013**



TEST FORMS

Antimicrobial susceptibility testing of *Escherichia coli*, enterococci and staphylococci, and identification and typing of MRSA

Name:

Name of laboratory:

Name of institute:

City:

Country:

E-mail:

Fax:

Comments:

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013**



TEST FORM

Which method did you use for antimicrobial susceptibility testing of enterococci in this EQAS:

- MIC – Microtitre
 MIC – Agar dilution
 Strips – E-test
 Discs, tablets
 Rosco, Neo Sensitabs

Brand:

How many *Enterococcus* spp. isolates does your laboratory annually isolate:

How many *Enterococcus* spp. isolates does your laboratory annually test for antimicrobial susceptibility:

Comments or additional information:

Antimicrobial	General info		Zone diameter (mm)		
	The relevant information in the two columns below should be reported		Please, report breakpoint information <i>only</i> if you did not use the cut-off values recommended in the protocol		
	Disk content (µg)	Test-range for MIC (µg/mL)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Ampicillin AMP			≤		≥
Chloramphenicol, CHL			≤		≥
Ciprofloxacin, CIP			≤		≥
Daptomycin, DAP			≤		≥
Erythromycin, ERY			≤		≥
Gentamicin, GEN			≤		≥
Linezolid, LZD			≤		≥
Quin.-Dalf. (Synercid), SYN			≤		≥
Teicoplanin, TEI			≤		≥
Tetracycline, TET			≤		≥
Tigecycline, TGC			≤		≥
Vancomycin, VAN			≤		≥

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013**



TEST FORM

Which method did you use for antimicrobial susceptibility testing of staphylococci in this EQAS:

- MIC – Microtitre
 MIC – Agar dilution
 Strips – E-test
 Discs, tablets
 Rosco, Neo Sensitabs

Brand:

How many *Staphylococcus* spp. isolates does your laboratory annually isolate:

How many *Staphylococcus* spp. isolates does your laboratory annually test for antimicrobial susceptibility:

Comments or additional information:

Antimicrobial	General info		Zone diameter (mm)		
	The relevant information in the two columns below should be reported		Please, report breakpoint information <i>only</i> if you did not use the cut-off values recommended in the protocol		
	Disk content (µg)	Test-range for MIC (µg/mL)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Cefoxitin, FOX			≤		≥
Chloramphenicol, CHL			≤		≥
Ciprofloxacin, CIP			≤		≥
Clindamycin, CLN			≤		≥
Erythromycin, ERY			≤		≥
Florfenicol, FFN			≤		≥
Gentamicin, GEN			≤		≥
Linezolid, LNZ			≤		≥
Mupirocin, MUP			≤		≥
Penicillin, PEN			≤		≥
Quin.-Dalf. (Synercid), SYN			≤		≥
Sulphonamides, SMX			≤		≥
Tetracycline, TET			≤		≥
Trimethoprim, TMP			≤		≥
Vancomycin, VAN			≤		≥

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013**



TEST FORM

Which method did you use for antimicrobial susceptibility testing of *E. coli* in this EQAS:

- MIC – Microtitre
 MIC – Agar dilution
 Strips – E-test
 Discs, tablets
 Rosco, Neo Sensitabs

Brand:

Incubation conditions: °C/ h

How many *E. coli* isolates does your laboratory annually isolate:

How many *E. coli* isolates does your laboratory annually test for antimicrobial susceptibility:

Comments or additional information:

Antimicrobial	General info		Zone diameter (mm)		
	The relevant information in the two columns below should be reported		Please, report breakpoint information <i>only</i> if you did not use the cut-off values recommended in the protocol		
	Disk content (µg)	Test-range for MIC (µg/mL)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Ampicillin, AMP			≤		≥
Cefotaxime, CTX			≤		≥
Cefoxitin, FOX			≤		≥
Ceftazidime, CAZ			≤		≥
Ceftiofur, XNL			≤		≥
Chloramphenicol, CHL			≤		≥
Ciprofloxacin CIP			≤		≥
Colistin, COL			≤		≥
Florfenicol, FFN			≤		≥
Gentamicin, GEN			≤		≥
Meropenem, MER			≤		≥
Nalidixic acid, NAL			≤		≥
Sulphonamides, SMX			≤		≥
Tetracycline, TET			≤		≥
Trimethoprim, TMP			≤		≥

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013



TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ >	Zone diameter (mm) or MIC-value (µg/ml)	S / R
Enterococci EURL ENT. 7.X <input type="checkbox"/> <i>E. faecium</i> <input type="checkbox"/> <i>E. faecalis</i>	Ampicillin AMP			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptomycin, DAP			
	Erythromycin, ERY			
	Gentamicin, GEN			
	Linezolid, LZD			
	Quin.-Dalf. (Synercid), SYN			
	Teicoplanin, TEI			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

Strain	Antimicrobial	Results and interpretation		
		≤ >	Zone diameter (mm) or MIC-value (µg/ml)	S / R
Enterococci EURL ENT. 7.X <input type="checkbox"/> <i>E. faecium</i> <input type="checkbox"/> <i>E. faecalis</i>	Ampicillin AMP			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptomycin, DAP			
	Erythromycin, ERY			
	Gentamicin, GEN			
	Linezolid, LZD			
	Quin.-Dalf. (Synercid), SYN			
	Teicoplanin, TEI			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013



TEST FORM

Antimicrobial susceptibility testing of reference strain *Enterococcus faecalis* ATCC 29212

Strain	Antimicrobial	Zone diameter (mm) or MIC-value (µg/ml)
<i>E. faecalis</i> ATCC 29212	Ampicillin, AMP	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Daptomycin, DAP	
	Erythromycin, ERY	
	Gentamicin, GEN	
	Linezolid, LZD	
	Quinupristin-Dalfopristin (Synercid), SYN	
	Teicoplanin, TEI	
	Tetracycline, TET	
	Tigecycline, TIG	
	Vancomycin, VAN	

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013



TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ >	Zone diameter (mm) or MIC-value (µg/ml)	S / R
<i>S. aureus</i> EURL ST 7.X	Cefoxitin, FOX			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Clindamycin, CLN			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LNZ			
	Mupirocin, MUP			
	Penicillin, PEN			
	Quino-dalfo (Synercid), SYN			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
Vancomycin, VAN				

Methicillin resistance (MRSA)	<input type="checkbox"/> Positive	<input type="checkbox"/> Negative
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EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013



TEST FORM

Antimicrobial susceptibility testing of reference strain *S. aureus* ATCC 29213 (MIC) / 25923 (disk diffusion)

Strain	Antimicrobial	Zone diameter (mm) or MIC-value (µg/ml)
Please mark the tested strain <input type="checkbox"/> <i>S. aureus</i> ATCC 29213 <input type="checkbox"/> <i>S. aureus</i> ATCC 25923	Cefoxitin, FOX	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Clindamycin	
	Erythromycin, ERY	
	Florfenicol, FFN	
	Gentamicin, GEN	
	Linezolid, LNZ	
	Mupirocin, MUP	
	Penicillin, PEN	
	Quino-dalfo (Synercid), SYN	
	Sulphonamides, SMX	
	Tetracycline, TET	
	Trimethoprim, TMP	

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013**



TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ >	Zone diameter (mm) or MIC-value (µg/ml)	S / R
<i>E. coli</i> EURL EC 7.X	Ampicillin, AMP			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Meropenem, MER			
	Nalidixic acid, NAL			
	Sulphonamides, SMX			
	Tetracycline, TET			
Trimethoprim, TMP				

All strains resistant to cefotaxime (CTX), ceftazidime (CAZ) or meropenem (MER) should be included for testing in the second panel confirmatory tests for ESBL or carbapenemase production. See further description of confirmatory tests in the protocol section '3.3 *E. coli*'.

	MIC, value or ratio		Disks, zone diameter or increase
CTX/CL : CTX MIC ratio	<input type="checkbox"/> MIC ratio ≥ 8 (synergy) <input type="checkbox"/> MIC ratio < 8 <input type="checkbox"/> Phantom zone (synergy) <input type="checkbox"/> Deformation (synergy) <input type="checkbox"/> Not determinable	Incr. in zone diam	<input type="checkbox"/> Incr. ≥ 5 mm (synergy) <input type="checkbox"/> Incr. < 5 mm
CAZ/CL : CAZ MIC ratio	<input type="checkbox"/> MIC ratio ≥ 8 (synergy) <input type="checkbox"/> MIC ratio < 8 <input type="checkbox"/> Phantom zone (synergy) <input type="checkbox"/> Deformation (synergy) <input type="checkbox"/> Not determinable	Incr. in zone diam	<input type="checkbox"/> Incr. ≥ 5 mm (synergy) <input type="checkbox"/> Incr. < 5 mm
Cefoxitin, FOX MIC value	<input type="checkbox"/> MIC value > 8 <input type="checkbox"/> MIC value ≤ 8	Zone diameter	<input type="checkbox"/> D ≤ 18 mm <input type="checkbox"/> D > 18 mm
Cefepime, FEP MIC value	<input type="checkbox"/> MIC value > 0,125 <input type="checkbox"/> MIC value ≤ 0,125	Zone diameter	<input type="checkbox"/> D ≤ 18 mm <input type="checkbox"/> D > 18 mm
Imipenem, IMI MIC value	<input type="checkbox"/> MIC value > 1 <input type="checkbox"/> MIC value ≤ 1	Zone diameter	<input type="checkbox"/> D ≤ 23 mm <input type="checkbox"/> D > 23 mm
Ertapenem, ERP MIC value	<input type="checkbox"/> MIC value > 1 <input type="checkbox"/> MIC value ≤ 1	Zone diameter	<input type="checkbox"/> D ≤ 22mm <input type="checkbox"/> D > 22 mm
<input type="checkbox"/> Presumptive ESBL <input type="checkbox"/> Presumptive pAmpC <input type="checkbox"/> Unusual phenotype <input type="checkbox"/> Presumptive ESBL+ pAmpC <input type="checkbox"/> Presumptive carbapenemase			

Comments (include genotype or other results):

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2013



TEST FORM

Antimicrobial susceptibility testing of reference strain *E. coli* ATCC 25922

Strain	Antimicrobial	Zone diameter (mm) or MIC-value (µg/ml)
<i>E. coli</i> ATCC 25922	Amoxicillin, AMX	
	Ampicillin, AMP	
	Cefotaxime, CTX	
	Cefoxitin, FOX	
	Ceftazidime, CAZ	
	Ceftiofur, XNL	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin	
	Florfenicol, FFN	
	Gentamicin, GEN	
	Meropenem	
	Nalidixic acid, NAL	
	Streptomycin, STR	
	Sulfisoxazole, FIS*	
	Tetracycline, TET	
Trimethoprim, TMP		
Imipenem, IMI		

*The antimicrobial which is mentioned in the CLSI M100 performance standard as representative for the sulfonamides concerning acceptable limits for quality control strains (CLSI M100, Table 3)



INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Manual from Czech Collection of Microorganisms (CCM)
Masaryk University
Tvrdeho 14
602 00 BRNO
Czech Republic

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

- a. Check the number of the culture on the label inside the ampoule
- b. Make a file cut on the ampoule near the middle of the plug
- c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end
- d. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule
- e. Remove the pointed end of the ampoule into disinfectant
- f. Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media
- g. Incubate the inoculated medium at appropriate conditions for several days
- h. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

Please note that:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue
- Cultures may need at least one subculturing before they can be optimally used in experiments
- Unopened ampoules should be kept in a dark and cool place!



SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1.1 Purpose

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has published a guideline for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test results.

1.2 References

M100-S21, January 2011 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A8, January 2009 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard)

1.3 Definition of Terms

Reference Culture: A reference culture is a microorganism preparation that is acquired from a culture type collection.

Reference Stock Culture: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

Working Stock Cultures: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

Subcultures (Passages): A subculture is simply the transfer of established microorganism growth on media to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time

1.4 Important Considerations

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC
- CLSI requires that QC be performed either on the same day or weekly (only after 30 day QC validation)
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides
- Periodically perform colony counts to check the inoculum preparation procedure



- Ideally, test values should be in the middle of the acceptable range
- Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems

1.5 Storage of Reference Strains

Preparation of stock cultures

- Use a suitable stabilizer such as 50% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen. (Alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

Working cultures

- Set up on agar slants with appropriate medium, store at 4-8°C and subculture weekly.
- Replace the working strain with a stock culture at least monthly.
- If a change in the organisms inherent susceptibility occurs, obtain a fresh stock culture or a new strain from a reference culture collection e.g. ATCC.

1.6 Frequency of Testing

Weekly vs. daily testing

Weekly testing is possible if the lab can demonstrate satisfactory performance with daily testing as follows:

- Documentation showing reference strain results from 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more than 3 out of 30 MIC values may be outside the acceptable range.

When the above are fulfilled, each quality control strain may be tested once a week and whenever any reagent component is changed.

