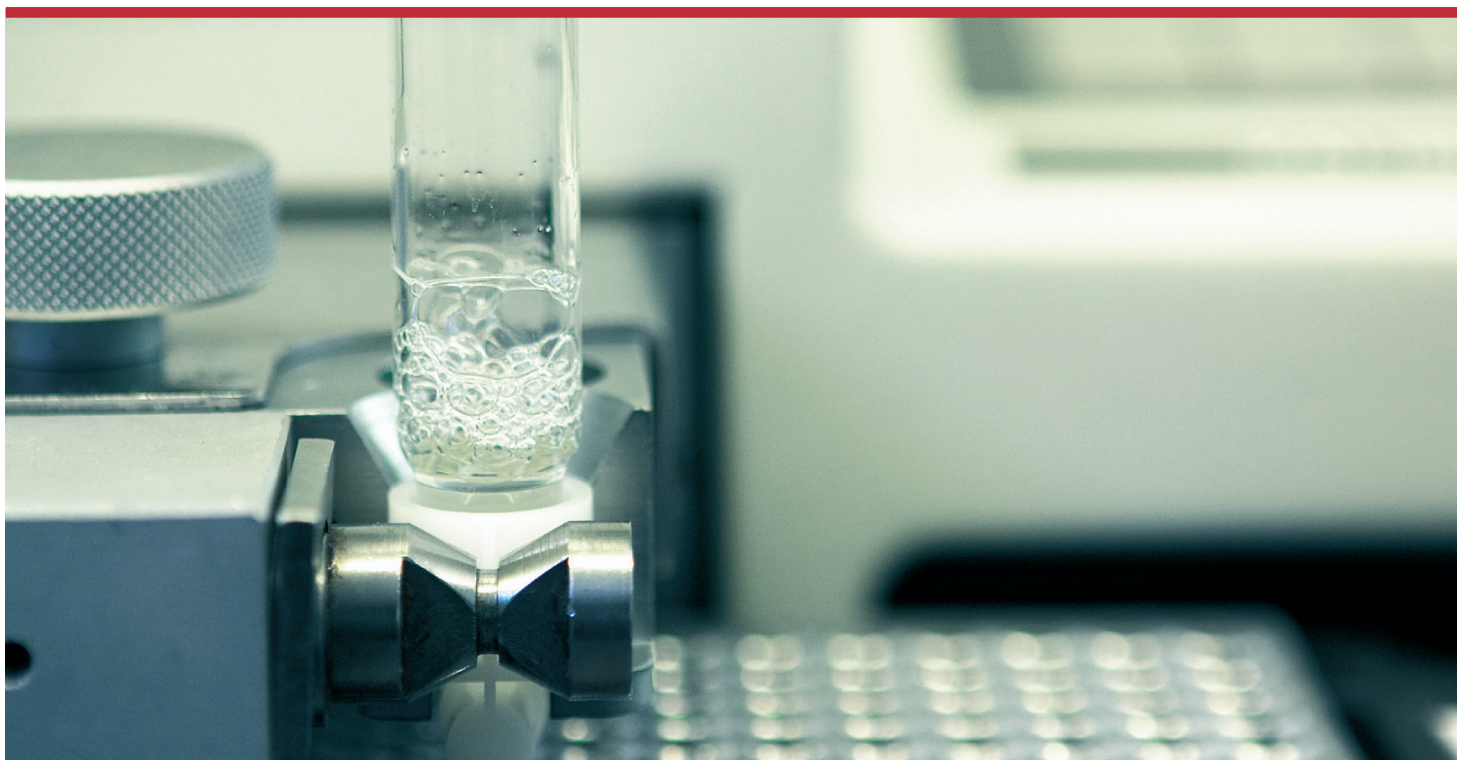


The 12th EURL-AR Proficiency Test - enterococci, staphylococci and *E. coli* 2012



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



DTU Food
National Food Institute

THE 12th EURL-AR Proficiency Test
Enterococci, Staphylococci and *Escherichia coli* 2012

1. edition, July 2013

Copyright: National Food Institute, Technical University of Denmark

Photo: Mikkel Adsbøl

ISBN: 978-87-92763-79-2

The report is available at
www.food.dtu.dk

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



Index

1. Introduction.....	3
2. Materials and Methods.....	4
2.1 Participants in EQAS 2012	4
2.2 Strains	5
2.3 Antimicrobials	5
2.4 Distribution	5
2.5 Procedure.....	5
3. Results.....	7
3.1 EQAS 2012 compared to previous EQAS iterations	7
3.2 Deviations from expected results divided by species tested and AST method used	7
3.3 Deviations from expected results analyzed by participating laboratory	13
3.4 Deviations from expected results for the reference strains	17
4. Discussion	20
4.1 General overview	20
4.2 Enterococci trial.....	20
4.3 Staphylococci trial	22
4.4 <i>Escherichia coli</i> trial	22
5. Conclusions	23
Appendix 1. Pre-notification EURL-AR EQAS 2012 – Enterococci, staphylococci and <i>E. coli</i>	
Appendix 2. List of participants	
Appendix 3a. Expected results for the enterococci trial	
Appendix 3b. Expected results for the staphylococci trial	
Appendix 3c. Expected results for the <i>Escherichia coli</i> trial	
Appendix 4. Protocol EQAS 2012 – Enterococci, staphylococci and <i>E. coli</i>	
Appendix 5a. Breakpoints used routinely in disk diffusion - Enterococci	
Appendix 5b. Breakpoints used routinely in disk diffusion - Staphylococci	
Appendix 5c. Breakpoints used routinely in disk diffusion – <i>Escherichia coli</i>	
Appendix 6. Acceptable quality control ranges for the ATCC reference strains	
Appendix 6a. Test results from QC strain <i>E. faecalis</i> ATCC 29212	
Appendix 6b. Test results from QC strain <i>S. aureus</i> ATCC 29213 and ATCC 25923	
Appendix 6c. Test results from QC strain <i>E. coli</i> ATCC 25922	
Appendix 7a. Summary of obtained and expected results in the enterococci trial	
Appendix 7b. Summary of obtained and expected results in the staphylococci trial	
Appendix 7c. Summary of obtained and expected results in the <i>Escherichia coli</i> trial	
Appendix 8a. Deviations from expected results in the enterococci trial	
Appendix 8b. Deviations from expected results in the staphylococci trial	
Appendix 8c. Deviations from expected results in the <i>Escherichia coli</i> trial	



1. Introduction

This report describes the results of a proficiency test defined as External Quality Assurance System (EQAS) 2012 for antimicrobial susceptibility testing (AST) of enterococci, staphylococci and *Escherichia coli*. The results discussed in this report were obtained by National Reference Laboratories for Antimicrobial Resistance (NRL-AR) in Member States (MS) and in affiliated non-Member States of the European Union.

This is the 12th EQAS organized by the National Food Institute at the Technical University of Denmark (DTU Food) since its appointment as European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) by the European Commission (EC) in 2006. 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).

This EQAS aims to: i) monitor the quality of AST results produced by 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 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 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) 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.

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

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 publicly 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.

2. Materials and Methods

2.1 Participants in EQAS 2012

In March 2012, a pre-notification to announce the EQAS 2012 on antimicrobial susceptibility testing of enterococci, staphylococci and *E. coli* was sent by e-mail to the 32 European NRLs for antimicrobial resistance designated by the MS (App. 1). Nine additional laboratories (one

from each of the following countries: Croatia, Iceland, Norway, Romania, Serbia, Spain, Switzerland, The Netherlands and Turkey) were invited to take part in the EQAS 2012 on the basis of their participation in previous EQAS iterations and/or affiliation to the EU. Participants represented all EU countries except Luxembourg (App. 2). Among the

Figure 1 European map showing the countries participating in EQAS 2012





designated NRLs, 25 submitted results for the enterococci strains, 29 submitted results for the staphylococci and 29 for the *E. coli* strains. The level of participation was similar to EQAS 2011 in which 24, 29 and 29 laboratories submitted results for enterococci, staphylococci and *E. coli*, respectively. In addition, this report includes results from one laboratory for each of the following EU-affiliated country non-MS: Croatia, Norway, Serbia, Switzerland and Turkey (Figure 1).

In total, this report includes AST results of enterococci strains submitted by 29 laboratories, and AST results of staphylococci strains submitted by 34 laboratories and *E. coli* strains submitted by 33 laboratories.

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.

Antimicrobial susceptibility testing of the EQAS strains was performed at DTU-Food by MIC determination using the Sensititre system 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. 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 are listed in Table 1.

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.

All the above-mentioned choices were made on the basis of agreements reached by NRL participants at EURL-AR workshops in previous years.

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 2012, 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.

2.5 Procedure

Participants were instructed to keep the agar stab cultures refrigerated until performance of antimicrobial susceptibility tests (App. 4). In addition, instructions for interpretation of antimicrobial susceptibility test results were provided. For interpretation of MIC determination results, cut-off values were reported in the protocol (App. 4: Tables 1, 2 and



Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2012 component

Enterococci trial	Staphylococci trial*	<i>Escherichia coli</i> trial
Ampicillin [†]	Cefoxitin	Ampicillin [†]
Chloramphenicol [†]	Chloramphenicol	Cefotaxime [†]
Ciprofloxacin	Ciprofloxacin	Cefoxitin
Erythromycin [†]	Erythromycin	Ceftazidime
Gentamicin [†]	Florfenicol	Ceftiofur
Linezolid [†]	Gentamicin	Chloramphenicol [†]
Streptomycin [†]	Penicillin	Ciprofloxacin [†]
Quinupristin-dalfopristin [†]	Streptomycin	Florfenicol
Tetracycline [†]	Sulphonamides	Gentamicin [†]
Vancomycin [†]	Tetracycline	Nalidixic acid [†]
	Trimethoprim	Streptomycin [†]
		Sulphonamides [†]
		Tetracycline [†]
		Trimethoprim [†]

[†]Antimicrobials recommended by EFSA for monitoring antimicrobial resistance in Europe

*No specific recommendations have been suggested by EFSA for monitoring resistance in staphylococci

3). For interpretation of disk-diffusion (DD) method results, participants were advised to use interpretive breakpoints as in their routine methods (App. 5). 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.

Of note, the terms ‘susceptible’, ‘intermediate’ and ‘resistant’ should be reserved to categorize strains in relation to the therapeutic application of antimicrobial agents, while interpretation of AST results based on epidemiological cut-off values should result in categorization of bacterial strains in ‘wild-type’ or ‘non-wild-type’. However, due to different AST methods used by the participants and to simplify the interpretation of results, we will use the terms susceptible and resistant throughout this report even in the cases in which we refer 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 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-S22 (2012) (App. 6).

The website was inaccessible from the 10th of September to the 8th of October due to issues related to an upgrading. The database was finally closed and evaluations were made available to participants on the 8th of October 2012.

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

3.1 EQAS 2012 compared to previous EQAS iterations

In EQAS 2012, the overall percentages of deviations from expected results were 4.6 %, 2.0 % and 1.6 % for enterococci, staphylococci and *E. coli*, respectively (Figure 2). These percentages were slightly lower for enterococci, the staphylococci and *E. coli* trials when compared to the ones observed in 2011. The internal control strains (ENT-6.2, ST-6.8 and EC-6.4) followed the same decreasing pattern with 5.0 %, 1.4 % and 0.5 % deviating from the expected values. (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).

3.2 Deviations from expected results divided by species tested and AST method used

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 giving results corresponding to the minimum concentration of an antimicrobial which inhibits growth 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.

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 line.