Corrective Actions

If an MIC is outside the range in weekly testing, corrective action is required as follows:

- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

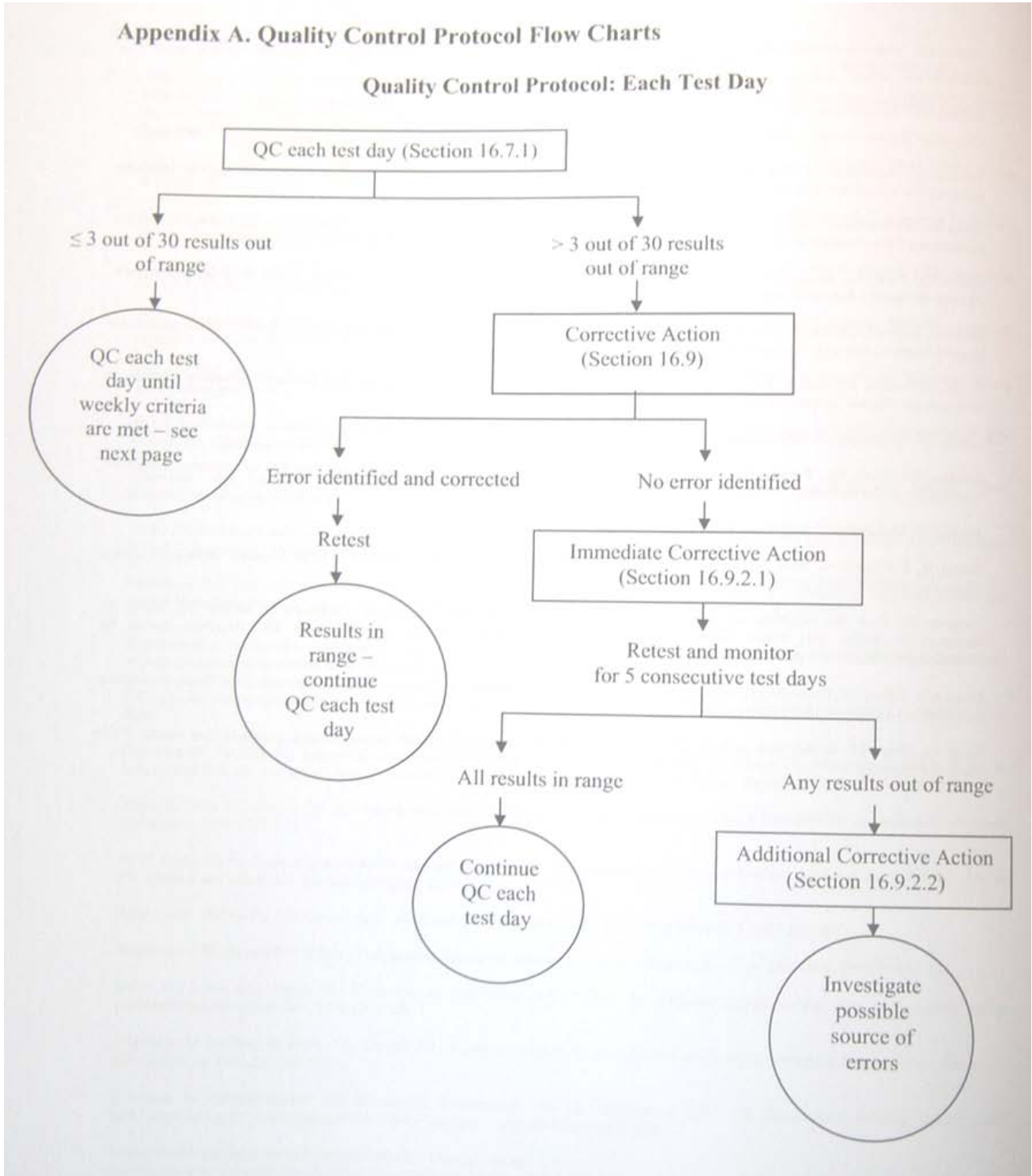
The problem is considered resolved only after the reference strain is tested for 5 consecutive days and each drug/organism result is within specification on each day.

If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.



DAILY MIC QC CHART

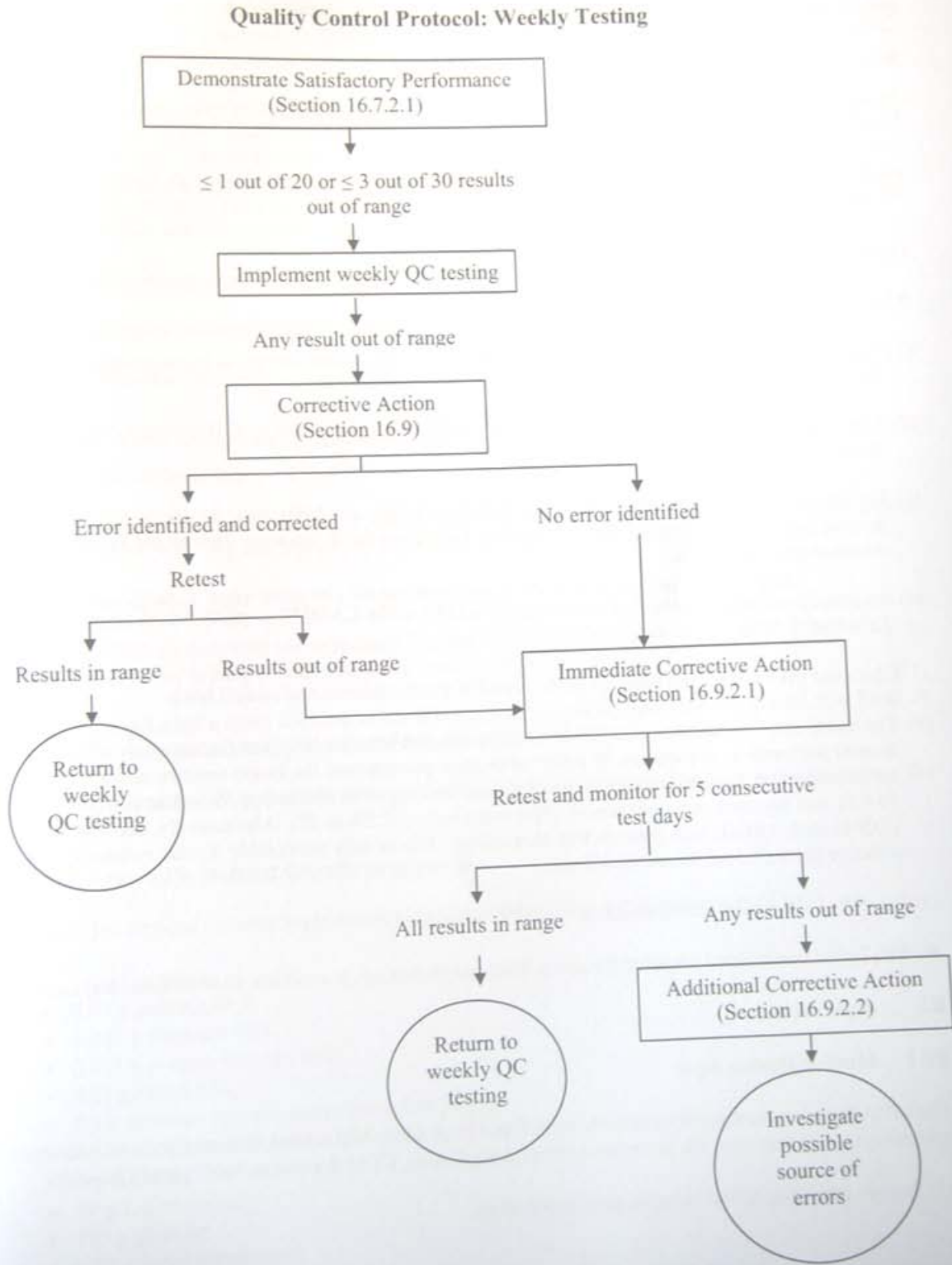


Reference: CLSI M7-A8, page 44



WEEKLY MIC QC CHART

Appendix A. (Continued)



Reference: CLSI M7-A8, page 45

Appendix 5- Quality control ranges for ATCC QC strains

Escherichia coli ATCC 25922

Antimicrobial	Disk diffusion	MIC
Ampicillin, AMP	16 - 22	2 - 8
Cefotaxime, CTX	29 - 35	0.03 - 0.12
Cefoxitin, FOX	23-29	2-8
Ceftazidime, CAZ	25 - 32	0.06 - 0.5
Chloramphenicol, CHL	21 - 27	2 - 8
Ciprofloxacin, CIP	30 - 40	0.004 - 0.015
Colistin, COL	11-17	0,25-2
Florfenicol, FFN	22-28	2-8
Gentamicin, GEN	19 - 26	0.25 - 1
Meropenem, MER	28-34	0.008-0.06
Nalidixic acid, NAL	22 - 28	1 - 4
Sulphonamides, SMX	15 - 23	8 - 32
Tetracycline, TET	18 - 25	0.5 - 2
Trimethoprim, TMP	21 - 28	0.5 - 2

Enterococcus faecalis ATCC 29212- MIC method

Antimicrobial	Low limit	High limit
Ampicillin , AMP	0,5	2
Chloramphenicol, CHL	4	16
Ciprofloxacin , CIP	0,25	2
Daptomycin, DAP	1	4
Erythromycin, ERY	1	4
Gentamicin, GEN	4	16
Linezolid, LZD	1	4
Quinu-dalfo-pristin, Q-D	2	8
Teicoplanin, TEI	0,25	1
Tetracycline, TET	8	32
Tigecycline, TIG	0,03	0,12
Vancomycin, VAN	1	4

Staphylococcus aureus ATCC 29213- MIC method

Antimicrobial	Low limit	High limit
Cefoxitin, FOX	1	4
Chloramphenicol, CHL	2	16
Ciprofloxacin, CIP	0,12	0,5
Clindamycin, CLN	0,06	0,25
Erythromycin, ERY	0,25	1
Florfenicol, FFN	2	8
Gentamicin, GEN	0,12	1
Linezolid, LZD	1	4

Mupirocin, MUP		
Penicillin, PEN	0,25	2
Quinupristin-dalfopristin, SYN	0,25	1
Tetracycline, TET	0,12	1
Trimethoprim, TMP	1	4
Vancomycin, VAN	0,5	2

Staphylococcus aureus ATCC 25923- Disk Diffusion

Antimicrobial	Concentration	Low limit	High limit
Cefoxitin, FOX	30	23	29
Chloramphenicol, CHL	30	19	26
Ciprofloxacin, CIP	5	22	30
Clindamycin, CLN	2	24	30
Erythromycin, ERY	15	22	30
Florfenicol, FFN	30	22	29
Gentamicin, GEN	10	19	27
Linezolid, LZD	30	25	32
Mupirocin, MUP	Na		
Penicillin, PEN	10	26	37
Quinupristin-dalfopristin, SYN	15	21	28
Sulfisoxazole, FIS	250-300	24	34
Tetracycline, TET	30	24	30
Trimethoprim, TMP	5	19	26
Vancomycin, VAN	30	17	21

S. aureus ATCC 25923 – Rosco tablets

Antimicrobial	Concentration	Low limit	High limit
Cefoxitin, FOX	30	23	29
Chloramphenicol, CHL	30	19	26
Ciprofloxacin, CIP	5	22	30
Clindamycin CLN	2	24	30
Erythromycin, ERY	15	22	30
Florfenicol, FFN	na	0	256
Gentamicin, GEN	10	19	27
Linezolid, LZD	30	25	32
Mupirocin, MUP	10	21	26
Quinupristin-dalfopristin, SYN	15	21	28
Sulfisoxazole, FIS	240	23	33
Tetracycline, TET	30	24	30
Trimethoprim, TMP	5	19	26
Vancomycin, VAN	30	17	21

Appendix 6a- Test results from reference strain *Enterococcus faecalis* ATCC 29212

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
1	Ampicillin , AMP	<=	2	0.5	2	1	MIC
1	Chloramphenicol, CHL	=	4	4	16	1	MIC
1	Ciprofloxacin , CIP	<=	0.5	0.25	2	1	MIC
1	Erythromycin, ERY	=	1	1	4	1	MIC
1	Gentamicin, GEN	<=	16	4	16	1	MIC
1	Linezolid, LZD	=	1	1	4	1	MIC
1	Quin.-Dalf. (Synercid), SYN	=	8	2	8	1	MIC
1	Teicoplanin, TEI	<=	0.25	0.25	1	1	MIC
1	Tetracycline, TET	=	16	8	32	1	MIC
1	Tigecycline, TGC	=	0.06	0.03	0.12	1	MIC
1	Vancomycin, VAN	=	2	1	4	1	MIC
2	Ampicillin , AMP	=	1	0.5	2	1	MIC
2	Chloramphenicol, CHL	=	8	4	16	1	MIC
2	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
2	Daptomycin, DAP	=	2	1	4	1	MIC
2	Erythromycin, ERY	=	2	1	4	1	MIC
2	Gentamicin, GEN	=	8	4	16	1	MIC
2	Linezolid, LZD	=	2	1	4	1	MIC
2	Teicoplanin, TEI	<=	0.25	0.25	1	1	MIC
2	Tetracycline, TET	=	16	8	32	1	MIC
2	Tigecycline, TGC	=	0.06	0.03	0.12	1	MIC
2	Vancomycin, VAN	=	2	1	4	1	MIC
6	Chloramphenicol, CHL	=	8	4	16	1	MIC
6	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
6	Daptomycin, DAP	=	2	1	4	1	MIC
6	Erythromycin, ERY	=	1	1	4	1	MIC
6	Linezolid, LZD	=	2	1	4	1	MIC
6	Quin.-Dalf. (Synercid), SYN	=	8	2	8	1	MIC
6	Tetracycline, TET	=	16	8	32	1	MIC
6	Tigecycline, TGC	=	0.06	0.03	0.12	1	MIC
6	Vancomycin, VAN	=	4	1	4	1	MIC
9	Ampicillin , AMP	=	1	0.5	2	1	MIC
9	Chloramphenicol, CHL	=	8	4	16	1	MIC
9	Ciprofloxacin , CIP	=	0.5	0.25	2	1	MIC
9	Erythromycin, ERY	=	2	1	4	1	MIC
9	Gentamicin, GEN	=	8	4	16	1	MIC
9	Linezolid, LZD	=	2	1	4	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
9	Quin.-Dalf. (Synercid), SYN	=	4	2	8	1	MIC
9	Tetracycline, TET	=	16	8	32	1	MIC
9	Vancomycin, VAN	=	2	1	4	1	MIC
11	Ampicillin , AMP	=	0.5	0.5	2	1	MIC
11	Chloramphenicol, CHL	=	4	4	16	1	MIC
11	Erythromycin, ERY	=	2	1	4	1	MIC
11	Gentamicin, GEN	=	8	4	16	1	MIC
11	Linezolid, LZD	=	1	1	4	1	MIC
11	Tetracycline, TET	=	16	8	32	1	MIC
11	Vancomycin, VAN	<=	1	1	4	1	MIC
12	Ampicillin , AMP	=	1	0.5	2	1	MIC
12	Chloramphenicol, CHL	=	8	4	16	1	MIC
12	Erythromycin, ERY	=	4	1	4	1	MIC
12	Gentamicin, GEN	=	8	4	16	1	MIC
12	Linezolid, LZD	=	2	1	4	1	MIC
12	Tetracycline, TET	=	16	8	32	1	MIC
12	Vancomycin, VAN	=	2	1	4	1	MIC
16	Ampicillin , AMP	=	1	0.5	2	1	MIC
16	Chloramphenicol, CHL	=	8	4	16	1	MIC
16	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
16	Daptomycin, DAP	=	2	1	4	1	MIC
16	Erythromycin, ERY	=	2	1	4	1	MIC
16	Gentamicin, GEN	=	16	4	16	1	MIC
16	Linezolid, LZD	=	2	1	4	1	MIC
16	Quin.-Dalf. (Synercid), SYN	=	8	2	8	1	MIC
16	Tetracycline, TET	=	32	8	32	1	MIC
16	Tigecycline, TGC	=	0.25	0.03	0.12	0	MIC
16	Vancomycin, VAN	=	4	1	4	1	MIC
17	Ampicillin , AMP	=	1	0.5	2	1	MIC
17	Chloramphenicol, CHL	=	8	4	16	1	MIC
17	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
17	Erythromycin, ERY	=	1	1	4	1	MIC
17	Gentamicin, GEN	=	16	4	16	1	MIC
17	Linezolid, LZD	=	2	1	4	1	MIC
17	Quin.-Dalf. (Synercid), SYN	=	4	2	8	1	MIC
17	Tetracycline, TET	=	16	8	32	1	MIC
17	Vancomycin, VAN	=	4	1	4	1	MIC
20	Ampicillin , AMP	=	2	0.5	2	1	MIC
20	Chloramphenicol, CHL	=	16	4	16	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
20	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
20	Erythromycin, ERY	=	2	1	4	1	MIC
20	Gentamicin, GEN	=	8	4	16	1	MIC
20	Linezolid, LZD	=	2	1	4	1	MIC
20	Quin.-Dalf. (Synercid), SYN	=	4	2	8	1	MIC
20	Tetracycline, TET	=	32	8	32	1	MIC
20	Vancomycin, VAN	=	2	1	4	1	MIC
21	Chloramphenicol, CHL	=	4	4	16	1	MIC
21	Ciprofloxacin , CIP	=	2	0.25	2	1	MIC
21	Erythromycin, ERY	=	1	1	4	1	MIC
21	Gentamicin, GEN	=	8	4	16	1	MIC
21	Linezolid, LZD	=	2	1	4	1	MIC
21	Quin.-Dalf. (Synercid), SYN	=	4	2	8	1	MIC
21	Tetracycline, TET	=	32	8	32	1	MIC
22	Ampicillin , AMP	=	1	0.5	2	1	MIC
22	Chloramphenicol, CHL	=	8	4	16	1	MIC
22	Erythromycin, ERY	=	2	1	4	1	MIC
22	Gentamicin, GEN	=	8	4	16	1	MIC
22	Linezolid, LZD	=	2	1	4	1	MIC
22	Tetracycline, TET	=	16	8	32	1	MIC
22	Vancomycin, VAN	=	2	1	4	1	MIC
23	Chloramphenicol, CHL	=	8	4	16	1	MIC
23	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
23	Erythromycin, ERY	=	2	1	4	1	MIC
23	Tetracycline, TET	=	32	8	32	1	MIC
23	Vancomycin, VAN	=	4	1	4	1	MIC
25	Ampicillin , AMP	<=	1	0.5	2	1	MIC
25	Chloramphenicol, CHL	=	8	4	16	1	MIC
25	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
25	Erythromycin, ERY	=	4	1	4	1	MIC
25	Gentamicin, GEN	=	16	4	16	1	MIC
25	Linezolid, LZD	=	2	1	4	1	MIC
25	Quin.-Dalf. (Synercid), SYN	=	16	2	8	0	MIC
25	Tetracycline, TET	=	32	8	32	1	MIC
25	Vancomycin, VAN	=	4	1	4	1	MIC
26	Chloramphenicol, CHL	=	8	4	16	1	MIC
26	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
26	Daptomycin, DAP	=	4	1	4	1	MIC
26	Erythromycin, ERY	=	1	1	4	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
26	Gentamicin, GEN	<=	128	4	16	1	MIC
26	Linezolid, LZD	=	2	1	4	1	MIC
26	Quin.-Dalf. (Synercid), SYN	=	8	2	8	1	MIC
26	Tetracycline, TET	=	16	8	32	1	MIC
26	Tigecycline, TGC	=	0.06	0.03	0.12	1	MIC
26	Vancomycin, VAN	=	4	1	4	1	MIC
29	Ampicillin , AMP	=	1	0.5	2	1	MIC
29	Chloramphenicol, CHL	=	4	4	16	1	MIC
29	Erythromycin, ERY	=	4	1	4	1	MIC
29	Gentamicin, GEN	=	4	4	16	1	MIC
29	Tetracycline, TET	=	16	8	32	1	MIC
29	Vancomycin, VAN	=	2	1	4	1	MIC
30	Ampicillin , AMP	=	1	0.5	2	1	MIC
30	Chloramphenicol, CHL	=	8	4	16	1	MIC
30	Ciprofloxacin , CIP	<=	1	0.25	2	1	MIC
30	Daptomycin, DAP	=	2	1	4	1	MIC
30	Erythromycin, ERY	=	2	1	4	1	MIC
30	Gentamicin, GEN	=	8	4	16	1	MIC
30	Linezolid, LZD	=	2	1	4	1	MIC
30	Quin.-Dalf. (Synercid), SYN	>	4	2	8	1	MIC
30	Tetracycline, TET	>	16	8	32	1	MIC
30	Tigecycline, TGC	=	0.06	0.03	0.12	1	MIC
30	Vancomycin, VAN	=	2	1	4	1	MIC
32	Chloramphenicol, CHL	=	8	4	16	1	MIC
32	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
32	Daptomycin, DAP	=	2	1	4	1	MIC
32	Erythromycin, ERY	=	1	1	4	1	MIC
32	Gentamicin, GEN	<=	128	4	16	1	MIC
32	Linezolid, LZD	=	2	1	4	1	MIC
32	Quin.-Dalf. (Synercid), SYN	=	8	2	8	1	MIC
32	Tetracycline, TET	=	16	8	32	1	MIC
32	Tigecycline, TGC	=	0.06	0.03	0.12	1	MIC
32	Vancomycin, VAN	=	4	1	4	1	MIC
33	Ampicillin , AMP	=	1	0.5	2	1	MIC
33	Chloramphenicol, CHL	=	4	4	16	1	MIC
33	Erythromycin, ERY	=	2	1	4	1	MIC
33	Gentamicin, GEN	=	8	4	16	1	MIC
33	Linezolid, LZD	=	2	1	4	1	MIC
33	Tetracycline, TET	=	32	8	32	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
33	Vancomycin, VAN	=	2	1	4	1	MIC
34	Ampicillin , AMP	=	1	0.5	2	1	MIC
34	Chloramphenicol, CHL	=	8	4	16	1	MIC
34	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
34	Erythromycin, ERY	=	1	1	4	1	MIC
34	Gentamicin, GEN	=	8	4	16	1	MIC
34	Linezolid, LZD	=	2	1	4	1	MIC
34	Quin.-Dalf. (Synercid), SYN	>	4	2	8	1	MIC
34	Tetracycline, TET	=	32	8	32	1	MIC
34	Vancomycin, VAN	=	4	1	4	1	MIC
36	Ampicillin , AMP	=	1	0.5	2	1	MIC
36	Chloramphenicol, CHL	=	4	4	16	1	MIC
36	Erythromycin, ERY	=	4	1	4	1	MIC
36	Gentamicin, GEN	=	8	4	16	1	MIC
36	Linezolid, LZD	=	1	1	4	1	MIC
36	Tetracycline, TET	=	32	8	32	1	MIC
36	Vancomycin, VAN	<=	1	1	4	1	MIC
37	Ampicillin , AMP	=	1	0.5	2	1	AGA
37	Chloramphenicol, CHL	=	4	4	16	1	AGA
37	Ciprofloxacin , CIP	=	0.5	0.25	2	1	AGA
37	Erythromycin, ERY	=	1	1	4	1	AGA
37	Gentamicin, GEN	=	4	4	16	1	AGA
37	Tetracycline, TET	=	16	8	32	1	AGA
37	Vancomycin, VAN	=	2	1	4	1	AGA
39	Ampicillin , AMP	=	1	0.5	2	1	MIC
39	Chloramphenicol, CHL	=	4	4	16	1	MIC
39	Erythromycin, ERY	=	1	1	4	1	MIC
39	Gentamicin, GEN	=	4	4	16	1	MIC
39	Linezolid, LZD	<=	0.5	1	4	0	MIC
39	Tetracycline, TET	=	16	8	32	1	MIC
39	Vancomycin, VAN	=	2	1	4	1	MIC
40	Ampicillin , AMP	=	22	None	None	*	DD
40	Chloramphenicol, CHL	=	24	None	None	*	DD
40	Ciprofloxacin , CIP	=	23	None	None	*	DD
40	Erythromycin, ERY	=	23	None	None	*	DD
40	Linezolid, LZD	=	24	None	None	*	DD
40	Tetracycline, TET	=	19	None	None	*	DD
40	Vancomycin, VAN	=	20	None	None	*	DD
41	Ampicillin , AMP	=	1	0.5	2	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
41	Chloramphenicol, CHL	=	8	4	16	1	MIC
41	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
41	Daptomycin, DAP	=	2	1	4	1	MIC
41	Erythromycin, ERY	=	1	1	4	1	MIC
41	Gentamicin, GEN	=	8	4	16	1	MIC
41	Linezolid, LZD	=	2	1	4	1	MIC
41	Quin.-Dalf. (Synercid), SYN	=	2	2	8	1	MIC
41	Tetracycline, TET	=	8	8	32	1	MIC
41	Tigecycline, TGC	=	0.12	0.03	0.12	1	MIC
41	Vancomycin, VAN	=	2	1	4	1	MIC
42	Ampicillin , AMP	<=	2	0.5	2	1	MIC
42	Chloramphenicol, CHL	=	8	4	16	1	MIC
42	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
42	Erythromycin, ERY	=	2	1	4	1	MIC
42	Gentamicin, GEN	<=	128	4	16	1	MIC
42	Linezolid, LZD	=	2	1	4	1	MIC
42	Quin.-Dalf. (Synercid), SYN	=	8	2	8	1	MIC
42	Tetracycline, TET	=	16	8	32	1	MIC
42	Vancomycin, VAN	=	4	1	4	1	MIC
45	Ampicillin , AMP	=	26.2	None	None	*	DD
45	Chloramphenicol, CHL	=	21.5	None	None	*	DD
45	Ciprofloxacin , CIP	=	21.7	None	None	*	DD
45	Erythromycin, ERY	=	18.1	None	None	*	DD
45	Gentamicin, GEN	=	12.1	None	None	*	DD
45	Linezolid, LZD	=	24.3	None	None	*	DD
45	Teicoplanin, TEI	=	17.3	None	None	*	DD
45	Tetracycline, TET	=	12.3	None	None	*	DD
45	Tigecycline, TGC	=	20.1	None	None	*	DD
45	Vancomycin, VAN	=	17.6	None	None		DD
46	Ampicillin , AMP	>	8	0.5	2	0	AGA
46	Chloramphenicol, CHL	=	4	4	16	1	AGA
46	Ciprofloxacin , CIP	=	8	0.25	2	0	AGA
46	Daptomycin, DAP	=	4	1	4	1	AGA
46	Erythromycin, ERY	=	1	1	4	1	AGA
46	Gentamicin, GEN	=	8	4	16	1	AGA
46	Linezolid, LZD	=	2	1	4	1	AGA
46	Quin.-Dalf. (Synercid), SYN	=	2	2	8	1	AGA
46	Teicoplanin, TEI	>	32	0.25	1	0	AGA
46	Tetracycline, TET	>	8	8	32	1	AGA

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
46	Tigecycline, TGC	=	0.032	0.03	0.12	1	AGA
46	Vancomycin, VAN	>	32	1	4	0	AGA
58	Ampicillin , AMP	=	1	0.5	2	1	MIC
58	Chloramphenicol, CHL	=	8	4	16	1	MIC
58	Ciprofloxacin , CIP	=	1	0.25	2	1	MIC
58	Daptomycin, DAP	=	2	1	4	1	MIC
58	Erythromycin, ERY	=	2	1	4	1	MIC
58	Gentamicin, GEN	=	8	4	16	1	MIC
58	Linezolid, LZD	=	2	1	4	1	MIC
58	Quin.-Dalf. (Synecid), SYN	=	8	2	8	1	MIC
58	Tetracycline, TET	=	16	8	32	1	MIC
58	Tigecycline, TGC	=	0.12	0.03	0.12	1	MIC
58	Vancomycin, VAN	=	4	1	4	1	MIC

- CLSI has not published a QC ranges for DD of strain ATCC 29212, therefore the results submitted by the laboratories using DD were not evaluated.