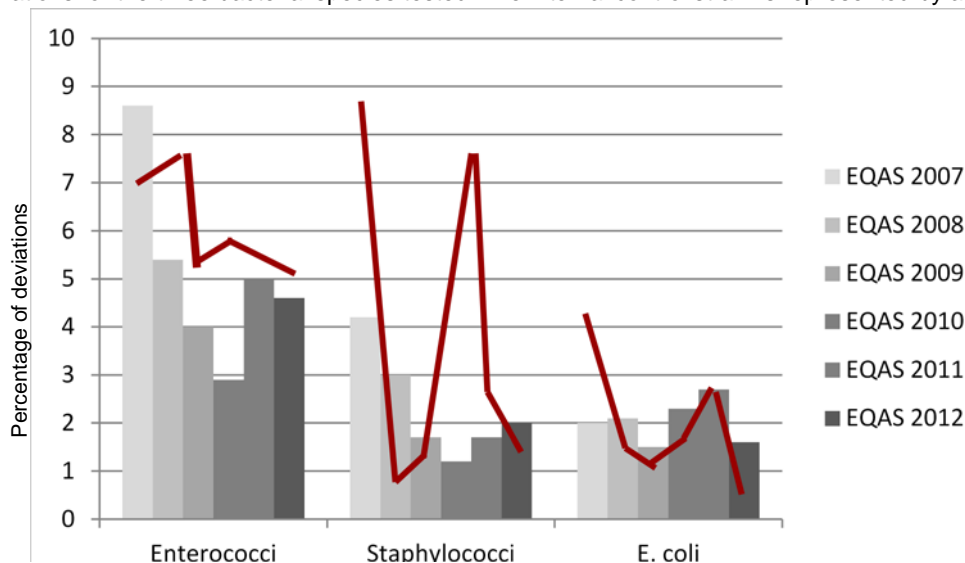


Table 2. Total number of antimicrobial susceptibility tests (AST) performed for each EQAS 2012 strain and percentage (%) of correct results

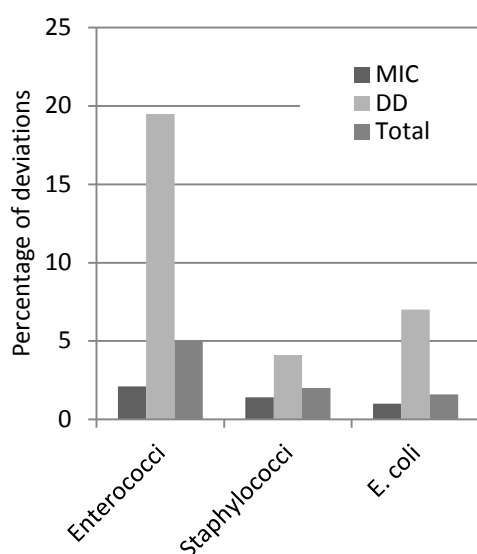
Strain*	No. AST	No correct	% correct	Strain*	No. AST	No correct	% correct	Strain*	No. AST	No correct	% correct
ENT-6.1	259	243	93,8%	ST-6.1	287	283	98.6%	EC 6.1	383	375	97.9%
ENT-6.2	259	246	95,0%	ST-6.2	319	305	95.6%	EC 6.2	383	371	96.9%
ENT-6.3	247	236	95,5%	ST-6.3	319	309	96.9%	EC 6.3	383	379	99.0%
ENT-6.4	245	232	94,7%	ST-6.4	318	315	99.1%	EC 6.4	383	381	99.5%
ENT-6.5	245	233	95,1%	ST-6.5	320	316	98.8%	EC 6.5	383	380	99.2%
ENT-6.6	245	238	97,1%	ST-6.6	320	316	98.8%	EC 6.6	383	376	98.2%
ENT-6.7	245	234	95,5%	ST-6.7	319	313	98.1%	EC 6.7	350	345	98.6%
ENT-6.8	244	234	95,9%	ST-6.8	287	283	98.6%	EC 6.8	384	376	97.9%

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

Higher percentages of deviations from expected results were obtained by performing AST by DD methods as compared to MIC determinations (Figure 3), as observed in previous EQAS iterations. Indeed, the percentage of deviations from expected results was 10 times higher for results obtained by DD compared to MIC in the enterococci trial.

In EQAS 2012, 25, 26 and 29 participants

Figure 3 EQAS 2012: results deviating from the expected interpretation subdivided by tested species and antimicrobial susceptibility test method used.



performed AST by MIC determination for enterococci, staphylococci and *E. coli*, respectively, and four, eight and four participants performed AST by DD for enterococci, staphylococci and *E. coli*, respectively.

Overall, the percentage of results in agreement with the expected values ranged from a minimum of 93.8 % (strain ENT 6.1) to a maximum of 99.5 % (strains EC 6.4), as shown in Table 2. The *E. coli* trial resulted in the highest percentages of results in agreement with the expected values.

Detailed analysis of the results obtained for each species and strain tested in EQAS 2012 are reported in the following paragraphs.

3.2.1 Enterococci trial

As mentioned in the introduction, the EURL-AR network established that data should be individually 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.

No results were omitted from the enterococci trial, as the combination with lowest percentage of correct results ENT-6.1-erythromycin resulted in a percentage of 76 % correct results and was therefore included in the report.

Analysis of results deviating from expected

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

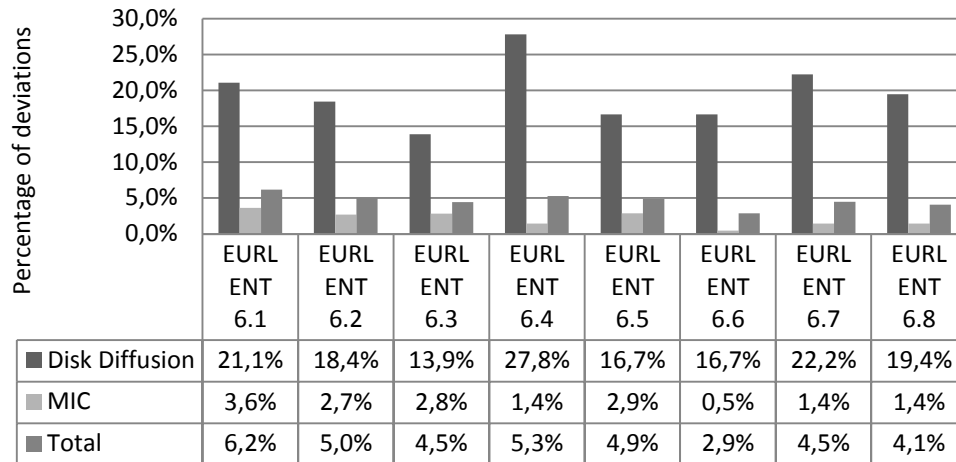
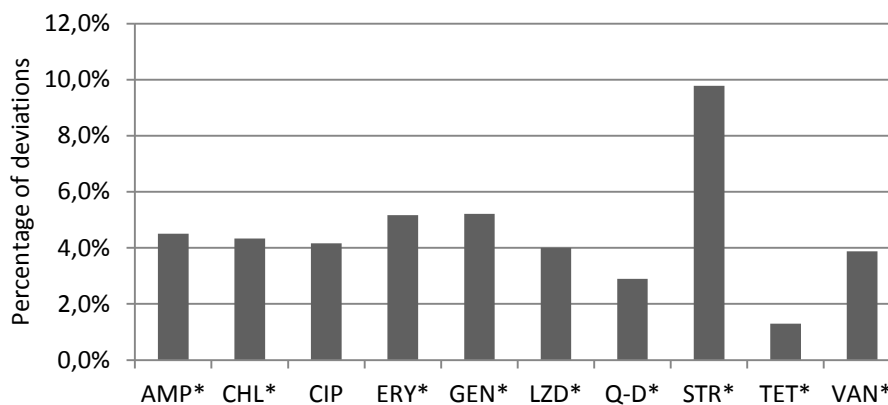


Figure 5. Enterococci trial: results deviating from expected interpretation according to the tested antimicrobials.



*Antimicrobials recommended by EFSA for antimicrobial resistance monitoring within EU

interpretation subdivided by strain showed that percentage of deviations from expected results ranged from 2.9 % (ENT 6.6) to 6.2 % (ENT 6.1) (Figure 4). The lowest percentage of deviation (2.9 %) was observed for ENT 6.6 (Figure 4). Laboratories performing AST by DD reported results highly deviating from the expected categories ranging from 13.9 % (for ENT-6.3) to 27.8 % (for ENT-6.4), as shown in Figure 4. Out of 29 laboratories participating in the enterococci trial, only four 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 streptomycin (9.8 %), gentamicin and erythromycin (5.2 % for both) (Figure 5). Of note, streptomycin, gentamicin and erythromycin are among the EFSA-recommended antimicrobials (Table 1). An overview of obtained and expected results is reported in Appendix 7a.

3.2.2 Staphylococci trial

Analysis of the different strain/antimicrobial combinations showed that ST-6.1/ciprofloxacin



and ST6.8/ciprofloxacin were categorized in agreement with the expected category by only 57.1% and 34.5% of the participants, respectively. According to the decision established by the EURL-AR network, further analysis was performed to examine the reason of these unsatisfactory results.

The expected MIC of ST 6.1 was 1 µg/ml, which results in categorization of the strain as susceptible. However, this value is one-step dilution just below the cut-off value (please refer to protocol, App. 4). Participants obtaining a MIC of 2 µg/ml, which is acceptable as it is within one-fold dilution difference from the expected value, categorized the strain as resistant and this was evaluated as an error. Fourteen participants obtained an MIC of 0.5-1 and classified the strain as susceptible, while thirteen participants obtained an MIC of >1 to 4 µg/ml and classified it incorrectly as resistant. Among the seven participants performing DD, two erroneously categorized the strain as resistant. All these results have been subtracted from the main analysis reported in this evaluation report since they cannot be representative of the quality of performance of the different participants in AST.

Regarding ST6.8/ciprofloxacin, the results were analyzed in a similar way. The expected MIC for this strain/antimicrobial combination was 2 µg/ml and the strain was expected to be classified as resistant. However, this value is one-step dilution above the cut-off value (please refer to protocol, App. 4) and participants obtaining an MIC of 1 µg/ml, which is acceptable as it is within one-fold dilution difference from the expected value, categorized the strain as susceptible and this was evaluated as an error. Thirteen participants obtained an MIC of 0.25-1 µg/ml and classified the strain incorrectly as susceptible, while only nine participants obtained an MIC of >1 to 4 µg/ml and classified it correctly as resistant. Among the seven participants performing DD, six erroneously categorized the strain as

susceptible and only one assigned it correctly as resistant.

All the results from both of these strain/antimicrobial combinations (ST 6.1/ciprofloxacin and ST6.8/ciprofloxacin) have been subtracted from the main analysis reported in this evaluation report.

Analysis of results deviating from expected interpretation subdivided by strain showed that percentage of deviations from expected results ranged from 0,9 % to 4,4 % (Figure 6). The highest percentage (4.4 %) of disagreement with expected results was obtained for ST-6.2 (Figure 6). Percentage of disagreement with expected results was 0.9 % for strain ST 6.4 (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, eight 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 sulfamethoxazole (6.4 %), streptomycin (4.8 %) and trimethoprim (4.7 %) (Figure 7). Gentamicin resulted in a deviation percentage of 2.7 % and tetracycline 1.1 % while the remaining antimicrobials lead to less than 1.0 % of the results deviating from the expected (Figure 7).

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

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

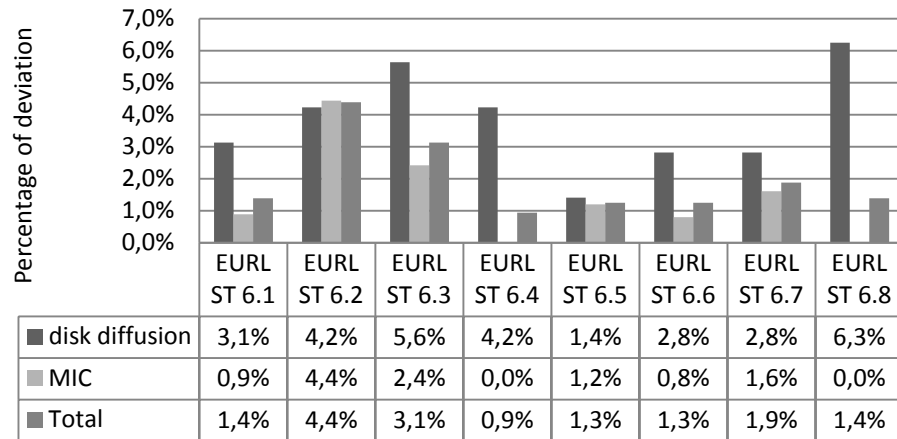
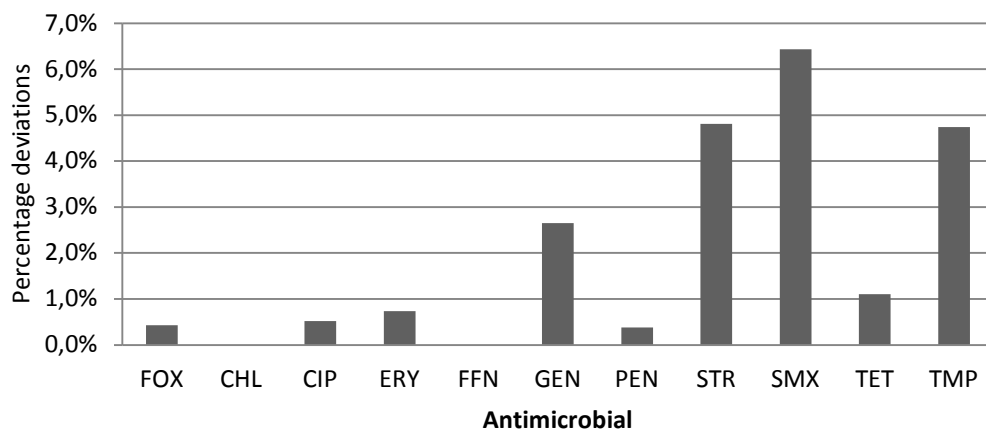


Figure 7. Staphylococci trial: results deviating from the expected interpretation according to tested antimicrobial.