Appendix 6b- Test results from QC reference strain *Staphylococcus aureus*Test results from reference strain *Staphylococcus aureus* ATCC 29213-MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
1	Cefoxitin, FOX	=	2	1	4	1	MIC
1	Chloramphenicol, CHL	=	8	2	16	1	MIC
1	Ciprofloxacin, CIP	=	0.25	0.12	0.5	1	MIC
1	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
1	Florfenicol, FFN	=	4	2	8	1	MIC
1	Gentamicin, GEN	<=	0.25	0.12	1	1	MIC
1	Penicillin, PEN	=	0.5	0.25	2	1	MIC
1	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
1	Trimethoprim, TMP	=	1	1	4	1	MIC
2	Cefoxitin, FOX	=	4	1	4	1	MIC
2	Chloramphenicol, CHL	=	8	2	16	1	MIC
2	Ciprofloxacin, CIP	<=	0.25	0.12	0.5	1	MIC
2	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
2	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
2	Gentamicin, GEN	<=	1	0.12	1	1	MIC
2	Linezolid, LZD	=	2	1	4	1	MIC
2	Mupirocin, MUP	<=	0.5	0	256	1	MIC
2	Penicillin, PEN	=	0.5	0.25	2	1	MIC
2	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
2	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
2	Trimethoprim, TMP	<=	2	1	4	1	MIC
2	Vancomycin, VAN	<=	1	0.5	2	1	MIC
6	Cefoxitin, FOX	=	2	1	4	1	MIC
6	Chloramphenicol, CHL	=	8	2	16	1	MIC
6	Ciprofloxacin, CIP	<	0.25	0.12	0.5	1	MIC
6	Clindamycin, CLN	=	0.12	0.06	0.25	1	MIC
6	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
6	Gentamicin, GEN	<	1	0.12	1	1	MIC
6	Linezolid, LZD	=	2	1	4	1	MIC
6	Mupirocin, MUP	<	0.5	0	256	1	MIC
6	Penicillin, PEN	=	0.25	0.25	2	1	MIC
6	Quin.-Dalf. (Synercid), SYN	<	0.5	0.25	1	1	MIC
6	Sulfisoxazole, FIS	<	64	32	128	1	MIC
6	Tetracycline, TET	<	0.5	0.12	1	1	MIC
6	Trimethoprim, TMP	<	2	1	4	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
6	Vancomycin, VAN	<	1	0.5	2	1	MIC
9	Cefoxitin, FOX	=	4	1	4	1	MIC
9	Chloramphenicol, CHL	=	4	2	16	1	MIC
9	Ciprofloxacin, CIP	=	0.25	0.12	0.5	1	MIC
9	Clindamycin, CLN	=	0.12	0.06	0.25	1	MIC
9	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
9	Florfenicol, FFN	=	4	2	8	1	MIC
9	Gentamicin, GEN	=	1	0.12	1	1	MIC
9	Linezolid, LZD	=	2	1	4	1	MIC
9	Penicillin, PEN	=	0.5	0.25	2	1	MIC
9	Quin.-Dalf. (Synercid), SYN	=	0.5	0.25	1	1	MIC
9	Sulfisoxazole, FIS	=	64	32	128	1	MIC
9	Tetracycline, TET	=	0.5	0.12	1	1	MIC
9	Trimethoprim, TMP	=	2	1	4	1	MIC
9	Vancomycin, VAN	=	1	0.5	2	1	MIC
11	Chloramphenicol, CHL	=	8	2	16	1	MIC
11	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
11	Clindamycin, CLN	<=	0.25	0.06	0.25	1	MIC
11	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
11	Gentamicin, GEN	<=	0.5	0.12	1	1	MIC
11	Penicillin, PEN	=	1	0.25	2	1	MIC
11	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
11	Trimethoprim, TMP	=	2	1	4	1	MIC
12	Cefoxitin, FOX	=	4	1	4	1	MIC
12	Chloramphenicol, CHL	=	8	2	16	1	MIC
12	Ciprofloxacin, CIP	=	0.25	0.12	0.5	1	MIC
12	Clindamycin, CLN	<=	0.25	0.06	0.25	1	MIC
12	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
12	Gentamicin, GEN	<=	0.5	0.12	1	1	MIC
12	Penicillin, PEN	=	0.5	0.25	2	1	MIC
12	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
12	Trimethoprim, TMP	=	2	1	4	1	MIC
17	Cefoxitin, FOX	=	4	1	4	1	MIC
17	Chloramphenicol, CHL	=	8	2	16	1	MIC
17	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
17	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
17	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
17	Gentamicin, GEN	<=	1	0.12	1	1	MIC
17	Linezolid, LZD	=	4	1	4	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
17	Mupirocin, MUP	<=	0.5	0	256	1	MIC
17	Penicillin, PEN	=	1	0.25	2	1	MIC
17	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
17	Sulfisoxazole, FIS	<=	64	32	128	1	MIC
17	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
17	Trimethoprim, TMP	=	4	1	4	1	MIC
17	Vancomycin, VAN	<=	1	0.5	2	1	MIC
19	Cefoxitin, FOX	=	2	1	4	1	MIC
19	Chloramphenicol, CHL	=	8	2	16	1	MIC
19	Ciprofloxacin, CIP	<=	0.25	0.12	0.5	1	MIC
19	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
19	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
19	Gentamicin, GEN	<=	1	0.12	1	1	MIC
19	Linezolid, LZD	=	2	1	4	1	MIC
19	Mupirocin, MUP	<=	0.5	0	256	1	MIC
19	Penicillin, PEN	=	0.25	0.25	2	1	MIC
19	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
19	Sulfisoxazole, FIS	<=	64	32	128	1	MIC
19	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
19	Trimethoprim, TMP	<=	2	1	4	1	MIC
19	Vancomycin, VAN	<=	1	0.5	2	1	MIC
20	Cefoxitin, FOX	=	2	1	4	1	MIC
20	Chloramphenicol, CHL	=	16	2	16	1	MIC
20	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
20	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
20	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
20	Florfenicol, FFN	<=	8	2	8	1	MIC
20	Gentamicin, GEN	<=	1	0.12	1	1	MIC
20	Linezolid, LZD	=	2	1	4	1	MIC
20	Mupirocin, MUP	<=	0.5	0	256	1	MIC
20	Penicillin, PEN	=	0.25	0.25	2	1	MIC
20	Quin.-Dalf. (Synercid), SYN	=	1	0.25	1	1	MIC
20	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
20	Trimethoprim, TMP	<=	2	1	4	1	MIC
20	Vancomycin, VAN	<=	1	0.5	2	1	MIC
21	Cefoxitin, FOX	=	2	1	4	1	MIC
21	Chloramphenicol, CHL	=	8	2	16	1	MIC
21	Ciprofloxacin, CIP	=	0.25	0.12	0.5	1	MIC
21	Clindamycin, CLN	=	0.12	0.06	0.25	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
21	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
21	Gentamicin, GEN	=	1	0.12	1	1	MIC
21	Linezolid, LZD	=	2	1	4	1	MIC
21	Mupirocin, MUP	=	0.5	0	256	1	MIC
21	Penicillin, PEN	=	0.25	0.25	2	1	MIC
21	Quin.-Dalf. (Synercid), SYN	=	0.5	0.25	1	1	MIC
21	Sulfisoxazole, FIS	=	64	32	128	1	MIC
21	Tetracycline, TET	=	0.5	0.12	1	1	MIC
21	Trimethoprim, TMP	=	2	1	4	1	MIC
21	Vancomycin, VAN	=	1	0.5	2	1	MIC
22	Cefoxitin, FOX	=	2	1	4	1	MIC
22	Chloramphenicol, CHL	=	8	2	16	1	MIC
22	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
22	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
22	Gentamicin, GEN	=	1	0.12	1	1	MIC
22	Penicillin, PEN	=	0.5	0.25	2	1	MIC
22	Tetracycline, TET	=	1	0.12	1	1	MIC
22	Trimethoprim, TMP	=	2	1	4	1	MIC
23	Cefoxitin, FOX	=	4	1	4	1	MIC
23	Chloramphenicol, CHL	=	8	2	16	1	MIC
23	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
23	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
23	Gentamicin, GEN	<	1	0.12	1	1	MIC
23	Penicillin, PEN	=	0.25	0.25	2	1	MIC
23	Sulfisoxazole, FIS	<	64	32	128	1	MIC
23	Tetracycline, TET	<	0.5	0.12	1	1	MIC
23	Trimethoprim, TMP	<	2	1	4	1	MIC
25	Clindamycin, CLN	=	0.25	0.06	0.25	1	MIC
25	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
25	Penicillin, PEN	=	1	0.25	2	1	MIC
25	Tetracycline, TET	=	0.5	0.12	1	1	MIC
26	Cefoxitin, FOX	=	2	1	4	1	MIC
26	Chloramphenicol, CHL	=	8	2	16	1	MIC
26	Ciprofloxacin, CIP	=	0.25	0.12	0.5	1	MIC
26	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
26	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
26	Florfenicol, FFN	=	4	2	8	1	MIC
26	Gentamicin, GEN	=	0.5	0.12	1	1	MIC
26	Linezolid, LZD	=	2	1	4	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
26	Mupirocin, MUP	<=	0.5	0	256	1	MIC
26	Penicillin, PEN	=	0.25	0.25	2	1	MIC
26	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
26	Sulfisoxazole, FIS	<=	32	32	128	1	MIC
26	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
26	Trimethoprim, TMP	=	1	1	4	1	MIC
26	Vancomycin, VAN	<=	1	0.5	2	1	MIC
29	Chloramphenicol, CHL	=	2	2	16	1	MIC
29	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
29	Erythromycin, ERY	=	0.25	0.25	1	1	MIC
29	Gentamicin, GEN	=	0.5	0.12	1	1	MIC
29	Penicillin, PEN	=	0.5	0.25	2	1	MIC
29	Tetracycline, TET	=	0.5	0.12	1	1	MIC
29	Trimethoprim, TMP	=	1	1	4	1	MIC
30	Cefoxitin, FOX	=	4	1	4	1	MIC
30	Chloramphenicol, CHL	=	8	2	16	1	MIC
30	Ciprofloxacin, CIP	<=	0.25	0.12	0.5	1	MIC
30	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
30	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
30	Gentamicin, GEN	<=	1	0.12	1	1	MIC
30	Linezolid, LZD	=	2	1	4	1	MIC
30	Mupirocin, MUP	<=	0.5	0	256	1	MIC
30	Penicillin, PEN	=	0.25	0.25	2	1	MIC
30	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
30	Sulfisoxazole, FIS	<=	64	32	128	1	MIC
30	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
30	Trimethoprim, TMP	<=	2	1	4	1	MIC
30	Vancomycin, VAN	<=	1	0.5	2	1	MIC
31	Cefoxitin, FOX	<=	4	1	4	1	MIC
31	Chloramphenicol, CHL	<=	16	2	16	1	MIC
31	Ciprofloxacin, CIP	<=	0.12	0.12	0.5	1	MIC
31	Clindamycin, CLN	<=	0.25	0.06	0.25	1	MIC
31	Erythromycin, ERY	<=	0.5	0.25	1	1	MIC
31	Florfenicol, FFN	<=	8	2	8	1	MIC
31	Gentamicin, GEN	<=	2	0.12	1	1	MIC
31	Linezolid, LZD	<=	2	1	4	1	MIC
31	Mupirocin, MUP	<=	1	0	256	1	MIC
31	Penicillin, PEN	<=	0.5	0.25	2	1	MIC
31	Quin.-Dalf. (Synercid), SYN	<=	1	0.25	1	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
31	Sulfisoxazole, FIS	<=	128	32	128	1	MIC
31	Tetracycline, TET	<=	1	0.12	1	1	MIC
31	Trimethoprim, TMP	<=	2	1	4	1	MIC
31	Vancomycin, VAN	<=	1	0.5	2	1	MIC
33	Cefoxitin, FOX	=	4	1	4	1	MIC
33	Chloramphenicol, CHL	=	8	2	16	1	MIC
33	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
33	Clindamycin, CLN	<=	0.25	0.06	0.25	1	MIC
33	Erythromycin, ERY	=	1	0.25	1	1	MIC
33	Gentamicin, GEN	=	1	0.12	1	1	MIC
33	Penicillin, PEN	=	1	0.25	2	1	MIC
33	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
33	Trimethoprim, TMP	=	2	1	4	1	MIC
34	Cefoxitin, FOX	=	4	1	4	1	MIC
34	Chloramphenicol, CHL	=	16	2	16	1	MIC
34	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
34	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
34	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
34	Gentamicin, GEN	<=	1	0.12	1	1	MIC
34	Linezolid, LZD	=	4	1	4	1	MIC
34	Mupirocin, MUP	<=	0.5	0	256	1	MIC
34	Penicillin, PEN	=	1	0.25	2	1	MIC
34	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
34	Sulfisoxazole, FIS	<=	64	32	128	1	MIC
34	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
34	Trimethoprim, TMP	<=	2	1	4	1	MIC
34	Vancomycin, VAN	<=	1	0.5	2	1	MIC
35	Cefoxitin, FOX	=	4	1	4	1	MIC
36	Chloramphenicol, CHL	=	4	2	16	1	MIC
36	Ciprofloxacin, CIP	=	0.25	0.12	0.5	1	MIC
36	Clindamycin, CLN	<=	0.25	0.06	0.25	1	MIC
36	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
36	Gentamicin, GEN	=	1	0.12	1	1	MIC
36	Penicillin, PEN	=	1	0.25	2	1	MIC
36	Tetracycline, TET	=	1	0.12	1	1	MIC
36	Trimethoprim, TMP	=	2	1	4	1	MIC
37	Cefoxitin, FOX	=	4	1	4	1	AGA
37	Chloramphenicol, CHL	=	4	2	16	1	AGA
37	Ciprofloxacin, CIP	=	0.25	0.12	0.5	1	AGA

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
37	Erythromycin, ERY	=	0.5	0.25	1	1	AGA
37	Gentamicin, GEN	=	0.25	0.12	1	1	AGA
37	Penicillin, PEN	=	0.25	0.25	2	1	AGA
37	Tetracycline, TET	=	0.25	0.12	1	1	AGA
37	Trimethoprim, TMP	=	1	1	4	1	AGA
39	Chloramphenicol, CHL	=	8	2	16	1	MIC
39	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
39	Clindamycin, CLN	=	8	0.06	0.25	0	MIC
39	Erythromycin, ERY	=	1	0.25	1	1	MIC
39	Gentamicin, GEN	=	1	0.12	1	1	MIC
39	Penicillin, PEN	=	1	0.25	2	1	MIC
39	Tetracycline, TET	=	1	0.12	1	1	MIC
39	Trimethoprim, TMP	=	2	1	4	1	MIC
41	Cefoxitin, FOX	=	4	1	4	1	MIC
41	Chloramphenicol, CHL	=	8	2	16	1	MIC
41	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
41	Clindamycin, CLN	=	0.25	0.06	0.25	1	MIC
41	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
41	Florfenicol, FFN	=	4	2	8	1	MIC
41	Gentamicin, GEN	<=	1	0.12	1	1	MIC
41	Linezolid, LZD	=	2	1	4	1	MIC
41	Mupirocin, MUP	=	1	0	256	1	MIC
41	Penicillin, PEN	<=	0.12	0.25	2	0	MIC
41	Quin.-Dalf. (Synercid), SYN	=	1	0.25	1	1	MIC
41	Tetracycline, TET	=	0.5	0.12	1	1	MIC
41	Trimethoprim, TMP	=	2	1	4	1	MIC
41	Vancomycin, VAN	=	2	0.5	2	1	MIC
42	Cefoxitin, FOX	=	4	1	4	1	MIC
42	Chloramphenicol, CHL	=	8	2	16	1	MIC
42	Ciprofloxacin, CIP	<=	0.25	0.12	0.5	1	MIC
42	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
42	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
42	Gentamicin, GEN	<=	1	0.12	1	1	MIC
42	Linezolid, LZD	=	2	1	4	1	MIC
42	Mupirocin, MUP	<=	0.5	0	256	1	MIC
42	Penicillin, PEN	=	0.25	0.25	2	1	MIC
42	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
42	Sulfisoxazole, FIS	<=	64	32	128	1	MIC
42	Tetracycline, TET	<=	0.5	0.12	1	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
42	Trimethoprim, TMP	<=	2	1	4	1	MIC
42	Vancomycin, VAN	<=	1	0.5	2	1	MIC
56	Cefoxitin, FOX	=	4	1	4	1	MIC
56	Chloramphenicol, CHL	=	16	2	16	1	MIC
56	Ciprofloxacin, CIP	<=	0.25	0.12	0.5	1	MIC
56	Clindamycin, CLN	<=	0.12	0.06	0.25	1	MIC
56	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
56	Gentamicin, GEN	<=	1	0.12	1	1	MIC
56	Linezolid, LZD	=	4	1	4	1	MIC
56	Mupirocin, MUP	<=	0.5	0	256	1	MIC
56	Penicillin, PEN	=	0.5	0.25	2	1	MIC
56	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
56	Sulfisoxazole, FIS	=	128	32	128	1	MIC
56	Tetracycline, TET	=	1	0.12	1	1	MIC
56	Trimethoprim, TMP	<=	2	1	4	1	MIC
56	Vancomycin, VAN	<=	1	0.5	2	1	MIC
58	Cefoxitin, FOX	=	4	1	4	1	MIC
58	Chloramphenicol, CHL	=	8	2	16	1	MIC
58	Ciprofloxacin, CIP	=	0.5	0.12	0.5	1	MIC
58	Clindamycin, CLN	<=	0.125	0.06	0.25	1	MIC
58	Erythromycin, ERY	=	0.5	0.25	1	1	MIC
58	Gentamicin, GEN	<=	1	0.12	1	1	MIC
58	Linezolid, LZD	=	2	1	4	1	MIC
58	Mupirocin, MUP	<=	0.5	0	256	1	MIC
58	Penicillin, PEN	=	0.25	0.25	2	1	MIC
58	Quin.-Dalf. (Synercid), SYN	<=	0.5	0.25	1	1	MIC
58	Tetracycline, TET	<=	0.5	0.12	1	1	MIC
58	Trimethoprim, TMP	<=	2	1	4	1	MIC
58	Vancomycin, VAN	<=	1	0.5	2	1	MIC