Methicillin-resistant strains

Strains ST6.1, ST6.6, ST6.7 and ST6.8 were methicillin-resistant. Among 34 participants testing staphylococci strains, one (lab # 57) did not report results concerning methicillin resistance.

One participant (lab #4) failed in detecting methicillin resistance in strain ST6.1.

All remaining results were correct.

3.2.3 *Escherichia coli* trial

Analysis of the different strain/antimicrobial combinations showed that EC6.7/streptomycin was categorized in agreement with the expected category by only 70 % of the participants. According to the decision established by the EURL-AR network, further analysis was performed to examine the reason of this unsatisfactory result.

The expected MIC was 16 µg/ml, which in categorized the strain as resistant. However,

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

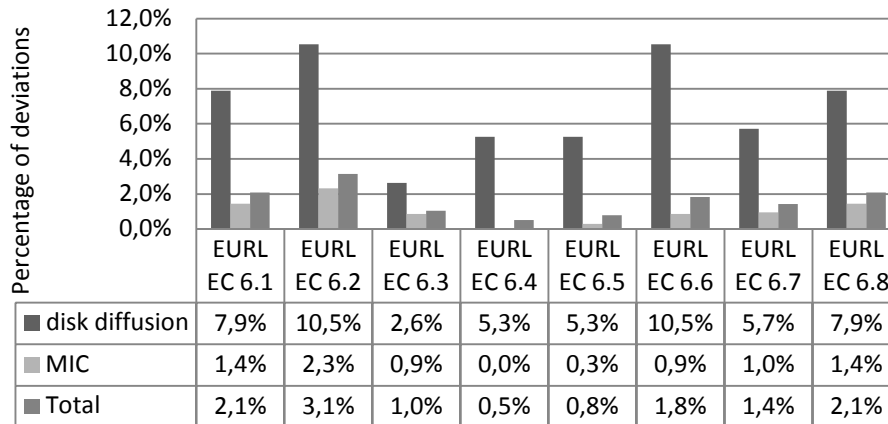
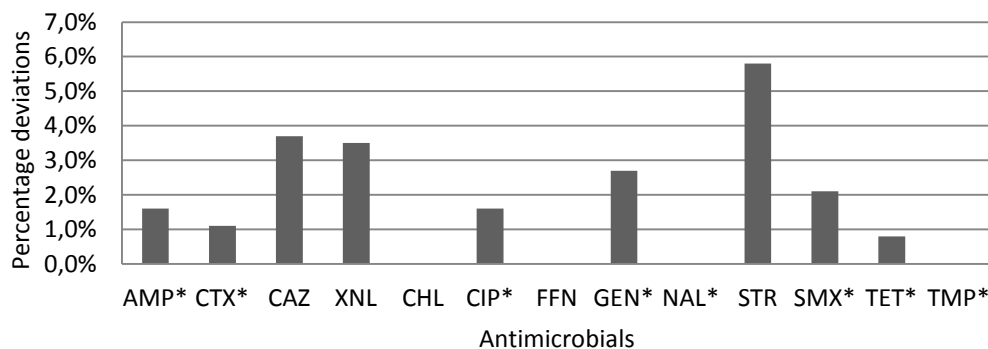


Figure 9. *Escherichia coli* trial: results deviating from expected interpretation according to the tested antimicrobial



*Antimicrobials recommended by EFSA for antimicrobial resistance monitoring within EU

this value is one-step dilution above of the cut-off value (please refer to protocol, App. 4) and participants obtaining a MIC of 8 µg/ml, which is acceptable as it is within one-fold dilution difference from the expected value, categorized the strain as susceptible and this was evaluated as an error. Six participants obtained a MIC of 8 µg/ml and two participants obtained a MIC of ≤16 µg/ml, fourteen participants obtained an MIC of 16 µg/ml (one of these classified as susceptible). The isolate exhibits resistance (borderline) to this drug. From the three participating laboratories performing DD, one erroneously categorized the strain as susceptible. All these results have been subtracted from the main analysis reported.

Analysis of results deviating from expected interpretation subdivided by strain showed that percentage of deviations from all expected results ranged from 0.5 % to 3,1 % (Figure 8). The highest percentage (3.1 %) of disagreement with expected results was obtained for EC6.2 (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 2.6 %- 10.5 % which corresponds to three to 18 times higher percentages of deviations in AST performed by DD compared to MIC (Figure 8). Out of 33 laboratories participating in the *E. coli*



trial, four 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 streptomycin (5.8 %), (Figure 9). ceftazidime (3.7%) and ceftiofur (3.5%), gentamicin (2.7 %) and sulfametoxazole (2.1 %), while tests of susceptibility to the remaining antimicrobials resulted in less than 2.0 % results deviating from the expected (Figure 9). No deviations were observed for chloramphenicol, florfenicol and trimethoprim susceptibility testing (Figure 9).

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

Beta-lactamase-producing *E. coli*

As described in the EQAS-protocol, MIC values and related interpretations for cefotaxime, ceftazidime and ceftiofur should be reported as found according to EUCAST expert rules. Cefotaxime susceptibility testing was performed 98,9 % correctly by the participants who tested this antimicrobial, while 3.5 % and 3.7 % of results were in disagreement with the expected values for ceftiofur and ceftazidime, respectively.

Confirmation of beta-lactamase production is a mandatory component of this EQAS. All *E. coli* strains resistant to cefotaxime, ceftazidime and/or ceftiofur should undergo confirmatory tests for beta-lactamase production. Participant # 57 did not perform this component.

EC6.4 and EC6.7 were extended-spectrum beta-lactamase (ESBL) producers and EC6.1 and EC6.8 were AmpC-producers.

Deviations from expected results were obtained as follows.

Two participants (lab #54 and lab #57) did not identify EC6.4 and two participants (lab #39 and

#54) and did not identify EC6.7 as ESBL producers as they did not obtain signs of synergy (please refer to protocol, App.4) by testing cefotaxime and cefotaxime+clavulanic acid.

Participants #39, #54 and #57 did not identify both EC6.1 and EC6.8 as AmpC producers. Overall, these participants performed correct procedures except for the fact that the participant # 57 did not test for ceftiofur resistance and labs #39 and #54 did not classify the strain as AmpC despite finding they were resistant to ceftiofur and not showing synergy and finding the resistance to cefotaxime and ceftazidime in the AST panel whereas lab #57 did not perform confirmation of either ESBL or AmpC production.

One of the participants (lab # 22) identified both EC6.1 and EC6.8 as AmpC producer and ESBL producers since they observed synergy in the test with ceftazidime and cefotaxime+clavulanic acid, for both strains and for cefotaxime and clavulanic acid for strain EC6.8 since an increase of the inhibition zone diameter ≥ 5 mm was observed. Furthermore they observed a reduced diameter zone for ceftiofur and therefore classified the strains also as AmpC producers, which is correct.

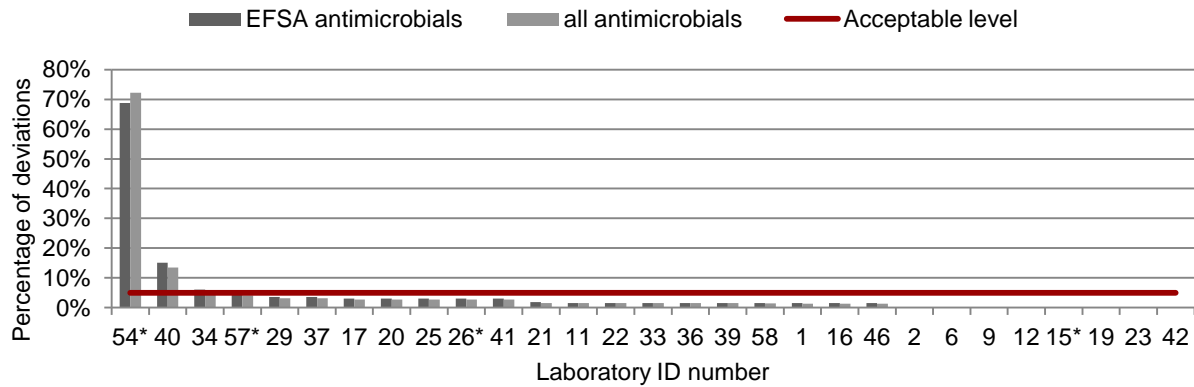
Finally, one participant (lab # 39) classified EC-6.3 erroneously as ESBL producer. In this case, participant # 39 categorized the strain as cefotaxime and ceftazidime resistant, performed confirmatory test and found synergy by testing cefotaxime and cefotaxime+clavulanic acid.

3.3 Deviations from expected results analyzed by participating laboratory

3.3.1 Enterococci trial

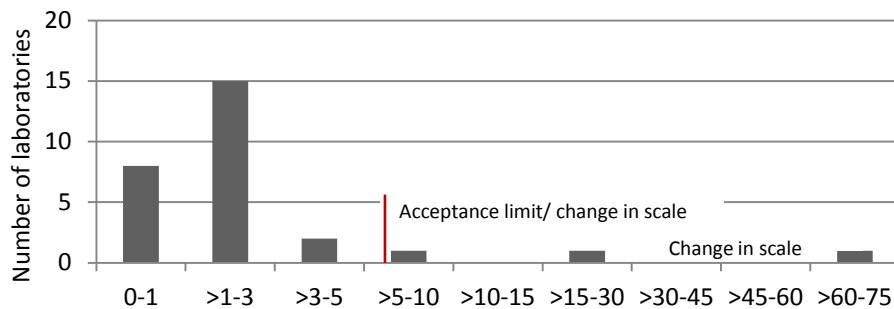
Analysis of laboratory performance of AST restricted to EFSA-recommended antimicrobials showed that three out of 29 participants

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 including EFSA-recommended antimicrobials only.



*Laboratories performing AST by disk diffusion

Figure 11. Number of laboratories categorized according to the percentage of deviations from expected results obtained by testing enterococci strains for susceptibility to EFSA-recommended antimicrobials.



obtained a percentage of deviations from expected results higher than 5 % (Figure 10). Also by including all antimicrobials tested, the same participants obtained a percentage of deviations from expected results higher than 5 %.

Participant #54 obtained the largest number of deviations (72 % when considering all antimicrobials and 68.8% when considering the EFSA antimicrobials). These deviations from expected results were obtained by testing all reported antimicrobials and were all due to reporting as resistant strain/antimicrobial combinations which should have considered as susceptible. This was probably due to the DD

method used, which lead to reduced diameter zones observed.

Participant # 40 obtained 13.5% deviations from expected results mainly in testing gentamicin, quinupristin-dalfopristin and streptomycin (susceptible strains classified as resistant).

Participant #34 reported 4 deviations including two for erythromycin (susceptible strains reported as resistant) and two for streptomycin (deviations observed in both directions regarding two strains).

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

In summary, 26 of 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 three participants who did not meet the acceptance level, one was considered an outlier (lab # 54) (Figure 11).

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

3.3.2 Staphylococci trial

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 12). One out of seven participants performing AST by DD obtained more than 5.0 % deviations from expected results (Figure 12).