Appendix 6c- Test results from QC reference strain *Escherichia coli* ATCC 25922

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
1	Ampicillin, AMP	=	4	2	8	1	MIC
1	Cefotaxime, CTX	<=	0.125	0.03	0.125	1	MIC
1	Chloramphenicol, CHL	=	4	2	8	1	MIC
1	Ciprofloxacin, CIP	<=	0.015	0.04	0.016	1	MIC
1	Colistin, COL	<=	1	0.25	2	1	MIC
1	Florfenicol, FFN	=	4	2	8	1	MIC
1	Gentamicin, GEN	<=	0.5	0.25	1	1	MIC
1	Nalidixic acid, NAL	<=	4	1	4	1	MIC
1	Tetracycline, TET	<=	2	0.5	2	1	MIC
1	Trimethoprim, TMP	<=	1	0.5	2	1	MIC
2	Ampicillin, AMP	=	4	2	8	1	MIC
2	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
2	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
2	Chloramphenicol, CHL	=	8	2	8	1	MIC
2	Ciprofloxacin, CIP	=	0.016	0.04	0.016	1	MIC
2	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
2	Meropenem, MER	=	0.03	0.008	0.06	1	MIC
2	Nalidixic acid, NAL	<=	2	1	4	1	MIC
2	Tetracycline, TET	=	2	0.5	2	1	MIC
2	Trimethoprim, TMP	=	1	0.5	2	1	MIC
4	Ampicillin, AMP	=	2	2	8	1	MIC
4	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
4	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
4	Chloramphenicol, CHL	=	8	2	8	1	MIC
4	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC
4	Colistin, COL	=	2	0.25	2	1	MIC
4	Florfenicol, FFN	=	4	2	8	1	MIC
4	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
4	Nalidixic acid, NAL	=	4	1	4	1	MIC
4	Sulfisoxazole, FIS	=	16	8	32	1	MIC
4	Tetracycline, TET	=	1	0.5	2	1	MIC
4	Trimethoprim, TMP	=	1	0.5	2	1	MIC
6	Ampicillin, AMP	=	2	2	8	1	MIC
6	Cefotaxime, CTX	<	0.06	0.03	0.125	1	MIC
6	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
6	Chloramphenicol, CHL	=	4	2	8	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
6	Ciprofloxacin, CIP	<	0.008	0.04	0.016	1	MIC
6	Florfenicol, FFN	=	4	2	8	1	MIC
6	Gentamicin, GEN	=	0.25	0.25	1	1	MIC
6	Nalidixic acid, NAL	<	4	1	4	1	MIC
6	Tetracycline, TET	<	1	0.5	2	1	MIC
6	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
9	Ampicillin, AMP	=	4	2	8	1	MIC
9	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
9	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
9	Chloramphenicol, CHL	=	4	2	8	1	MIC
9	Ciprofloxacin, CIP	=	0.008	0.04	0.016	1	MIC
9	Colistin, COL	=	0.25	0.25	2	1	MIC
9	Florfenicol, FFN	=	4	2	8	1	MIC
9	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
9	Meropenem, MER	=	0.03	0.008	0.06	1	MIC
9	Nalidixic acid, NAL	=	4	1	4	1	MIC
9	Sulfisoxazole, FIS	=	16	8	32	1	MIC
9	Tetracycline, TET	=	1	0.5	2	1	MIC
9	Trimethoprim, TMP	=	1	0.5	2	1	MIC
11	Ampicillin, AMP	=	4	2	8	1	MIC
11	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
11	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
11	Chloramphenicol, CHL	<=	2	2	8	1	MIC
11	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
11	Colistin, COL	<=	0.5	0.25	2	1	MIC
11	Florfenicol, FFN	<=	4	2	8	1	MIC
11	Gentamicin, GEN	=	1	0.25	1	1	MIC
11	Nalidixic acid, NAL	=	2	1	4	1	MIC
11	Sulfisoxazole, FIS	=	16	8	32	1	MIC
11	Tetracycline, TET	<=	1	0.5	2	1	MIC
11	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
12	Ampicillin, AMP	=	2	2	8	1	MIC
12	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
12	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
12	Chloramphenicol, CHL	=	4	2	8	1	MIC
12	Ciprofloxacin, CIP	=	0.03	0.04	0.016	0	MIC
12	Colistin, COL	<=	0.5	0.25	2	1	MIC
12	Florfenicol, FFN	<=	4	2	8	1	MIC
12	Gentamicin, GEN	=	1	0.25	1	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
12	Nalidixic acid, NAL	=	2	1	4	1	MIC
12	Sulfisoxazole, FIS	=	16	8	32	1	MIC
12	Tetracycline, TET	<=	1	0.5	2	1	MIC
12	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
14	Ampicillin, AMP	=	4	2	8	1	MIC
14	Cefotaxime, CTX	=	0.03	0.03	0.125	1	MIC
14	Ciprofloxacin, CIP	=	0.008	0.04	0.016	1	MIC
14	Colistin, COL	<=	0.5	0.25	2	1	MIC
14	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
14	Meropenem, MER	<=	0.12	0.008	0.06	1	MIC
14	Nalidixic acid, NAL	=	4	1	4	1	MIC
14	Sulfisoxazole, FIS	=	16	8	32	1	MIC
14	Tetracycline, TET	=	2	0.5	2	1	MIC
14	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
15	Ceftazidime, CAZ	=	32	25	32	1	DD
15	Chloramphenicol, CHL	=	25	21	27	1	DD
15	Gentamicin, GEN	=	26	19	26	1	DD
15	Nalidixic acid, NAL	=	25	22	28	1	DD
15	Tetracycline, TET	=	25	18	25	1	DD
15	Trimethoprim, TMP	=	26	21	28	1	DD
16	Ampicillin, AMP	=	4	2	8	1	MIC
16	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
16	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
16	Chloramphenicol, CHL	<=	4	2	8	1	MIC
16	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
16	Colistin, COL	<=	0.5	0.25	2	1	MIC
16	Gentamicin, GEN	<=	0.5	0.25	1	1	MIC
16	Meropenem, MER	<=	0.12	0.008	0.06	1	MIC
16	Nalidixic acid, NAL	<=	2	1	4	1	MIC
16	Tetracycline, TET	<=	1	0.5	2	1	MIC
16	Trimethoprim, TMP	=	1	0.5	2	1	MIC
17	Ampicillin, AMP	=	4	2	8	1	MIC
17	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
17	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
17	Chloramphenicol, CHL	=	4	2	8	1	MIC
17	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC
17	Colistin, COL	<=	2	0.25	2	1	MIC
17	Florfenicol, FFN	=	4	2	8	1	MIC
17	Gentamicin, GEN	=	0.5	0.25	1	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
17	Nalidixic acid, NAL	<=	4	1	4	1	MIC
17	Sulfisoxazole, FIS	=	32	8	32	1	MIC
17	Tetracycline, TET	<=	1	0.5	2	1	MIC
17	Trimethoprim, TMP	=	1	0.5	2	1	MIC
18	Ampicillin, AMP	=	4	2	8	1	MIC
18	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
18	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
18	Chloramphenicol, CHL	=	4	2	8	1	MIC
18	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
18	Colistin, COL	<=	2	0.25	2	1	MIC
18	Florfenicol, FFN	=	4	2	8	1	MIC
18	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
18	Nalidixic acid, NAL	=	4	1	4	1	MIC
18	Sulfisoxazole, FIS	=	16	8	32	1	MIC
18	Tetracycline, TET	=	1	0.5	2	1	MIC
18	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
19	Ampicillin, AMP	=	4	2	8	1	MIC
19	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
19	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
19	Chloramphenicol, CHL	=	4	2	8	1	MIC
19	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
19	Colistin, COL	<=	2	0.25	2	1	MIC
19	Florfenicol, FFN	=	8	2	8	1	MIC
19	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
19	Nalidixic acid, NAL	<=	4	1	4	1	MIC
19	Sulfisoxazole, FIS	=	32	8	32	1	MIC
19	Tetracycline, TET	<=	1	0.5	2	1	MIC
19	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
20	Ampicillin, AMP	=	4	2	8	1	MIC
20	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
20	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
20	Chloramphenicol, CHL	=	4	2	8	1	MIC
20	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
20	Colistin, COL	<=	2	0.25	2	1	MIC
20	Florfenicol, FFN	=	4	2	8	1	MIC
20	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
20	Meropenem, MER	<=	1	0.008	0.06	1	MIC
20	Nalidixic acid, NAL	<=	4	1	4	1	MIC
20	Sulfisoxazole, FIS	=	32	8	32	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
20	Tetracycline, TET	<=	1	0.5	2	1	MIC
20	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
21	Ampicillin, AMP	=	4	2	8	1	MIC
21	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
21	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
21	Chloramphenicol, CHL	=	4	2	8	1	MIC
21	Ciprofloxacin, CIP	=	0.008	0.04	0.016	1	MIC
21	Colistin, COL	=	2	0.25	2	1	MIC
21	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
21	Nalidixic acid, NAL	=	4	1	4	1	MIC
21	Sulfisoxazole, FIS	=	32	8	32	1	MIC
21	Tetracycline, TET	=	1	0.5	2	1	MIC
21	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
22	Ampicillin, AMP	=	4	2	8	1	MIC
22	Cefotaxime, CTX	<	0.06	0.03	0.125	1	MIC
22	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
22	Chloramphenicol, CHL	=	4	2	8	1	MIC
22	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC
22	Florfenicol, FFN	=	4	2	8	1	MIC
22	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
22	Nalidixic acid, NAL	<	4	1	4	1	MIC
22	Sulfisoxazole, FIS	=	16	8	32	1	MIC
22	Tetracycline, TET	=	1	0.5	2	1	MIC
22	Trimethoprim, TMP	=	1	0.5	2	1	MIC
23	Ampicillin, AMP	=	2	2	8	1	MIC
23	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
23	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
23	Chloramphenicol, CHL	=	4	2	8	1	MIC
23	Ciprofloxacin, CIP	=	0.008	0.04	0.016	1	MIC
23	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
23	Nalidixic acid, NAL	=	4	1	4	1	MIC
23	Sulfisoxazole, FIS	=	32	8	32	1	MIC
23	Tetracycline, TET	=	1	0.5	2	1	MIC
23	Trimethoprim, TMP	=	1	0.5	2	1	MIC
25	Ampicillin, AMP	=	8	2	8	1	MIC
25	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
25	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
25	Chloramphenicol, CHL	=	8	2	8	1	MIC
25	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
25	Colistin, COL	<=	2	0.25	2	1	MIC
25	Florfenicol, FFN	=	8	2	8	1	MIC
25	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
25	Nalidixic acid, NAL	<=	4	1	4	1	MIC
25	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
25	Tetracycline, TET	<=	1	0.5	2	1	MIC
25	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
26	Ampicillin, AMP	=	4	2	8	1	MIC
26	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
26	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
26	Chloramphenicol, CHL	=	4	2	8	1	MIC
26	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
26	Colistin, COL	<=	2	0.25	2	1	MIC
26	Florfenicol, FFN	<=	2	2	8	1	MIC
26	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
26	Nalidixic acid, NAL	<=	4	1	4	1	MIC
26	Tetracycline, TET	<=	1	0.5	2	1	MIC
26	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
29	Ampicillin, AMP	=	4	2	8	1	MIC
29	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
29	Ceftazidime, CAZ	=	0.06	0.06	0.5	1	MIC
29	Chloramphenicol, CHL	=	2	2	8	1	MIC
29	Ciprofloxacin, CIP	=	0.016	0.04	0.016	1	MIC
29	Florfenicol, FFN	=	8	2	8	1	MIC
29	Gentamicin, GEN	=	1	0.25	1	1	MIC
29	Nalidixic acid, NAL	=	2	1	4	1	MIC
29	Tetracycline, TET	=	1	0.5	2	1	MIC
29	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
30	Ampicillin, AMP	=	2	2	8	1	MIC
30	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
30	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
30	Chloramphenicol, CHL	=	4	2	8	1	MIC
30	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
30	Colistin, COL	<=	2	0.25	2	1	MIC
30	Florfenicol, FFN	<=	2	2	8	1	MIC
30	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
30	Meropenem, MER	<=	1	0.008	0.06	1	MIC
30	Nalidixic acid, NAL	<=	4	1	4	1	MIC
30	Sulfisoxazole, FIS	=	16	8	32	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
30	Tetracycline, TET	<=	1	0.5	2	1	MIC
30	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
32	Ampicillin, AMP	=	2	2	8	1	MIC
32	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
32	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
32	Chloramphenicol, CHL	=	4	2	8	1	MIC
32	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
32	Colistin, COL	<=	2	0.25	2	1	MIC
32	Florfenicol, FFN	=	4	2	8	1	MIC
32	Gentamicin, GEN	=	1	0.25	1	1	MIC
32	Nalidixic acid, NAL	<=	4	1	4	1	MIC
32	Sulfisoxazole, FIS	=	32	8	32	1	MIC
32	Tetracycline, TET	<=	1	0.5	2	1	MIC
32	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
33	Ampicillin, AMP	=	4	2	8	1	MIC
33	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
33	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
33	Chloramphenicol, CHL	=	4	2	8	1	MIC
33	Ciprofloxacin, CIP	=	0.03	0.04	0.016	0	MIC
33	Colistin, COL	=	1	0.25	2	1	MIC
33	Florfenicol, FFN	<=	4	2	8	1	MIC
33	Gentamicin, GEN	=	1	0.25	1	1	MIC
33	Nalidixic acid, NAL	=	2	1	4	1	MIC
33	Sulfisoxazole, FIS	=	32	8	32	1	MIC
33	Tetracycline, TET	<=	1	0.5	2	1	MIC
33	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
34	Ampicillin, AMP	=	4	2	8	1	MIC
34	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
34	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
34	Chloramphenicol, CHL	=	4	2	8	1	MIC
34	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	MIC
34	Colistin, COL	<=	2	0.25	2	1	MIC
34	Florfenicol, FFN	=	4	2	8	1	MIC
34	Gentamicin, GEN	=	1	0.25	1	1	MIC
34	Nalidixic acid, NAL	<=	4	1	4	1	MIC
34	Sulfisoxazole, FIS	=	16	8	32	1	MIC
34	Tetracycline, TET	<=	1	0.5	2	1	MIC
34	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
36	Ampicillin, AMP	=	4	2	8	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
36	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
36	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
36	Chloramphenicol, CHL	=	4	2	8	1	MIC
36	Ciprofloxacin, CIP	=	0.03	0.04	0.016	0	MIC
36	Colistin, COL	<=	0.5	0.25	2	1	MIC
36	Florfenicol, FFN	<=	4	2	8	1	MIC
36	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
36	Nalidixic acid, NAL	=	2	1	4	1	MIC
36	Sulfisoxazole, FIS	=	32	8	32	1	MIC
36	Tetracycline, TET	<=	1	0.5	2	1	MIC
36	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
37	Ampicillin, AMP	=	4	2	8	1	AGA
37	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	AGA
37	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	AGA
37	Chloramphenicol, CHL	=	4	2	8	1	AGA
37	Ciprofloxacin, CIP	<=	0.008	0.04	0.016	1	AGA
37	Gentamicin, GEN	=	0.5	0.25	1	1	AGA
37	Nalidixic acid, NAL	<=	2	1	4	1	AGA
37	Tetracycline, TET	=	1	0.5	2	1	AGA
37	Trimethoprim, TMP	=	0.5	0.5	2	1	AGA
39	Ampicillin, AMP	=	2	2	8	1	MIC
39	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
39	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
39	Chloramphenicol, CHL	=	8	2	8	1	MIC
39	Ciprofloxacin, CIP	=	0.16	0.04	0.016	0	MIC
39	Colistin, COL	<=	0.5	0.25	2	1	MIC
39	Florfenicol, FFN	=	8	2	8	1	MIC
39	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
39	Nalidixic acid, NAL	=	2	1	4	1	MIC
39	Tetracycline, TET	<=	1	0.5	2	1	MIC
39	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
40	Ampicillin, AMP	=	21	16	22	1	DD
40	Cefotaxime, CTX	=	32	29	35	1	DD
40	Ceftazidime, CAZ	=	28	25	32	1	DD
40	Chloramphenicol, CHL	=	24	21	27	1	DD
40	Ciprofloxacin, CIP	=	34	30	40	1	DD
40	Gentamicin, GEN	=	22	19	26	1	DD
40	Meropenem, MER	=	30	28	34	1	DD
40	Nalidixic acid, NAL	=	27	22	28	1	DD

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
40	Sulfisoxazole, FIS	=	21	15	23	1	DD
40	Tetracycline, TET	=	22	18	25	1	DD
40	Trimethoprim, TMP	=	27	21	28	1	DD
41	Ampicillin, AMP	=	4	2	8	1	MIC
41	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
41	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
41	Chloramphenicol, CHL	=	8	2	8	1	MIC
41	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC
41	Colistin, COL	=	2	0.25	2	1	MIC
41	Florfenicol, FFN	=	4	2	8	1	MIC
41	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
41	Nalidixic acid, NAL	=	4	1	4	1	MIC
41	Tetracycline, TET	=	1	0.5	2	1	MIC
41	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
42	Ampicillin, AMP	=	8	2	8	1	MIC
42	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
42	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
42	Chloramphenicol, CHL	=	4	2	8	1	MIC
42	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC
42	Colistin, COL	<=	2	0.25	2	1	MIC
42	Florfenicol, FFN	=	8	2	8	1	MIC
42	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
42	Meropenem, MER	<=	1	0.008	0.06	1	MIC
42	Nalidixic acid, NAL	<=	4	1	4	1	MIC
42	Sulfisoxazole, FIS	=	32	8	32	1	MIC
42	Tetracycline, TET	=	2	0.5	2	1	MIC
42	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
45	Ampicillin, AMP	=	0	16	22	0	DD
45	Cefotaxime, CTX	=	32.1	29	35	1	DD
45	Ceftazidime, CAZ	=	29.2	25	32	1	DD
45	Chloramphenicol, CHL	=	22.9	21	27	1	DD
45	Ciprofloxacin, CIP	=	34.0	30	40	1	DD
45	Colistin, COL	=	15.4	11	17	1	DD
45	Gentamicin, GEN	=	19.3	19	26	1	DD
45	Meropenem, MER	=	31.5	28	34	1	DD
45	Nalidixic acid, NAL	=	26.9	22	28	1	DD
45	Sulfisoxazole, FIS	=	19	15	23	1	DD
45	Tetracycline, TET	=	23.1	18	25	1	DD
45	Trimethoprim, TMP	=	25.8	21	28	1	DD

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
56	Ampicillin, AMP	=	4	2	8	1	MIC
56	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
56	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
56	Chloramphenicol, CHL	=	4	2	8	1	MIC
56	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC
56	Colistin, COL	<=	2	0.25	2	1	MIC
56	Florfenicol, FFN	=	4	2	8	1	MIC
56	Gentamicin, GEN	<=	0.25	0.25	1	1	MIC
56	Nalidixic acid, NAL	<=	4	1	4	1	MIC
56	Sulfisoxazole, FIS	=	32	8	32	1	MIC
56	Tetracycline, TET	<=	1	0.5	2	1	MIC
56	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
58	Ampicillin, AMP	=	8	2	8	1	MIC
58	Cefotaxime, CTX	=	0.125	0.03	0.125	1	MIC
58	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
58	Chloramphenicol, CHL	=	8	2	8	1	MIC
58	Ciprofloxacin, CIP	=	0.015	0.04	0.016	1	MIC
58	Colistin, COL	<=	2	0.25	2	1	MIC
58	Florfenicol, FFN	=	8	2	8	1	MIC
58	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
58	Meropenem, MER	=	0.016	0.008	0.06	1	MIC
58	Nalidixic acid, NAL	<=	4	1	4	1	MIC
58	Sulfisoxazole, FIS	=	32	8	32	1	MIC
58	Tetracycline, TET	<=	1	0.5	2	1	MIC
58	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC


Appendix 7a- Summary of results Enterococci trial

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Ampicillin , AMP	EURL ENT 7.1	S	0	100	24	0
	EURL ENT 7.2	*	61	39	*	
	EURL ENT 7.3	*	71	29	*	
	EURL ENT 7.4	S	0	100	24	0
	EURL ENT 7.5	S	4	96	23	1
	EURL ENT 7.6	S	0	100	24	0
	EURL ENT 7.7	S	17	83	20	4
	EURL ENT 7.8	R	100	0	24	0
Chloramphenicol, CHL	EURL ENT 7.1	S	0	100	29	0
	EURL ENT 7.2	S	0	100	29	0
	EURL ENT 7.3	S	0	100	29	0
	EURL ENT 7.4	S	0	100	29	0
	EURL ENT 7.5	S	0	100	29	0
	EURL ENT 7.6	R	100	0	28	0
	EURL ENT 7.7	S	0	100	29	0
	EURL ENT 7.8	S	0	100	29	0
Ciprofloxacin , CIP	EURL ENT 7.1	S	0	100	24	0
	EURL ENT 7.2	S	0	100	24	0
	EURL ENT 7.3	S	0	100	24	0
	EURL ENT 7.4	R	96	4	22	1
	EURL ENT 7.5	S	0	100	24	0
	EURL ENT 7.6	S	4	96	23	1
	EURL ENT 7.7	S	0	100	24	0
	EURL ENT 7.8	R	100	0	23	0
Daptomycin, DAP	EURL ENT 7.1	S	0	100	11	0
	EURL ENT 7.2	S	18	82	9	2
	EURL ENT 7.3	S	0	100	11	0
	EURL ENT 7.4	S	0	100	11	0
	EURL ENT 7.5	S	0	100	11	0
	EURL ENT 7.6	S	0	100	11	0
	EURL ENT 7.7	S	0	100	11	0
	EURL ENT 7.8	S	27	73	8	3

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Erythromycin, ERY	EURL ENT 7.1	S	0	100	29	0
	EURL ENT 7.2	R	93	7	27	2
	EURL ENT 7.3	R	100	0	29	0
	EURL ENT 7.4	S	3	97	28	1
	EURL ENT 7.5	R	97	3	28	1
	EURL ENT 7.6	R	100	0	29	0
	EURL ENT 7.7	S	3	97	28	1
	EURL ENT 7.8	R	100	0	29	0
Gentamicin, GEN	EURL ENT 7.1	S	8	92	22	2
	EURL ENT 7.2	S	4	96	23	1
	EURL ENT 7.3	S	4	96	23	1
	EURL ENT 7.4	S	4	96	23	1
	EURL ENT 7.5	R	96	4	27	1
	EURL ENT 7.6	R	100	0	27	0
	EURL ENT 7.7	S	8	92	22	2
	EURL ENT 7.8	R	100	0	28	0
Linezolid, LZD	EURL ENT 7.1	S	0	100	27	0
	EURL ENT 7.2	S	0	100	27	0
	EURL ENT 7.3	S	0	100	27	0
	EURL ENT 7.4	S	0	100	27	0
	EURL ENT 7.5	S	0	100	27	0
	EURL ENT 7.6	S	0	100	27	0
	EURL ENT 7.7	S	0	100	27	0
	EURL ENT 7.8	S	0	100	27	0
Quin.-Dalf. (Synercid), SYN	EURL ENT 7.2	*	25	75	*	
	EURL ENT 7.3	*	71	29	*	
	EURL ENT 7.4	S	0	100	18	0
	EURL ENT 7.7	S	0	100	17	0
	EURL ENT 7.8	S	0	100	18	0
Teicoplanin, TEI	EURL ENT 7.1	S	0	100	5	0
	EURL ENT 7.2	S	0	100	6	0
	EURL ENT 7.3	R	100	0	5	0
	EURL ENT 7.4	S	0	100	5	0
	EURL ENT 7.5	S	0	100	6	0
	EURL ENT 7.6	S	0	100	6	0
	EURL ENT 7.7	R	100	0	5	0
	EURL ENT 7.8	S	0	100	6	0

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Tetracycline, TET	EURL ENT 7.1	S	0	100	29	0
	EURL ENT 7.2	S	0	100	29	0
	EURL ENT 7.3	R	97	3	28	1
	EURL ENT 7.4	S	0	100	29	0
	EURL ENT 7.5	R	100	0	29	0
	EURL ENT 7.6	R	100	0	29	0
	EURL ENT 7.7	R	100	0	29	0
	EURL ENT 7.8	S	0	100	29	0
Tigecycline, TGC	EURL ENT 7.2	S	0	100	12	0
	EURL ENT 7.3	S	0	100	13	0
	EURL ENT 7.4	S	0	100	13	0
	EURL ENT 7.7	S	0	100	13	0
	EURL ENT 7.8	S	0	100	13	0
Vancomycin, VAN	EURL ENT 7.1	S	0	100	29	0
	EURL ENT 7.2	S	0	100	29	0
	EURL ENT 7.3	R	100	0	29	0
	EURL ENT 7.4	S	0	100	29	0
	EURL ENT 7.5	S	3	97	28	1
	EURL ENT 7.6	S	3	97	28	1
	EURL ENT 7.7	R	100	0	29	0
	EURL ENT 7.8	S	0	100	29	0

- Combinations subtracted before evaluation due to breakpoint issues leading to deviation level of 25 or above.

 Combination ENT 7.8/daptomycin subtracted from report as it caused more than 25% deviation.

Appendix 7b- Summary of results Staphylococci trial

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Cefoxitin, FOX	EURL ST 7.1	R	97	3	29	1
	EURL ST 7.2	S	3	97	29	1
	EURL ST 7.3	R	97	3	29	1
	EURL ST 7.4	R	100	0	30	0
	EURL ST 7.5	R	100	0	30	0
	EURL ST 7.6	S	3	97	28	1
	EURL ST 7.7	R	100	0	30	0
	EURL ST 7.8	R	100	0	30	0
Chloramphenicol, CHL	EURL ST 7.1	S	0	100	32	0
	EURL ST 7.2	S	0	100	32	0
	EURL ST 7.3	S	0	100	32	0
	EURL ST 7.4	S	0	100	32	0
	EURL ST 7.5	S	3	97	31	1
	EURL ST 7.6	S	0	100	32	0
	EURL ST 7.7	S	0	100	32	0
	EURL ST 7.8	S	0	100	32	0
Ciprofloxacin, CIP	EURL ST 7.1	S	3	97	32	1
	EURL ST 7.2	S	3	97	32	1
	EURL ST 7.3	S	0	100	33	0
	EURL ST 7.5	S	0	100	33	0
	EURL ST 7.6	S	3	97	32	1
	EURL ST 7.7	S	0	100	33	0
	EURL ST 7.8	S	0	100	33	0
Clindamycin, CLN	EURL ST 7.1	S	18	82	23	5
	EURL ST 7.2	S	7	93	26	2
	EURL ST 7.3	R	100	0	28	0
	EURL ST 7.4	S	14	86	24	4
	EURL ST 7.5	S	11	89	25	3
	EURL ST 7.6	R	96	4	27	1
	EURL ST 7.7	R	100	0	28	0
	EURL ST 7.8	S	11	89	24	3
Erythromycin, ERY	EURL ST 7.1	S	0	100	34	0
	EURL ST 7.2	S	0	100	34	0
	EURL ST 7.3	S	0	100	34	0
	EURL ST 7.4	S	3	97	33	1
	EURL ST 7.5	S	0	100	34	0
	EURL ST 7.6	R	97	3	33	1
	EURL ST 7.7	S	6	94	32	2
	EURL ST 7.8	S	0	100	34	0

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Florfenicol, FFN	EURL ST 7.1	S	0	100	11	0
	EURL ST 7.2	S	0	100	10	0
	EURL ST 7.3	S	0	100	10	0
	EURL ST 7.4	S	0	100	10	0
	EURL ST 7.5	S	0	100	10	0
	EURL ST 7.6	S	0	100	10	0
	EURL ST 7.7	S	0	100	10	0
	EURL ST 7.8	S	0	100	10	0
Gentamicin, GEN	EURL ST 7.1	S	0	100	32	0
	EURL ST 7.2	S	0	100	33	0
	EURL ST 7.3	S	0	100	33	0
	EURL ST 7.4	R	100	0	33	0
	EURL ST 7.5	S	3	97	32	1
	EURL ST 7.6	R	97	3	32	1
	EURL ST 7.7	S	3	97	32	1
	EURL ST 7.8	S	0	100	33	0
Linezolid, LZD	EURL ST 7.1	S	0	100	23	0
	EURL ST 7.2	S	0	100	21	0
	EURL ST 7.3	S	0	100	23	0
	EURL ST 7.4	S	0	100	23	0
	EURL ST 7.5	S	0	100	23	0
	EURL ST 7.6	S	0	100	23	0
	EURL ST 7.7	S	0	100	23	0
	EURL ST 7.8	S	0	100	23	0
Mupirocin, MUP	EURL ST 7.1	S	6	94	17	1
	EURL ST 7.2	S	0	100	18	0
	EURL ST 7.3	S	0	100	18	0
	EURL ST 7.4	S	0	100	18	0
	EURL ST 7.5	S	0	100	18	0
	EURL ST 7.6	S	0	100	17	0
	EURL ST 7.7	S	0	100	18	0
	EURL ST 7.8	S	0	100	18	0
Penicillin, PEN	EURL ST 7.1	R	97	3	32	1
	EURL ST 7.2	R	100	0	33	0
	EURL ST 7.3	R	100	0	33	0
	EURL ST 7.4	R	100	0	33	0
	EURL ST 7.5	R	100	0	33	0
	EURL ST 7.6	R	100	0	33	0
	EURL ST 7.7	R	100	0	33	0
	EURL ST 7.8	R	97	3	32	1

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Quin.-Dalf. (Synercid), SYN	EURL ST 7.1	S	5	95	21	1
	EURL ST 7.2	S	0	100	22	0
	EURL ST 7.3	R	80	20	16	4
	EURL ST 7.4	S	5	95	21	1
	EURL ST 7.5	S	9	91	20	2
	EURL ST 7.7	R	80	20	16	4
	EURL ST 7.8	S	0	100	21	0
Sulfamethoxazole, SMX	EURL ST 7.1	S	4	96	25	1
	EURL ST 7.2	S	15	85	22	4
	EURL ST 7.3	S	0	100	26	0
	EURL ST 7.4	R	84	16	21	4
	EURL ST 7.5	S	0	100	26	0
	EURL ST 7.6	S	4	96	25	1
	EURL ST 7.7	S	0	100	26	0
	EURL ST 7.8	S	0	100	25	0
Tetracycline, TET	EURL ST 7.1	S	0	100	34	0
	EURL ST 7.2	S	3	97	33	1
	EURL ST 7.3	R	100	0	34	0
	EURL ST 7.4	R	100	0	34	0
	EURL ST 7.5	R	100	0	34	0
	EURL ST 7.6	R	100	0	34	0
	EURL ST 7.7	R	100	0	34	0
	EURL ST 7.8	R	100	0	34	0
Trimethoprim, TMP	EURL ST 7.1	S	0	100	32	0
	EURL ST 7.2	S	22	78	25	7
	EURL ST 7.3	R	100	0	32	0
	EURL ST 7.4	S	3	97	31	1
	EURL ST 7.5	S	3	97	31	1
	EURL ST 7.6	R	97	3	31	1
	EURL ST 7.7	R	100	0	32	0
	EURL ST 7.8	S	3	97	31	1
Vancomycin, VAN	EURL ST 7.1	S	0	100	22	0
	EURL ST 7.2	S	0	100	22	0
	EURL ST 7.3	S	0	100	22	0
	EURL ST 7.4	S	0	100	22	0
	EURL ST 7.5	S	0	100	22	0
	EURL ST 7.6	S	0	100	22	0
	EURL ST 7.7	S	0	100	22	0
	EURL ST 7.8	S	0	100	22	0

Appendix 7c- Summary of results *E.coli* trial

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Ampicillin, AMP	EURL EC 7.1	R	100	0	34	0
	EURL EC 7.2	R	100	0	34	0
	EURL EC 7.3	R	100	0	34	0
	EURL EC 7.4	R	100	0	34	0
	EURL EC 7.5	S	6	94	32	2
	EURL EC 7.6	R	100	0	34	0
	EURL EC 7.7	R	100	0	34	0
	EURL EC 7.8	R	100	0	34	0
Cefotaxime, CTX	EURL EC 7.1	S	0	100	34	0
	EURL EC 7.2	R	100	0	34	0
	EURL EC 7.3	R	100	0	34	0
	EURL EC 7.4	R	100	0	34	0
	EURL EC 7.5	S	0	100	34	0
	EURL EC 7.6	S	0	100	34	0
	EURL EC 7.7	S	0	100	34	0
	EURL EC 7.8	R	100	0	34	0
Ceftazidime, CAZ	EURL EC 7.1	S	0	100	32	0
	EURL EC 7.2	R	88	13	28	4
	EURL EC 7.3	R	100	0	33	0
	EURL EC 7.4	R	94	6	30	2
	EURL EC 7.5	S	0	100	32	0
	EURL EC 7.6	S	0	100	32	0
	EURL EC 7.7	S	3	97	31	1
	EURL EC 7.8	R	100	0	33	0
Chloramphenicol, CHL	EURL EC 7.1	S	0	100	33	0
	EURL EC 7.2	S	0	100	33	0
	EURL EC 7.3	S	0	100	33	0
	EURL EC 7.4	S	0	100	33	0
	EURL EC 7.5	S	0	100	33	0
	EURL EC 7.6	R	97	3	32	1
	EURL EC 7.7	R	100	0	33	0
	EURL EC 7.8	S	0	100	33	0