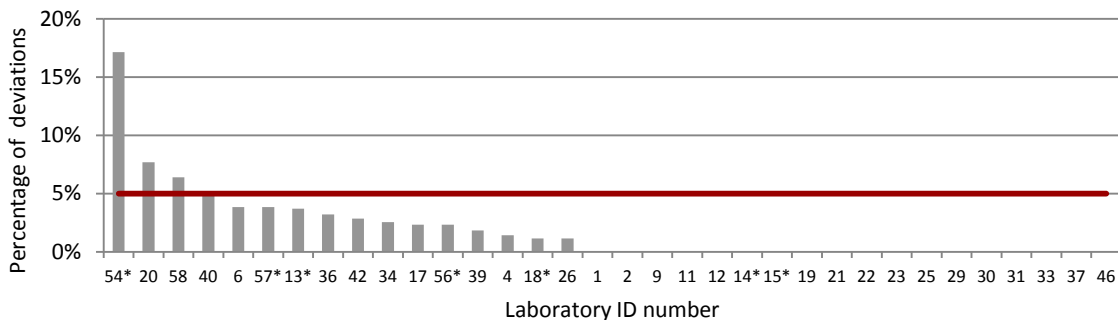
Participant #54 obtained deviations from

expected results mainly in testing gentamicin, streptomycin and ciprofloxacin (susceptible strains classified as resistant).

Participant #20 obtained deviations from expected results mainly in testing sulfamethoxazole, and trimethoprim (susceptible strains classified as resistant). Participant #58 obtained deviations from expected results for sulfamethoxazole, tetracycline and trimethoprim and both classification of susceptible strains as resistant and vice versa were observed (App. 8b).

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 12). One outlier (# 54) was identified among the three participants who did not meet the acceptance level (Figure 13). However, the

Figure 12. 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 disc diffusion

Figure 13. Number of laboratories categorized according to the percentage of deviations from expected results obtained by testing staphylococci strains for antimicrobial susceptibility

participant (# 57) who did not report any information concerning methicillin resistance will be contacted by the EURL-AR to agree on possible supportive actions.

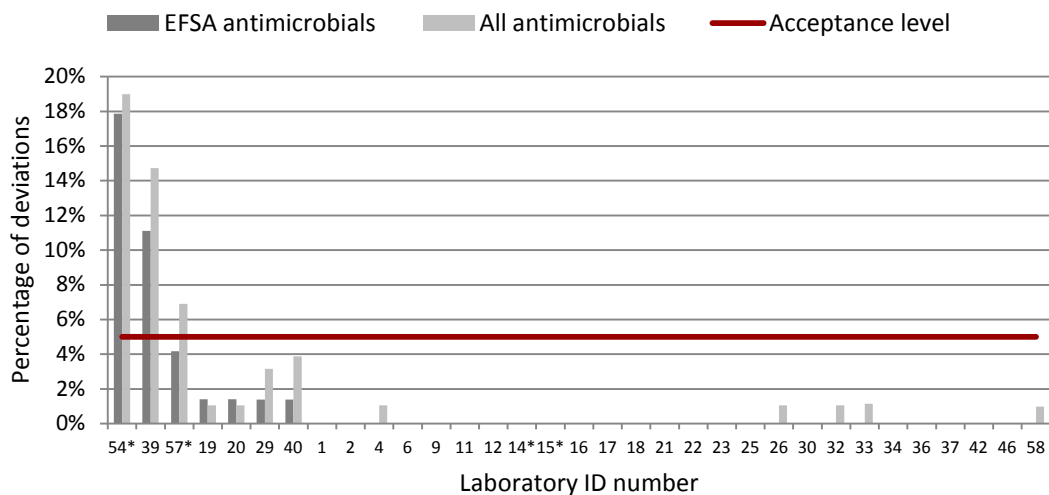
Deviations from expected results obtained by each participant in the staphylococci trial are reported in Appendix 8b.

3.3.3 *Escherichia coli* trial

Analysis of laboratory performance of AST restricted to EFSA-recommended antimicrobials showed that two out of 33 participants obtained a percentage of deviations from expected results higher than 5 % (Figure 14). By including all antimicrobials tested, three out of 33 participants obtained a percentage of deviations from expected results higher than 5 % (Figure 15).

Participant # 54 obtained deviations from

Figure 14 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



*Laboratories performing AST by disc diffusion

Figure 15. Number of laboratories categorized according to the percentage of deviations from expected results obtained by testing *Escherichia coli* strains for susceptibility to EFSA-recommended antimicrobials



expected results mainly in testing gentamicin, tetracycline and ampicillin (susceptible strains classified as resistant). Participant # 39 obtained deviations from expected results for different antimicrobials including cefotaxime, ampicillin, and sulfamethoxazole (susceptible strains classified as resistant). In the remaining case, deviations from expected results were observed for different antimicrobials and were represented both by classification of susceptible strains as resistant and vice-versa (App. 8c). In summary, 31 of 33 participants in the *E. coli* trial achieved the acceptance level by having less than 5 % of results deviating from the expected values when taking in account the EFSA recommended antimicrobials (Figure 14). Among the two participants who did not meet the acceptance level, both were considered outliers (Figure 15). One additional participant (# 57) reported results showing a percentage of deviations of 6.9 % when considering all antimicrobials, but had less than 5.0 % when considering only the EFSA recommended antimicrobials. Deviations from expected results obtained by each participant in the *E. coli* trial are reported in Appendix 8c.

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. 6).

3.4.1 *Enterococcus faecalis* ATCC 29212

Twenty-four participants performed AST of *E. faecalis* ATCC 29212 by MIC determination. No results were found outside of range. In summary, out of 188 tests performed all were correct (Table 3).

As CLSI has not published a QC range for *E. faecalis* ATCC 29212 using DD, the two laboratories (# 26 and # 57) that have entered data for the reference strain performing this method for AST could not be evaluated.

3.4.2a *Staphylococcus aureus* ATCC 25923

Six participants performed AST of *S. aureus* ATCC 25923 by DD (6). Two results outside of the QC range were obtained for ceftiofur, and tetracycline susceptibility tests and one result outside the QC range was obtained for gentamicin and penicillin susceptibility tests (Table 4). Per laboratory, the results show that one participant had four deviations (# 14) by measuring slightly larger diameter of the inhibition zones for ceftiofur, gentamicin, penicillin and tetracycline than the QC range. One laboratory (# 15) had a measurement of the inhibition zone for tetracycline above range and another (# 56) had a measurement of the inhibition zone for ceftiofur above range. In summary, out of 53 tests performed overall, 47 were correct.

One participant (# 4) performed AST of *S. aureus* ATCC 25923 by ROSCO method, and the results were not included in Table 4 because the quality control values were different from the ones used for DD. This participant (lab #4) obtained one results outside the QC range for ceftiofur.

3.4.2b *Staphylococcus aureus* ATCC 29213

Twenty-five participants performed AST of *S. aureus* ATCC 29213 by MIC determination (Table 5). No deviation was obtained. In summary, out of 207 tests performed, all were correct.



3.4.3 *Escherichia coli* ATCC 25922

Three participants performed AST of *E. coli* ATCC 25922 by DD. One of these laboratories had all results correct # 15, whereas the other laboratories # 14 and # 57, had two and four results outside of the QC range, respectively. The six deviations observed were one deviation each on the results for cefotaxime, ceftiofur, chloramphenicol, ciprofloxacin, sulfisoxazole and tetracycline (Table 6). In summary, out of 31 tests performed overall, 25 were correct. Twenty-eight participants performed AST of *E. coli* ATCC 25922 by MIC determination. Sixteen deviations were observed including five

deviations for the results for ciprofloxacin, three for gentamicin, and one deviation each for imipenem, nalidixic acid, sulfisoxazole and tetracycline (Table 7). Regarding the distribution per laboratories one laboratory (lab #40) reported seven results outside the range for the QC strain. This was probably due to a switch of the QC strain. Then, three laboratories (lab #4, and #36) reported two results out of range, three laboratories (labs #11, #23 and #33) reported one result out of range for this QC strain and the remaining did not have any deviations on the QC strain. In summary, out of 312 tests performed, 296 were correct.

Table 3. Antimicrobial susceptibility testing of *Enterococcus faecalis* ATCC 29212 by MIC determination: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

<i>Enterococcus faecalis</i> ATCC 29212				
Antimicrobial	QC range (MIC)	Min. value	Max. value	No. of deviations from expected result/ Total no. of tests
Ampicillin	0.5 - 2	0.5	2	0/19
Chloramphenicol	4-16	4	16	0/24
Ciprofloxacin	0.25 - 2	0.25	1	0/19
Erythromycin	1 - 4	1	4	0/24
Gentamicin	4 - 16	4	<=128	0/22
Linezolid	1 - 4	1	2	0/20
Quinu-dalfo-pristin	2 - 8	2	8	0/12
Streptomycin	n.a.*	16	1000	0/18
Tetracycline	8 - 32	8	32	0/24
Vancomycin	1 - 4	1	4	0/24

*n.a., not applicable

Table 4. Antimicrobial susceptibility testing of *Staphylococcus aureus* ATCC 25923 by disk diffusion: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

<i>Staphylococcus aureus</i> ATCC 25923				
Antimicrobial	QC range (DD)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Cefoxitin	23 – 29	26	32	2/6
Chloramphenicol	19 – 26	23	26	0/5
Ciprofloxacin	22 – 30	24	29	0/5
Erythromycin	22 – 30	25	30	0/6
Florfenicol	n. a.	24	27	0/3
Gentamicin	19 – 27	22	30	1/6
Penicillin	26 – 37	32	40	1/6
Streptomycin	14 – 22	15	22	0/3
Sulfisoxazole	24 – 34	24	30	0/4
Tetracycline	24 – 30	27	31	2/6
Trimethoprim	19 - 26	20	26	0/3

Table 5. Antimicrobial susceptibility testing of *Staphylococcus aureus* ATCC 29213 by MIC determination: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

<i>Staphylococcus aureus</i> ATCC 29213				
Antimicrobial	QC range (MIC)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Cefoxitin	1 - 4	2	4	0/19
Chloramphenicol	2 - 16	2	16	0/24
Ciprofloxacin	0.12 - 0.5	0.12	1	0/24
Erythromycin	0.25 - 1	0.25	1	0/25
Florfenicol	2 - 8	4	8	0/6
Gentamicin	0.12 - 1	<=0.25	<=2	0/24
Penicillin	0.25 - 2	0.25	2	0/25
Sulfisoxazole	32 - 128	<=64	128	0/12
Tetracycline	0.12 - 1	<=0.5	<=2	0/25
Trimethoprim	1 - 4	1	4	0/23

Table 6. Antimicrobial susceptibility testing of *Escherichia coli* ATCC 25922 by disk diffusion: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

<i>Escherichia coli</i> ATCC 25922				
Antimicrobial	QC range (DD)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Ampicillin	16 - 22	21	21	0/1
Cefotaxime	29 - 35	32	36	1/3
Cefoxitin	23 - 29	28	28	0/3
Ceftazidime	25 - 32	26	32	0/3
Ceftiofur	26 - 31	23	31	1/3
Chloramphenicol	21 - 27	26	32	1/2
Ciprofloxacin	30 - 40	25	37	1/2
Gentamicin	19 - 26	23	26	0/3
Imipenem	26 - 32	nt	nt	0/0
Nalidixic acid	22 - 28	25	28	0/3
Streptomycin	12 - 20	19	20	0/2
Sulfisoxazole	15 - 23	24	24	1/1
Tetracycline	18 - 25	23	26	1/3
Trimethoprim	21 - 28	25	27	0/2

Table 7. Antimicrobial susceptibility testing of *Escherichia coli* ATCC 25922 by MIC determination: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

<i>Escherichia coli</i> ATCC 25922				
Antimicrobial	QC range (MIC)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Ampicillin	2 - 8	1	8	1/28
Cefotaxime	0.03 - 0.125	≤0,06	0,25	1/28
Cefoxitin	2 - 8	≤4	8	0/9
Ceftazidime	0.06 - 0.5	≤0,25	1	1/25
Ceftiofur	0.25 - 1	0,25	1	0/4
Chloramphenicol	2 - 8	2	16	1/28
Ciprofloxacin	0.004 - 0.016	≤0,008	0,06	5/28
Gentamicin	0.25 - 1	0,5	4	3/28
Imipenem	0.06 - 0.25	0,12	0,5	1/5
Nalidixic acid	1 - 4	2	16	1/28
Streptomycin	4 - 16	≤4	16	0/27
Sulfisoxazole	8 - 32	≤8	256	1/19
Tetracycline	0.5 - 2	≤1	8	1/28
Trimethoprim	0.5 - 2	≤0,5	2	0/27

4. Discussion

4.1 General overview

In the overall analysis of results, it could be observed that the levels of deviations from the expected results were comparable to last year for AST of staphylococci and enterococci, while there was a decrease in deviations from the expected results for AST of *E. coli* (Figure 2). The percentage of deviations from the expected results for AST of the internal control strains followed a trend towards a decrease in all species tested (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. Results from five laboratories from EU-affiliated countries non-MS were included in this report, which is a larger number than compared to reports issued in previous years.