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Ciprofloxacin, CIP	EURL EC 7.1	R	94	6	31	2
	EURL EC 7.2	S	0	100	34	0
	EURL EC 7.3	S	0	100	34	0
	EURL EC 7.4	S	0	100	34	0
	EURL EC 7.5	S	0	100	34	0
	EURL EC 7.6	S	0	100	34	0
	EURL EC 7.7	S	0	100	34	0
	EURL EC 7.8	R	91	9	31	3
Colistin, COL	EURL EC 7.1	S	0	100	29	0
	EURL EC 7.2	S	3	97	28	1
	EURL EC 7.3	S	0	100	29	0
	EURL EC 7.4	S	0	100	29	0
	EURL EC 7.5	S	0	100	29	0
	EURL EC 7.6	S	0	100	29	0
	EURL EC 7.7	S	0	100	29	0
	EURL EC 7.8	S	0	100	29	0
Florfenicol, FFN	EURL EC 7.1	S	0	100	28	0
	EURL EC 7.2	S	0	100	28	0
	EURL EC 7.3	S	0	100	28	0
	EURL EC 7.4	S	0	100	28	0
	EURL EC 7.5	S	0	100	28	0
	EURL EC 7.6	S	7	93	26	2
	EURL EC 7.7	R	100	0	28	0
	EURL EC 7.8	S	0	100	28	0
Gentamicin, GEN	EURL EC 7.1	S	0	100	34	0
	EURL EC 7.2	S	0	100	34	0
	EURL EC 7.3	S	0	100	34	0
	EURL EC 7.4	S	0	100	34	0
	EURL EC 7.5	S	0	100	34	0
	EURL EC 7.6	S	0	100	34	0
	EURL EC 7.7	R	97	3	33	1
	EURL EC 7.8	S	0	100	34	0
Meropenem, MER	EURL EC 7.1	S	0	100	12	0
	EURL EC 7.2	S	0	100	13	0
	EURL EC 7.3	S	0	100	13	0
	EURL EC 7.4	S	0	100	13	0
	EURL EC 7.5	S	0	100	12	0
	EURL EC 7.6	S	0	100	12	0
	EURL EC 7.7	S	0	100	12	0
	EURL EC 7.8	S	0	100	13	0

Antimicrobial	Strain	Expected result	% R	% S	Number expected results	Number deviating results
Nalidixic acid, NAL	EURL EC 7.1	R	100	0	34	0
	EURL EC 7.2	S	0	100	34	0
	EURL EC 7.3	S	0	100	34	0
	EURL EC 7.4	S	0	100	34	0
	EURL EC 7.5	S	3	97	33	1
	EURL EC 7.6	S	3	97	33	1
	EURL EC 7.7	S	0	100	34	0
	EURL EC 7.8	R	100	0	34	0
Sulfamethoxazole, SMX	EURL EC 7.1	R	100	0	33	0
	EURL EC 7.2	R	100	0	33	0
	EURL EC 7.3	S	6	94	31	2
	EURL EC 7.4	S	0	100	33	0
	EURL EC 7.5	S	6	94	31	2
	EURL EC 7.6	S	3	97	31	1
	EURL EC 7.7	R	100	0	33	0
	EURL EC 7.8	S	6	94	31	2
Tetracycline, TET	EURL EC 7.1	R	100	0	34	0
	EURL EC 7.2	R	100	0	34	0
	EURL EC 7.3	S	0	100	34	0
	EURL EC 7.4	S	0	100	34	0
	EURL EC 7.5	S	0	100	34	0
	EURL EC 7.6	R	100	0	34	0
	EURL EC 7.7	S	0	100	33	0
	EURL EC 7.8	S	0	100	34	0
Trimethoprim, TMP	EURL EC 7.1	R	100	0	34	0
	EURL EC 7.2	R	100	0	34	0
	EURL EC 7.3	S	0	100	34	0
	EURL EC 7.4	S	0	100	34	0
	EURL EC 7.5	S	0	100	34	0
	EURL EC 7.6	S	3	97	33	1
	EURL EC 7.7	R	100	0	34	0
	EURL EC 7.8	S	0	100	34	0

Appendix 8a- Deviations of results Enterococci trial

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic
LAB. 006	EURL ENT 7.6	Ciprofloxacin , CIP	R	>4	S	1
	EURL ENT 7.6	Vancomycin, VAN	R	>32	S	1
	EURL ENT 7.8	Daptomycin, DAP	R	8	S	4
LAB. 011	EURL ENT 7.5	Ampicillin , AMP	R	8	S	<=2
	EURL ENT 7.5	Erythromycin, ERY	S	2	R	>32
	EURL ENT 7.5	Gentamicin, GEN	S	8	R	>1024
	EURL ENT 7.5	Vancomycin, VAN	R	>128	S	1
LAB. 012	EURL ENT 7.4	Erythromycin, ERY	R	8	S	2
LAB. 020	EURL ENT 7.7	Ampicillin , AMP	R	8	S	4
LAB. 022	EURL ENT 7.7	Ampicillin , AMP	R	=8	S	4
LAB. 025	EURL ENT 7.7	Ampicillin , AMP	R	8	S	4
LAB. 026	EURL ENT 7.1	Gentamicin, GEN	R	128	S	<=16
	EURL ENT 7.2	Gentamicin, GEN	R	128	S	<=16
	EURL ENT 7.3	Gentamicin, GEN	R	128	S	<=16
	EURL ENT 7.4	Gentamicin, GEN	R	128	S	<=16
	EURL ENT 7.7	Gentamicin, GEN	R	128	S	<=16
LAB. 029	EURL ENT 7.4	Ciprofloxacin , CIP	S	16mm	R	8
LAB. 032	EURL ENT 7.2	Daptomycin, DAP	R	<=8	S	4
	EURL ENT 7.8	Daptomycin, DAP	R	<=8	S	4
LAB. 039	EURL ENT 7.2	Erythromycin, ERY	S	4	R	>32
	EURL ENT 7.7	Ampicillin , AMP	R	8	S	4
LAB. 040	EURL ENT 7.2	Erythromycin, ERY	S	14	R	>32
LAB. 041	EURL ENT 7.2	Daptomycin, DAP	R	>4	S	4
	EURL ENT 7.7	Erythromycin, ERY	R	>4	S	1
	EURL ENT 7.7	Gentamicin, GEN	R	>16	S	<=16
	EURL ENT 7.8	Daptomycin, DAP	R	>4	S	4
LAB. 045	EURL ENT 7.1	Gentamicin, GEN	R	11.1	S	<=16
LAB. 046	EURL ENT 7.3	Tetracycline, TET	S	>8	R	>32

Combination ENT 7.8/daptomycin subtracted from report as it caused more than 25% deviation.

Appendix 8b- Deviations to results Staphylococci trial

Lab no.	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic
LAB. 002	Sulfamethoxazole, SMX	R	512	S	≤ 32
LAB. 004	Tetracycline, TET	R	25.77	S	≤ 0.5
	Trimethoprim, TMP	R	18.45	S	2
LAB. 006	Penicillin, PEN	S	2	R	0.5
	Cefoxitin, FOX	R	16	S	4
	Clindamycin, CLN	R	>4	S	0.12
	Erythromycin, ERY	R	>8	S	≤ 0.25
	Quin.-Dalf. (Synercid), SYN	R	2	S	0.5
	Trimethoprim, TMP	R	>32	S	≤ 0.5
	Clindamycin, CLN	R	>4	S	0.12
	Gentamicin, GEN	R	16	S	0.25
	Quin.-Dalf. (Synercid), SYN	R	2	S	0.5
	Trimethoprim, TMP	R	>32	S	≤ 1
	Sulfamethoxazole, SMX	R	256	S	≤ 32
	Quin.-Dalf. (Synercid), SYN	S	1	R	2
LAB. 011	Erythromycin, ERY	R	>32	S	0.5
	Gentamicin, GEN	R	64	S	≤ 0.25
LAB. 012	Trimethoprim, TMP	R	4	S	2
LAB. 015	Quin.-Dalf. (Synercid), SYN	S		R	2
	Quin.-Dalf. (Synercid), SYN	S		R	2
LAB. 017	Trimethoprim, TMP	R	4	S	2
	Clindamycin, CLN	R	0.5	S	0.12
LAB. 018	Quin.-Dalf. (Synercid), SYN	S	20	R	2
	Quin.-Dalf. (Synercid), SYN	R	24	S	0.5
	Quin.-Dalf. (Synercid), SYN	S	19	R	2
	Trimethoprim, TMP	R	6	S	1
LAB. 021	Penicillin, PEN	S	2	R	8
LAB. 022	Clindamycin, CLN	R	$=0.5$	S	0.12
	Trimethoprim, TMP	R	$=4$	S	2
LAB. 026	Clindamycin, CLN	R	1	S	0.12
	Mupirocin, MUP	R	2	S	0.06
	Quin.-Dalf. (Synercid), SYN	R	2	S	0.5
	Quin.-Dalf. (Synercid), SYN	S	1	R	2
LAB. 029	Sulfamethoxazole, SMX	S	27mm	R	256
LAB. 030	Cefoxitin, FOX	R	16	S	4
	Clindamycin, CLN	S	≤ 0.12	R	>256
	Erythromycin, ERY	S	≤ 0.25	R	>16
	Gentamicin, GEN	S	≤ 1	R	>16
	Trimethoprim, TMP	S	≤ 2	R	>32

Lab no.	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic
LAB. 033	Clindamycin, CLN	R	0.5	S	0.12
	Trimethoprim, TMP	R	4	S	2
LAB. 034	Trimethoprim, TMP	R	4	S	2
	Sulfamethoxazole, SMX	S	<=64	R	256
LAB. 036	Clindamycin, CLN	R	1	S	0.12
	Clindamycin, CLN	R	1	S	0.12
	Trimethoprim, TMP	R	4	S	2
	Clindamycin, CLN	R	0.5	S	0.12
	Clindamycin, CLN	R	0.5	S	0.12
LAB. 037	Sulfamethoxazole, SMX	S	128	R	256
LAB. 039	Ciprofloxacin, CIP	R	2	S	0.25
	Clindamycin, CLN	R	2	S	0.12
	Ciprofloxacin, CIP	R	2	S	0.5
	Clindamycin, CLN	R	2	S	0.12
	Clindamycin, CLN	R	2	S	0.12
	Clindamycin, CLN	R	2	S	0.12
	Ciprofloxacin, CIP	R	>4	S	0.5
	Erythromycin, ERY	R	2	S	0.5
	Clindamycin, CLN	R	2	S	0.12
LAB. 040	Quin.-Dalf. (Synercid), SYN	S	22	R	2
	Sulfamethoxazole, SMX	S	19	R	256
	Quin.-Dalf. (Synercid), SYN	S	20	R	2
LAB. 041	Sulfamethoxazole, SMX	R	256	S	<=32
	Clindamycin, CLN	R	1	S	0.12
LAB. 042	Sulfamethoxazole, SMX	R	>512	S	<=32
LAB. 045	Clindamycin, CLN	R	0	S	0.12
	Chloramphenicol, CHL	R	10.6	S	8
LAB. 046	Cefoxitin, FOX	S	4	R	8
	Cefoxitin, FOX	S	4	R	16
LAB. 056	Sulfamethoxazole, SMX	R	>512	S	<=32
	Sulfamethoxazole, SMX	R	>512	S	<=32

Appendix 8c- Deviations to results *E.coli* trial

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic
LAB. 004	EURL EC 7.5	Sulfamethoxazole, SMX	R	1024	S	<=16
LAB. 006	EURL EC 7.8	Sulfamethoxazole, SMX	R	<=8	S	<=16
LAB. 011	EURL EC 7.6	Chloramphenicol, CHL	S	<=2	R	32
LAB. 015	EURL EC 7.1	Ciprofloxacin, CIP	S		R	0.12
	EURL EC 7.2	Ceftazidime, CAZ	S	27	R	1
	EURL EC 7.8	Ciprofloxacin, CIP	S		R	0.25
LAB. 016	EURL EC 7.3	Sulfamethoxazole, SMX	R	>1024	S	<=16
LAB. 019	EURL EC 7.6	Florfenicol, FFN	R	32	S	16
LAB. 022	EURL EC 7.7	Gentamicin, GEN	S	=16	R	16
LAB. 023	EURL EC 7.2	Ceftazidime, CAZ	S	0.5	R	1
LAB. 034	EURL EC 7.6	Florfenicol, FFN	R	32	S	16
LAB. 039	EURL EC 7.2	Colistin, COL	R	4	S	<=1
LAB. 040	EURL EC 7.2	Ceftazidime, CAZ	S	27	R	1
	EURL EC 7.4	Ceftazidime, CAZ	S	23	R	2
LAB. 045	EURL EC 7.1	Ciprofloxacin, CIP	S	24.3	R	0.12
	EURL EC 7.2	Ceftazidime, CAZ	S	26.3	R	1
	EURL EC 7.4	Ceftazidime, CAZ	S	24.0	R	2
	EURL EC 7.5	Ampicillin, AMP	R	11.0	S	4
	EURL EC 7.5	Nalidixic acid, NAL	R	0	S	4
	EURL EC 7.6	Nalidixic acid, NAL	R	12.9	S	4
	EURL EC 7.7	Ceftazidime, CAZ	R	10.4	S	0.125
	EURL EC 7.8	Ciprofloxacin, CIP	S	22.3	R	0.25
LAB. 046	EURL EC 7.5	Ampicillin, AMP	R	16	S	4
	EURL EC 7.6	Sulfamethoxazole, SMX	R	>1024	S	<=16
	EURL EC 7.6	Trimethoprim, TMP	R	>32	S	<=1
	EURL EC 7.8	Ciprofloxacin, CIP	S	0.06	R	0.25
	EURL EC 7.8	Sulfamethoxazole, SMX	R	>1024	S	<=16
LAB. 056	EURL EC 7.3	Sulfamethoxazole, SMX	R	>1024	S	<=16
	EURL EC 7.5	Sulfamethoxazole, SMX	R	>1024	S	<=16

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

Tel. 35 88 70 00
Fax 35 88 70 01

www.food.dtu.dk

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