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. Of note, enterococci and *E. coli* AST performed by MIC determination resulted in significantly higher percentages of correct results compared to results obtained by DD over the different EQAS iterations.

4.2 Enterococci trial

The percentages of results deviating from the expected interpretations varied from circa 3 % to 6 % among the different test strains (Figure 4). The relatively high percentages of deviations from expected results were mainly generated by participants performing AST by DD (Figure



4).

Similar problems were observed last year in EQAS 2011 and previously. Enterococci appear to be quite difficult to test correctly by DD, and several different reasons may be found. Unsatisfactory performance may be due to factors related to the strains as certain enterococci strains may require incubation times longer than overnight incubation. In addition, inoculum size and density may also represent a source of errors in AST performance. The outcome of AST by DD is also influenced by factors related to the agar media like humidity, pH and volume. Finally, there may be factors related to the antimicrobial-containing disks like expiry date, humidity and concentration used. In addition, CLSI does not provide a QC range for the testing of the reference strain by DD, which does not allow to validate the data obtained by using this method, in the same way as it can be performed using MIC testing.

Susceptibility tests to streptomycin, erythromycin and gentamicin resulted in the highest percentages of results deviating from the expected interpretations (Figure 5).

For streptomycin the incorrect classification was represented both by susceptible strains reported as resistant and vice versa, which was reported by various participants including #34, #40, #41, #54 and #57 (App. 8a). Regarding gentamicin, the incorrect classification was represented by susceptible strains reported as resistant, which was mainly obtained by participants #40 and #54 (App. 8a). For erythromycin, as mentioned before, several laboratories have classified strain 6.1 as resistant to erythromycin and further deviations in other strains were only obtained by two participants (#34 and #54) which classified some of the remaining susceptible strains as resistant. Of note, erythromycin, gentamicin and streptomycin are among the EFSA-recommended antimicrobials, which implies that

it is important that each participant who submitted incorrect results takes corrective actions.

The number of participants submitting more than 5 % results deviating from the expected interpretation was three, which is three less than compared to last year (Figure 10). Of note, only one of these three participants performed testing by DD. However, there was a greater number of deviations occurring when performing DD, when compared to MIC methods.

Among the three participants who did not meet the 5.0 % acceptance threshold, one was considered an outlier with ca 72 % deviations, mainly due to susceptible strains classified as resistant for most antimicrobials. Another two participants reported ca 14 % and 6 % of results in disagreement with the expected values and was therefore considered as above the threshold but not considered as outliers (Figure 11). The three participants 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 8 (28 %), which is two laboratories less than last year.

AST of the quality control strain *E. faecalis* ATCC 29212 was excellent for the 22 participants that tested this strain by MIC determination (Table 3). In summary, out of 188 tests performed overall, all (100%) were correct. Of note, the outlier (#54) identified in the enterococci trial did report DD results for the quality control strain, which were therefore not evaluated and the other outlier (#40) reported MIC results for most antimicrobials, but not for quinupristin-dalfopristin, ampicillin and streptomycin.



4.3 Staphylococci trial

The percentages of results deviating from the expected interpretations ranged from 1 % to 4 % among the different test strains (Figure 6). The percentages of deviations from expected results generated by participants performing MIC determination and by participants performing DD were this time more differentiated, with better results being obtained by those performing MIC testing (Figure 7). The number of participants performing MIC determination increased from 25 (EQAS 2011) to 27 participants.

The overall satisfactory results obtained in the staphylococci trial show a successful implementation of the new method for AST.

Identification of methicillin-resistant strains was generally satisfactory, which demonstrated that laboratories within the EURL-AR network correctly identify MRSA. However, few improvements are necessary as participant # 57 did not report results concerning methicillin resistance, and participant # 4 reported strain ST 6.1 as methicillin susceptible.

The number of participants submitting more than 5 % results deviating from the expected interpretation was three (Figure 12), which is two more compared to last year. One outlier (#54) which obtained 17 % results deviating from expected values was identified in the staphylococci trial (Figure 13). 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 18 (53 %).

AST of the quality control strain *S. aureus* ATCC 25923 (for DD) resulted in 89 % correct tests (Table 4), and AST of the quality control strain *S. aureus* ATCC 29213 (for MIC determination) resulted in 100 % correct tests

(Table 5). Overall, this performance was quite satisfactory.

4.4 *Escherichia coli* trial

The percentages of results deviating from the expected interpretations varied from 0.5 % to 3.1 % among the different test strains (Figure 8). These relatively 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 streptomycin resulted in the highest percentages (6.8 %) of results deviating from the expected interpretations (Figure 9), and were mostly due to interpretation of susceptible strains as resistant. For ceftazidime, the incorrect classification was both represented by susceptible strains reported as resistant and vice versa, which was an issue for several participants (App. 8c). For ceftiofur, the incorrect classification was represented only by two resistant strains reported as susceptible by one participant # 29 (App. 8c). These results indicate that increased attention should be paid to correctly categorize strains according to susceptibility to critically important antimicrobials like ceftazidime and ceftiofur (3rd generation cephalosporins).

The number of participants submitting more than 5 % results deviating from the expected interpretation regarding EFSA recommended antimicrobials was three, which is lower than last year when seven participants performed outside the acceptance level (Figure 14). One out of four participants testing *E. coli* by DD obtained percentages of results deviating from the expected interpretations above the 5 % threshold for acceptable AST performance. Among the three participants who did not meet the 5 % acceptance threshold, two were considered an outliers (Figure 15). All three 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 26 (79 %).

Detection of beta-lactamases of the ESBL and AmpC-type should be further improved especially concerning identification of AmpC-type beta-lactamases. Participants did not show difficulties in correctly identifying cephalosporin resistance and a general improvement was observed compared to last year. However, there are limitations in the correct performance and interpretation of ESBL and AmpC confirmatory tests.

AST of the quality control strain *E. coli* ATCC

25922 resulted in 81 % and 95 % correct tests by DD and MIC determination, respectively (Tables 6 and 7). Overall, this performance was quite satisfactory. However, as for previous years the majority of deviations was observed for tests of ciprofloxacin and this results must be improved in future trials since ciprofloxacin is among the critically important antimicrobials as defined by the WHO. One of the participants mentioned above reporting more than 5 % incorrect results for specific antimicrobials (#39) had no deviations when testing the QC reference strain whereas the participant reporting the highest number of deviations in the *E. coli* trial (# 54) did not submit data for the reference strain testing.

5. Conclusions

The number of laboratories not performing AST within the acceptable level (i.e. > 5 % results deviating from the expected values) was relatively low. One participant was classified as an outlier both in the enterococci and in the staphylococci trial and two in the *E. coli* trial. 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 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.

Results obtained by MIC determination exhibited considerably higher level of agreement with the expected results compared to results obtained by DD for all EQAS trials. As this situation was observed also in previous EQAS iterations, the EURL-AR strongly encourages participants to perform AST by MIC

determination which seems to be more reliable and reproducible.

One participant did not provide data on methicillin resistance and the EURL-AR will also follow up on any needs regarding the implementation of the detection method.

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 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 our knowledge in antimicrobial resistance.

EURL-AR EQAS pre-notification

EQAS 2012 FOR *E. COLI*, STAPHYLOCOCCI AND ENTEROCOCCI

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. 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. Therefore, laboratories designated to be NRL-AR do not need to sign up to participate but are automatically regarded as participants. Participation is free of charge for all designated NRL-AR's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

The content of the parcel is "UN3373, Biological Substance Category B": eight *E. coli*, eight 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 2012. 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 7th of September 2012 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 2012

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

Sincerely,

Lina Cavaco

EURL-AR

Institute	Country	Ent	Staph	E.coli
National Food Institute	Denmark	x	x	x
Austrian Agency for Health and Food Safety	Austria	x	x	x
Institute of Public Health	Belgium	x	x	x
Veterinary Services	Cyprus	x	x	x
State Veterinary Institute Praha	Czech Republic	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 - LERQAP	France		x	
Agence nationale de sécurité sanitaire ANSES - Ploufragan - LERAP	France		x	x
Agence nationale de sécurité sanitaire ANSES - Lyon	France	x	x	x
Agence nationale de sécurité sanitaire ANSES - Fougères LERMVD	France	x		x
Federal Institute for Risk Assessment	Germany	x	x	x
Veterinary Laboratory of Chalkis	Greece	x	x	x
Central Agricultural Office Veterinary Diagnostic Directorate	Hungary	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
Food and Consumer Product Safety Authority (VWA)	Netherlands	x	x	x
Central Veterinary Institute of Wageningen UR	Netherlands	x	x	x
National Veterinary Research Institute	Poland	x	x	x
National Institute of Research-Development for Microbiology and Immunology "Cantacuzino"	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	x	x
Laboratorio Central de Sanidad, Animal de Algete	Spain	x	x	x
National Veterinary Institute, SVA	Sweden	x	x	x
The Veterinary Laboratory Agency	United Kingdom	x	x	x
VISAVET Health Surveillance Center, Complutense University	Spain	x	x	x
Veterinærinstituttet	Norway	x	x	x
Laboratorio Nacional de Investigação Veterinária	Portugal	x	x	x
Centro nacional de Alimentación. Agencia Española de Seguridad Alimentaria y Nutrición	Spain	x	x	x
Public Health Laboratory	Malta	x	x	x
Nacional Diagnostic and Research Veterinary Institute	Bulgaria	x	x	x
Institute for Hygiene and Veterinary Public Health	Romania	x	x	x
Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland	x	x	x
Centre for Infections Health Protection Agency	UK	x	x	x
Croatian Veterinary Institut	Croatia		X	
National Food Reference Laboratory	Turkey	x	x	x
Institute for Diagnosis and Animal Health	Romania	x	x	x
Institute of Veterinary Medicine of Serbia	Serbia	x	x	x

Designated NRL-AR by the competent authority of the Member state

Not a member state of the EU

Non- NRL-AR enrolled by the EURL

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

Strain nr	Species	Ampicillin AMP	Chloramphenicol CHL	Ciprofloxacin CIP	Erythromycin ERY	Gentamicin GEN	Linezolid LZD	Streptomycin STR	Quinopristin dalfopristin Q-D	Tetracycline TET	Vancomycin VAN
EURL ENT 6.1	<i>E. faecium</i>	<=2	8	<=0,5	2	<=16	2	<=64	4	<=1	<=1
EURL ENT 6.2	<i>E. faecium</i>	4	4	<=0,5	1	<=16	2	<=64	4	>32	>32
EURL ENT 6.3	<i>E. faecalis</i>	<=2	64	1	>32	>1024	2	>2048	na	>32	<=1
EURL ENT 6.4	<i>E. faecalis</i>	<=2	8	1	<=0,5	<=16	2	128	na	<=1	4
EURL ENT 6.5	<i>E. faecalis</i>	<=2	64	1	<=0,5	<=16	2	2048	na	>32	<=1
EURL ENT 6.6	<i>E. faecalis</i>	<=2	8	1	>32	<=16	1	>2048	na	>32	<=1
EURL ENT 6.7	<i>E. faecalis</i>	<=2	8	1	<=0,5	<=16	2	128	na	>32	<=1
EURL ENT 6.8	<i>E. faecalis</i>	<=2	8	1	>32	<=16	2	128	na	>32	<=1

Strain nr	Species	Amp	CHL	CIP	ERY	GEN	LZD	STR	Q-D	TET	VAN
EURL ENT 6.1	<i>E. faecium</i>	S	S	S	S	S	S	S	S	S	S
EURL ENT 6.2	<i>E. faecium</i>	S	S	S	S	S	S	S	S	R	R
EURL ENT 6.3	<i>E. faecalis</i>	S	R	S	R	R	S	R	na	R	S
EURL ENT 6.4	<i>E. faecalis</i>	S	S	S	S	S	S	S	na	S	S
EURL ENT 6.5	<i>E. faecalis</i>	S	R	S	S	S	S	R	na	R	S
EURL ENT 6.6	<i>E. faecalis</i>	S	S	S	R	S	S	R	na	R	S
EURL ENT 6.7	<i>E. faecalis</i>	S	S	S	S	S	S	S	na	R	S
EURL ENT 6.8	<i>E. faecalis</i>	S	S	S	R	S	S	S	na	R	S

 Resistant

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

Strain nr	Species	Chloramphenicol CHL	Ciprofloxacin CIP	Erythromycin ERY	Florfenicol FFN	Cefoxitin FOX	Gentamicin GEN	Penicillin PEN	Streptomycin STR	Sulfa methoxazole SMX	Tetracycline TET	Trimethro prim TMP	Methicillin- resistance mecA
EURL ST 6.1	<i>S. aureus</i>	8	1	>16	4	8	<=0,25	8	8	<=32	>32	1	POSITIVE
EURL ST 6.2	<i>S. aureus</i>	8	0,5	0,5	4	4	<=0,25	8	<=4	64	<=0,5	2	NEGATIVE
EURL ST 6.3	<i>S. aureus</i>	8	0,5	<=0,25	4	4	0,5	0,5	8	<=32	<=0,5	1	NEGATIVE
EURL ST 6.4	<i>S. aureus</i>	8	0,25	0,5	2	4	<=0,25	0,5	8	<=32	16	1	NEGATIVE
EURL ST 6.5	<i>S. aureus</i>	8	0,25	0,5	4	4	0,5	8	>64	<=32	32	1	NEGATIVE
EURL ST 6.6	<i>S. aureus</i>	8	0,5	0,5	4	16	<=0,25	8	>64	<=32	>32	>32	POSITIVE
EURL ST 6.7	<i>S. aureus</i>	8	0,25	0,5	4	8	0,5	8	<=4	<=32	>32	1	POSITIVE
EURL ST 6.8	<i>S. aureus</i>	8	2	0,25	4	8	>16	>16	>64	256	32	1	POSITIVE

Strain nr	Species	CHL	CIP	ERY	FFN	FOX	GEN	PEN	STR	SMX	TET	TMP	mecA
EURL ST 6.1	<i>S. aureus</i>	S	S	R	S	R	S	R	S	S	R	S	POSITIVE
EURL ST 6.2	<i>S. aureus</i>	S	S	S	S	S	S	R	S	S	S	S	NEGATIVE
EURL ST 6.3	<i>S. aureus</i>	S	S	S	S	S	S	R	S	S	S	S	NEGATIVE
EURL ST 6.4	<i>S. aureus</i>	S	S	S	S	S	S	R	S	S	R	S	NEGATIVE
EURL ST 6.5	<i>S. aureus</i>	S	S	S	S	S	S	R	R	S	R	S	NEGATIVE
EURL ST 6.6	<i>S. aureus</i>	S	S	S	S	R	S	R	R	S	R	R	POSITIVE
EURL ST 6.7	<i>S. aureus</i>	S	S	S	S	R	S	R	S	S	R	S	POSITIVE
EURL ST 6.8	<i>S. aureus</i>	S	R	S	S	R	R	R	R	R	R	S	POSITIVE

 Resistant

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

Strain nr	Species	Ampicilin Amp	Ceftazidime CAZ	Chloramphenicol CHL	Ciprofloxacin CIP	Cefotaxime CTX	Florfenicol FFN	Cefoxitine FOX	Gentamicin GEN	Nalidixic acid NAL	Sulfametoxazole SMX	Streptomycin STR	Tetracycline TET	Trimethoprim TMP	Ceftiofur XNL	CAZ/CLA	CTX/CLA	IP/PI	ESBL	AMP C	MBL
EURL EC 6.1	<i>E. coli</i>	>32	8	8	0,03	>4	4	>64	1	<=4	<=64	<=8	<=2	<=1	8	>4/16	>1/>16	<4/<1	No	Yes	No
EURL EC 6.2	<i>E. coli</i>	4	0,125	8	0,25	<=0,12	4	2	1	>64	<=64	<=8	>32	<=1	<=0,5	0,125/<=0,5	0,064/<0,25	<4/<1	No	No	No
EURL EC 6.3	<i>E. coli</i>	>32	0,125	4	<=0,015	<=0,12	4	2	<=0,5	<=4	>1024	>128	>32	>32	<=0,5	0,094/<=0,5	0,064/<0,25	<4/<1	No	No	No
EURL EC 6.4	<i>E. coli</i>	>32	2	4	<=0,015	>4	4	2	<=0,5	<=4	<=64	<=8	<=2	<=1	>8	phantom	0,064/>16 Syn	<4/<1	Yes	No	No
EURL EC 6.5	<i>E. coli</i>	2	0,06	4	4	<=0,12	4	2	1	>64	>1024	>128	<=2	<=1	<=0,5	0,064/<=0,5	0,032/<0,25	<4/<1	No	No	No
EURL EC 6.6	<i>E. coli</i>	4	0,25	8	<=0,015	<=0,12	8	2	<=0,5	<=4	<=64	<=8	<=2	<=1	<=0,5	0,125/<=0,5	0,032/<0,25	<4/<1	No	No	No
EURL EC 6.7	<i>E. coli</i>	>32	1	<=2	>4	>4	4	4	>16	>64	>1024	16	>32	>32	>8	0,094/<0,5	0,064/>16 Syn	<4/<1	Yes	No	No
EURL EC 6.8	<i>E. coli</i>	>32	8	8	0,03	>4	8	32	1	<=4	<=64	<=8	<=2	32	8	>4/>32	>1/>16	<4/<1	No	Yes	No

Strain nr	Species	Amp	CAZ	CHL	CIP	CTX	FFN	FOX	GEN	NAL	SMX	STR	TET	TMP	XNL	CAZ/CLA	CTX/CLA	IP/PI	ESBL	AMP C	MBL
EURL EC 6.1	<i>E. coli</i>	R	R	S	S	R	S	R	S	S	S	S	S	S	R	NO SYN	NO SYN	NO SYN	No	Yes	No
EURL EC 6.2	<i>E. coli</i>	S	S	S	R	S	S	S	S	R	S	S	R	S	S	NO SYN	NO SYN	NO SYN	No	No	No
EURL EC 6.3	<i>E. coli</i>	R	S	S	S	S	S	S	S	S	R	R	R	R	S	NO SYN	NO SYN	NO SYN	No	No	No
EURL EC 6.4	<i>E. coli</i>	R	R	S	S	R	S	S	S	S	S	S	S	S	R	Syn	Syn	NO SYN	Yes	No	No
EURL EC 6.5	<i>E. coli</i>	S	S	S	R	S	S	S	S	R	R	R	S	S	S	NO SYN	NO SYN	NO SYN	No	No	No
EURL EC 6.6	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	NO SYN	NO SYN	NO SYN	No	No	No
EURL EC 6.7	<i>E. coli</i>	R	R	S	R	R	S	S	R	R	R	R	R	R	R	NO SYN	SYN	NO SYN	Yes	No	No
EURL EC 6.8	<i>E. coli</i>	R	R	S	S	R	S	R	S	S	S	S	S	R	R	NO SYN	NO SYN	NO SYN	No	Yes	No

 Resistant

M00-06-001/01.12.2011

EURL-AR External Quality Assurance System (EQAS) 2012:*-Escherichia coli*, staphylococci and enterococci

Id: «Lab_no_»

«Name»

«Institute__»

«Country»

Lyngby, 6 June 2012

Dear «Name»

Please find enclosed the bacterial strains for the EURL-AR EQAS 2012. 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 and enterococci 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. 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: «Username»

Your password: «Password»

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

Results should be entered in the database no later than **7th September 2011**. 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

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012



PROTOCOL

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

1	INTRODUCTION.....	1
2	OBJECTIVES	2
3	OUTLINE OF THE EQAS 2012	2
3.1	Shipping, receipt and storage of strains	2
3.2	Suggested procedure for reconstitution of the lyophilised reference strains.....	2
3.3	Susceptibility testing.....	2
4	REPORTING OF RESULTS AND EVALUATION.....	5
5	HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE.....	6

1 INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *E. coli*, enterococci and staphylococci is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). The EC/Ent/Staph EQAS 2012 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).

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012



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.

2 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.

3 OUTLINE OF THE EC/ENT/STAPH EQAS 2012

3.1 Shipping, receipt and storage of strains

In June 2012, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight *E. coli*, eight enterococci and eight staphylococci strains from the National Food Institute, Denmark. 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) may 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.

3.2 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).

3.3 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.

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.**

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012



Participants using disk diffusion are recommended to interpret the results according to the breakpoints used routinely. 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**.

3.3.1 *E. coli*

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

Antimicrobials for <i>E. coli</i>	MIC ($\mu\text{g/mL}$) R is >
Ampicillin, AMP	8
Cefotaxime, CTX	0.25
Cefoxitin, FOX	8
Ceftazidime, CAZ	0.5
Ceftiofur, XNL	1
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.06
Florfenicol, FFN	16
Gentamicin, GEN	2
Nalidixic acid, NAL	16
Streptomycin, STR	8*
Sulfonamides, SMX	64
Tetracycline, TET	8
Trimethoprim, TMP	2

*Based on studies performed by the EURL-AR network (manuscript accepted for publication in Microbial Drug Resistance)

Important notes: *beta-lactam resistance*

Confirmatory tests for ESBL production is mandatory on all strains resistant to cefotaxime (CTX), ceftazidime (CAZ) and/or ceftiofur (XNL).

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.

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012



Confirmatory test for Metallo-beta-lactamase (MBL) production requires use of imipenem (IMI) and IMI/EDTA. Synergy is defined as a ≥ 3 twofold concentration decrease in the MIC for the combination IMI/EDTA vs. MIC for IMI alone (E-test 3 dilution steps difference, MIC IMI : IMI/EDTA ratio ≥ 8 ; CLSI M100, Table 2A; Enterobacteriaceae). The presence of synergy indicates MBL production.

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 should be verified by PCR and sequencing.

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

3.3.2 Enterococci

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

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
Erythromycin, ERY	4	4
Gentamicin, GEN	32	32
Linezolid, LZD	4	4
Streptomycin, STR	128	512
Quinupristin-dalfopristin (Synercid), SYN	4*	Not applicable
Tetracycline, TET	4	4
Vancomycin, VAN	4	4

*DANMAP 2009 (www.danmap.org)

Important notes: identity of the test strains

Please refer to the test forms for the species (*E. faecalis* or *E. faecium*) of the test strains.

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012



3.3.3 Staphylococci

Table 3: Antimicrobials recommended for AST of *Staphylococcus aureus* and interpretative breakpoints

Antimicrobials for <i>S. aureus</i>	MIC ($\mu\text{g/mL}$) R is >
Cefoxitin, FOX	4
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Erythromycin, ERY	1
Florfenicol, FFN	8
Gentamicin, GEN	2
Penicillin, PEN	0.125*
Streptomycin, STR	16
Sulfonamides, SMX	128
Tetracycline, TET	1
Trimethoprim, TMP	2

*CLSI M100 Table 2C

Important notes: *MRSA*

Some test strains may be methicillin-resistant. **Confirmation of *mecA* 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.

4 REPORTING OF RESULTS AND EVALUATION

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.**

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, 7th 2012.** After the deadline when all participants have uploaded results, you will be able to login to the database 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'.

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012



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.

For participants that have received additional strains as a retest for the 2012 Salm/Camp EQAS: Please send us the results by the document(s) 'Retest EQAS 2012, *Salmonella*' and/or 'Retest EQAS 2012, *Campylobacter*'.

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

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

Tel: +45 3588 6269

Fax: +45 3588 6341

E-mail: licav@food.dtu.dk

5 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 2012 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.

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

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012



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 *mecA*.

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.

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012**



DTU Food
National Food Institute

Enterococci, staphylococci and *Escherichia coli*

TEST FORMS

Name:
Name of laboratory:
Name of institute:
City:
Country:
E-mail:
Fax:

Comments:

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012**



DTU Food
National Food Institute

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			≤		≥
Erythromycin, ERY			≤		≥
Gentamicin, GEN			≤		≥
Linezolid, LZD			≤		≥
Streptomycin, STR			≤		≥
Quin.-Dalf. (Synercid), SYN			≤		≥
Tetracycline, TET			≤		≥
Vancomycin, VAN			≤		≥

EU Reference Laboratory for Antimicrobial Resistance External Quality Assurance System (EQAS) 2012

DTU Food
National Food Institute



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			≤		≥
Erythromycin, ERY			≤		≥
Florfenicol, FFN			≤		≥
Gentamicin, GEN			≤		≥
Penicillin, PEN			≤		≥
Streptomycin, STR			≤		≥
Sulphonamides, SMX			≤		≥
Tetracycline, TET			≤		≥
Trimethoprim, TMP			≤		≥

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012**



DTU Food
National Food Institute

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			≤		≥
Florfenicol, FFN			≤		≥
Gentamicin, GEN			≤		≥
Nalidixic acid, NAL			≤		≥
Streptomycin, STR			≤		≥
Sulphonamides, SMX			≤		≥
Tetracycline, TET			≤		≥
Trimethoprim, TMP			≤		≥

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012



TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ >	Zone diameter (mm) or MIC-value (µg/ml)	S / R
Enterococci EURL ENT. 6.X	Ampicillin AMP			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Quinupristin-Dalfopristin (Synercid), SYN			
	Tetracycline, TET			
	Vancomycin, VAN			

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	
	Erythromycin, ERY	
	Gentamicin, GEN	
	Linezolid, LZD	
	Streptomycin, STR	
	Quinupristin-Dalfopristin (Synercid), SYN	
	Tetracycline, TET	
	Vancomycin, VAN	

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012**



DTU Food
National Food Institute

TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ >	Zone diameter (mm) or MIC-value (µg/ml)	S / R
<i>S. aureus</i> EURL ST 6.x	Cefoxitin, FOX			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Methicillin resistance (MRSA)	<input type="checkbox"/> Positive	<input type="checkbox"/> Negative
-------------------------------	-----------------------------------	-----------------------------------

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012**



DTU Food
National Food Institute

TEST FORM

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

Strain	Antimicrobial	Zone diameter (mm) or MIC-value ($\mu\text{g/ml}$)
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	
	Erythromycin, ERY	
	Florfenicol, FFN	
	Gentamicin, GEN	
	Penicillin, PEN	
	Streptomycin, STR	
	Sulphonamides, SMX	
	Tetracycline, TET	
	Trimethoprim, TMP	

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012**



DTU Food
National Food Institute

TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ >	Zone diameter (mm) or MIC-value (µg/ml)	S / R
<i>E. coli</i> EURL EC 6.X	Ampicillin, AMP			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
Trimethoprim, TMP				

All strains resistant to cefotaxime (CTX), ceftazidime (CAZ) and/or ceftiofur (XNL) should be included for confirmatory tests for ESBL 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 ≤ 19 mm <input type="checkbox"/> D > 19 mm
Imipenem, IMI MIC value	<input type="checkbox"/> MIC value > 1 <input type="checkbox"/> MIC value ≤ 1	<input type="checkbox"/> Confirmed ESBL <input type="checkbox"/> Confirmed AmpC <input type="checkbox"/> Confirmed Metallo beta-lactamase	
IMI/E : IMI 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		

**EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012**



DTU Food
National Food Institute

Comments:

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	
	Florfenicol, FFN	
	Gentamicin, GEN	
	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

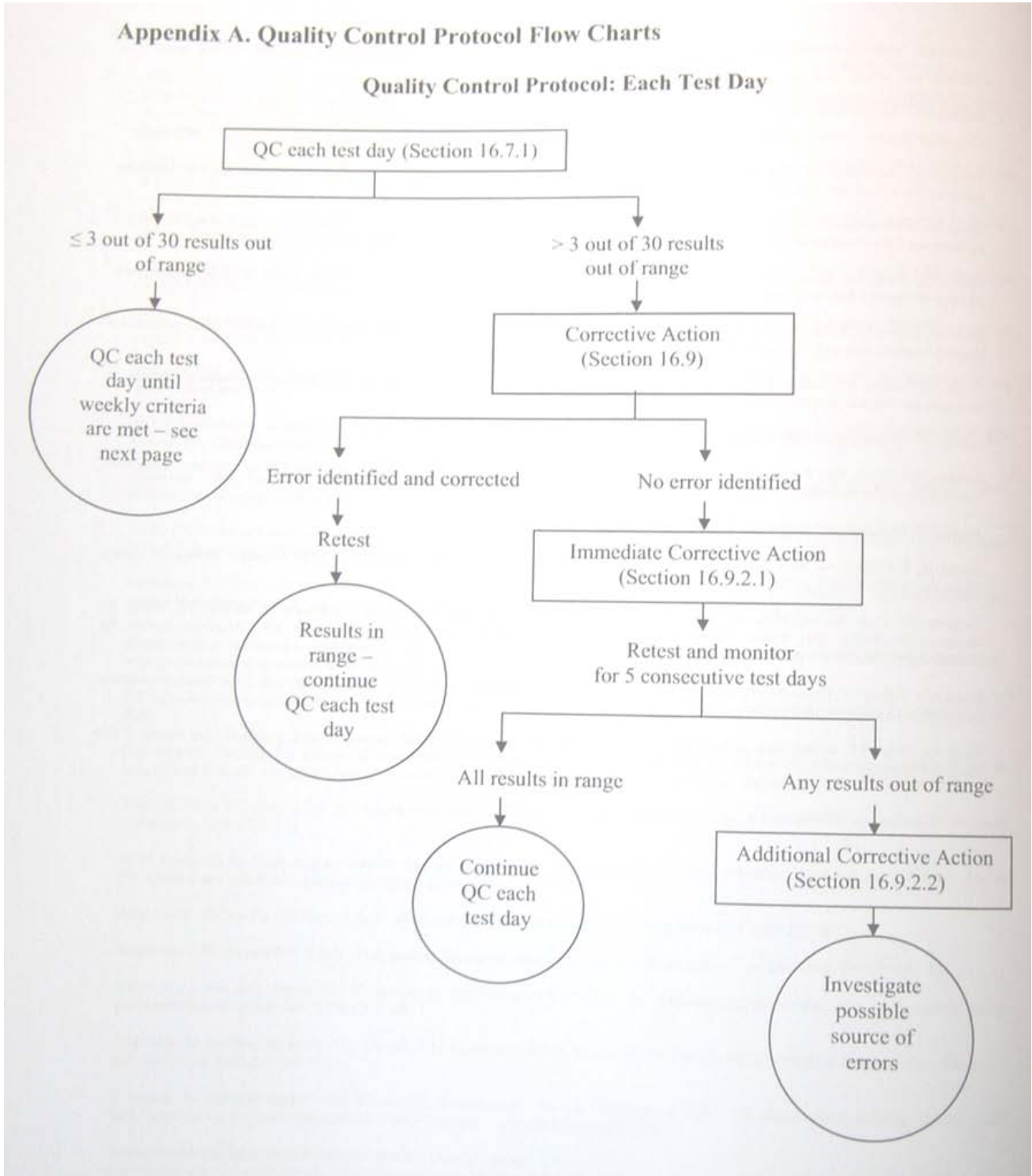
The problem is considered resolved only after the reference strain is tested for 5 consecutive days and each drug/organism result is within specification on each day.

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

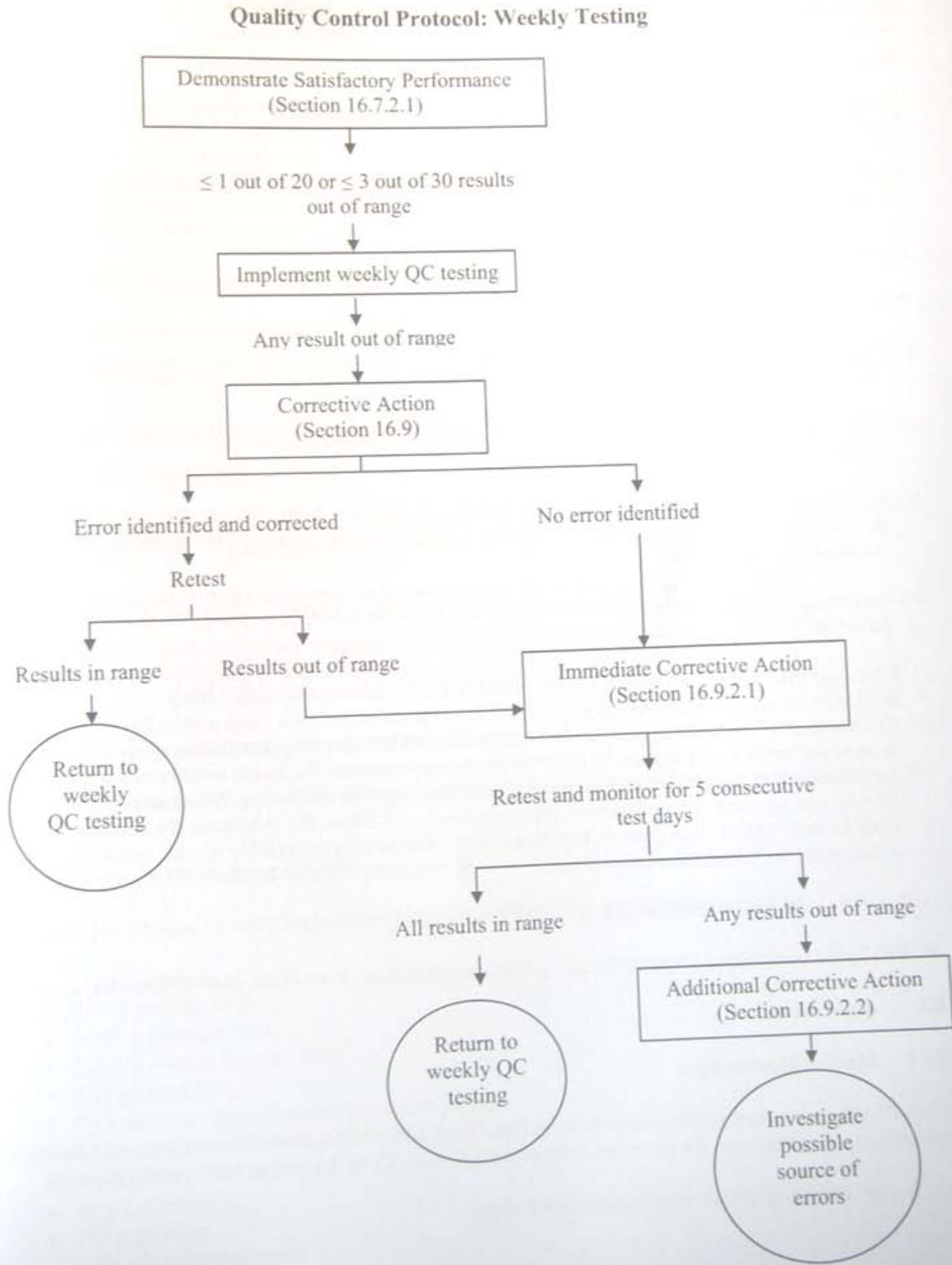


Reference: CLSI M7-A8, page 44



WEEKLY MIC QC CHART

Appendix A. (Continued)



Reference: CLSI M7-A8, page 45

Appendix 5a. Breakpoints used routinely in disk diffusion - Enterococci

Antibiotic	Lab nr	Disk content (µg)	R val ≤ (mm)	I val = (mm)	S val ≥ (mm)
Ampicillin, AMP	57	25	16		17
	26	10	16		17
	18	10	16		17
	54	10	16		17
Chloramphenicol, CHL	57	30	12	13-17	18
	26	30	12	13-17	18
	18	30	12		18
	54	30	12	13-17	18
Ciprofloxacin, CIP	57	10	15	16-20	21
	26	5	15	16-20	21
	18	5	15		21
	54	5	15	16-20	21
Erythromycin, ERY	57	30	13	14-22	23
	26	15	13	14-22	23
	18	15	13		23
	54	15	13	14-22	23
Gentamicin, GEN	57	30	12	13-14	15
	26	10	12	13-14	15
	18	10			
	54	10	12	13-14	15
Linezolid, LZD	57	30	20	21-22	23
	26	30	20	21-22	23
	18	30	20		23
	54	30	20	0	21
Quinu-dalfo-pristin, Q-D	57	15	15	16-18	19
	26	15	20		22
	18	15	15		19
Streptomycin, STR	57	25	11	12-14	15
	26	300	6	7-9	10
	18	10			
	54	10	11	12-14	15
Tetracycline, TET	57	30	14	15-18	19
	26	30	14	15-18	19
	18	30	14		19
	54	30	14	15-18	19
Vancomycin, VAN	57	30	14	15-16	17
	26	30	14	15-16	17
	18	30	14		17
	54	30	14	15-16	17

Appendix 5b. Breakpoints used routinely in disk diffusion – Staphylococci

Antibiotic	Lab nr	Disk content (µg)	R val ≤ (mm)	I val = (mm)	S val ≥ (mm)
Cefoxitin, FOX	57	30	21		22
	13	30	21		22
	18	30	22		22
	14				27
	56	30	19		20
	28	30	21	-	22
	54	30	14	15-17	18
	57	30	12	13-17	18
Chloramphenicol, CHL	18	30	18		18
	14	30			22
	56	30	12	13-17	18
	28	30	17	-	18
	15		18		22
	54	30	12	13-17	18
	57	10	15	16-20	21
Ciprofloxacin, CIP	13	5	15		21
	18	5	20		20
	14	5			22
	56	5	15	16-20	21
	28	5	19	-	20
	15		21		22
	54	5	15	16-20	21
	57	30	13	14-22	23
Erythromycin, ERY	13	15	13		23
	18	15	18		21
	14	15 UI			22
	56	15	13	14-22	23
	28	15	17	18 - 20	21
	15		16		22
	54	15	13	14-22	23
	57	30			
Florfenicol, FFN	18	30			
	56	30	14	15-18	19
	15		14		19
	54	30	14	15-17	19
	57	30	12	13-14	15
Gentamicin, GEN	13	10	12		15
	18	10	18		18
	14	15			20
	56	10	12	13-14	15
	28	10	17	-	18
	15		19		20
	54	10	12	13-14	15

Antibiotic	Lab nr	Disk content (µg)	R val ≤ (mm)	I val = (mm)	S val ≥ (mm)
Penicillin, PEN	57	10	28		29
	13	10units	28		29
	18	10	28		29
	14	6			29
	56	10	28		29
	28	1U	25	-	26
	15		28		29
	54	10U	28	-	29
	57	25	11	12-14	15
Streptomycin, STR	18	10			
	56	10	11	12-14	15
	15		12		15
	54	10	11	12-14	15
	57	25	12	13-16	17
Sulfamethoxazole, SMX	13	250	12		17
	18	300	12		17
	56	23,75	12	13-16	17
	15		11		17
	57	30	14	15-18	19
Tetracycline, TET	13	30	12		16
	18	30	19		22
	14	30 UI			19
	56	30	14	15-18	19
	28	30	18	19 - 21	22
	15		16		19
	54	30	14	15-18	19
	57	5	10	11-15	16
Trimethoprim, TMP	18	5	14		17
	56	5	10	11-15	16
	28	5	13	14 - 16	17
	15		11		16

Appendix 5c. Breakpoints used routinely in disk diffusion – *E. coli*

Antibiotic	Lab nr	Disk content (µg)	Test range for MIC (µg/mL)	R val	I val	S val
				<= (mm)	= (mm)	>= (mm)
Ampicillin, AMP	57	25		13	14-16	17
	28	10		13	14-16	17
	18	10		13	14-16	17
	54	10		13	14-16	17
	17	10		13	14-16	17
Cefotaxime, CTX	57	30		22	23-25	26
	14	30				26
	28	30		22	23-25	26
	15	30		22	23-25	26
	18	30		27		28
	54	30		14	15-22	23
	17	30		14	15-22	23
Ceftazidime, CAZ	57	30		17	18-20	21
	14	30				26
	28	30		17	18-20	21
	15	30		20	21-25	26
	18	30		22		23
	54	30		14	15-17	18
	17	30		14	15-17	18
Ceftiofur, XNL	57	30				
	14	30				21
	15	30		17	18-20	21
	18	30		17	18-20	21
	17	30		17	18-20	21
Chloramphenicol, CHL	57	30		12	13-17	18
	28	30		12	13-17	18
	15	30		18	19-21	22
	18	30		12	13-17	18
	54	30		12	13-17	18
	17	30		12	13-17	18
Ciprofloxacin, CIP	57	10		20	21-30	31
	14	5				25
	28	5		15	16-20	21
	18	5		29		30
	54	5		15	16-20	21
	17	5		15	16-20	21
Florphenicol, FFN	57	30				
	15	30		14	15-18	19
	18	30		14	15-18	19
	54	30		14	15-18	19
	17	30		14	15-18	19
Gentamicin, GEN	57	30		12	13-14	15
	14	15				18
	28	10		12	13-14	15
	15	15		15	16-17	18
	18	10		12	13-14	15
	54	10		12	13-14	15
	17	10		12	13-14	15
Nalidixic acid, NAL	57	30		13	14-18	19
	14	30				20
	28	30		13	14-18	19
	15	30		14	15-19	20
	18	30		13	14-18	19
	54	30		13	14-18	19
	17	30		13	14-18	19

Antibiotic	Lab nr	Disk content (µg)	Test range for MIC (µg/mL)	R val ≤ (mm)	I val = (mm)	S val ≥ (mm)
Streptomycin, STR	57	25		11	12-14	15
	28	10		11	12-14	15
	15	10		12	13-14	15
	18	10		11	12-14	15
	54	10		11	12-14	15
	17	10		11	12-14	15
Sulfamethoxazole, SMX	57	25		12	13-16	17
	28	250		12	13-16	17
	18	300		12	13-16	17
	17	300		12	13-16	17
Tetracycline, TET	57	30		11	12-14	15
	14	30UI				19
	28	30		11	12-14	15
	15	30		16	17-18	19
	18	30		11	12-14	15
	54	30		14	15-18	19
	17	30		11	12-14	15
Trimethoprim, TMP	57	5		10	11-15	16
	28	5		10	11-15	16
	15	5		11	12-15	16
	18	5		10	11-15	16
	17	1.25/23.75		10	11-15	16

Appendix 6. Acceptable ranges for the quality control strains

<i>Enterococcus faecalis</i> ATCC 29212	
Antimicrobial	MIC*
Ampicillin, AMP	0.5 - 2
Chloramphenicol, CHL	4 - 16
Ciprofloxacin, CIP	0.25 - 2
Erythromycin, ERY	1 - 4
Gentamicin, GEN	4 - 16
Linezolid, LZD	1 - 4
Quinupristin-dalfopristin, Q-D	2 - 8
Tetracycline, TET	8 - 32
Vancomycin, VAN	1 - 4

<i>Staphylococcus aureus</i>			
Antimicrobial	ATCC 25923		ATCC 29213
	Disk diffusion*	ROSCO	MIC*
Cefoxitin	23-29	23-29	1-4
Chloramphenicol, CHL	19 - 26	19-26	2 - 16
Ciprofloxacin, CIP	22 - 30	22-30	0.12 - 0.5
Erythromycin, ERY	22 - 30	22-30	0.25 - 1
Florfenicol, FFN	None	None	2 - 8
Gentamicin, GEN	19 - 27	19-27	0.12 - 1
Penicillin, PEN	26 - 37	26-37	0.25 - 2
Streptomycin, STR	14 - 22	14-22	None
Sulphonamides, SMX	24 - 34	23-33	32 - 128
Tetracycline, TET	24 - 30	24-30	0.12 - 1
Trimethoprim, TMP	19 - 26	19-26	1-4

<i>Escherichia coli</i> ATCC 25922		
Antimicrobial	Disk diffusion*	MIC*
Ampicillin, AMP	16 - 22	2 - 8
Cefotaxime, CTX	29 - 35	0.03 - 0.12
Cefoxitin	23-29	2-8
Ceftazidime, CAZ	25 - 32	0.06 - 0.5
Ceftiofur, XNL	26 - 31	0.25 - 1
Chloramphenicol, CHL	21 - 27	2 - 8
Ciprofloxacin, CIP	30 - 40	0.004 - 0.015
Gentamicin, GEN	19 - 26	0.25 - 1
Imipenem	26-32	0.06-0.25
Nalidixic acid, NAL	22 - 28	1 - 4
Streptomycin, STR	12-20	4 - 16
Sulphonamides, SMX	15 - 23	8 - 32
Tetracycline, TET	18 - 25	0.5 - 2
Trimethoprim, TMP	21 - 28	0.5 - 2

*MIC ranges (in µg/ml) and disk diffusion ranges (in mm) according to CLSI M100-S21 with the exception of the MIC range for streptomycin which is according to Sensititre. In addition, the range for ciprofloxacin is extended to include 0.016 µg/ml

Test results from reference strain *Enterococcus faecalis* ATCC 29212

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
1	Ampicillin , AMP	<=	2		2	1	MIC
1	Chloramphenicol, CHL	=	8	4	16	1	MIC
1	Ciprofloxacin , CIP	=	1		2	1	MIC
1	Erythromycin, ERY	=	1	1	4	1	MIC
1	Gentamicin, GEN	<=	16	4	16	1	MIC
1	Linezolid, LZD	=	2	1	4	1	MIC
1	Quinu-dalfo-pristin, Q-D	=	8	2	8	1	MIC
1	Streptomycin, STR	=	128	0	256	0	MIC
1	Tetracycline, TET	=	16	8	32	1	MIC
1	Vancomycin, VAN	=	4	1	4	1	MIC
2	Ampicillin , AMP	=	2		2	1	MIC
2	Chloramphenicol, CHL	=	8	4	16	1	MIC
2	Ciprofloxacin , CIP	=	1		2	1	MIC
2	Erythromycin, ERY	=	2	1	4	1	MIC
2	Gentamicin, GEN	=	16	4	16	1	MIC
2	Linezolid, LZD	=	2	1	4	1	MIC
2	Quinu-dalfo-pristin, Q-D	=	8	2	8	1	MIC
2	Streptomycin, STR	=	128	0	256	0	MIC
2	Tetracycline, TET	=	32	8	32	1	MIC
2	Vancomycin, VAN	=	4	1	4	1	MIC
6	Chloramphenicol, CHL	=	8	4	16	1	MIC
6	Ciprofloxacin , CIP	=	0.25		2	1	MIC
6	Erythromycin, ERY	=	1	1	4	1	MIC
6	Linezolid, LZD	=	2	1	4	1	MIC
6	Quinu-dalfo-pristin, Q-D	=	2	2	8	1	MIC
6	Tetracycline, TET	=	8	8	32	1	MIC
6	Vancomycin, VAN	=	1	1	4	1	MIC
9	Ampicillin , AMP	=	1		2	1	MIC
9	Chloramphenicol, CHL	=	8	4	16	1	MIC
9	Ciprofloxacin , CIP	=	0.5		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
9	Streptomycin, STR	=	64	0	256	0	MIC
9	Tetracycline, TET	=	16	8	32	1	MIC
9	Vancomycin, VAN	=	2	1	4	1	MIC
11	Ampicillin , AMP	=	1		2	1	MIC
11	Chloramphenicol, CHL	=	4	4	16	1	MIC
11	Erythromycin, ERY	=	1	1	4	1	MIC
11	Gentamicin, GEN	=	4	4	16	1	MIC
11	Linezolid, LZD	=	1	1	4	1	MIC
11	Streptomycin, STR	=	64	0	256	0	MIC
11	Tetracycline, TET	=	16	8	32	1	MIC
11	Vancomycin, VAN	=	2	1	4	1	MIC
12	Ampicillin , AMP	=	1		2	1	MIC
12	Chloramphenicol, CHL	=	4	4	16	1	MIC
12	Ciprofloxacin , CIP	=	1		2	1	MIC
12	Erythromycin, ERY	=	1	1	4	1	MIC
12	Gentamicin, GEN	=	8	4	16	1	MIC
12	Linezolid, LZD	=	2	1	4	1	MIC
12	Streptomycin, STR	=	64	0	256	0	MIC
12	Tetracycline, TET	=	16	8	32	1	MIC
12	Vancomycin, VAN	=	4	1	4	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
16	Ampicillin , AMP	=	1		2	1	MIC
16	Chloramphenicol, CHL	=	8	4	16	1	MIC
16	Ciprofloxacin , CIP	=	1		2	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	Quinu-dalfo-pristin, Q-D	=	8	2	8	1	MIC
16	Streptomycin, STR	=	128	0	256	0	MIC
16	Tetracycline, TET	=	16	8	32	1	MIC
16	Vancomycin, VAN	=	4	1	4	1	MIC
17	Ampicillin , AMP	=	2		2	1	MIC
17	Chloramphenicol, CHL	=	8	4	16	1	MIC
17	Ciprofloxacin , CIP	=	1		2	1	MIC
17	Erythromycin, ERY	=	1	1	4	1	MIC
17	Gentamicin, GEN	=	8	4	16	1	MIC
17	Linezolid, LZD	=	2	1	4	1	MIC
17	Quinu-dalfo-pristin, Q-D	>	4	2	8	1	MIC
17	Streptomycin, STR	=	128	0	256	0	MIC
17	Tetracycline, TET	=	32	8	32	1	MIC
17	Vancomycin, VAN	=	4	1	4	1	MIC
19	Chloramphenicol, CHL	=	8	4	16	1	MIC
19	Ciprofloxacin , CIP	=	1		2	1	MIC
19	Erythromycin, ERY	=	2	1	4	1	MIC
19	Gentamicin, GEN	=	16	4	16	1	MIC
19	Streptomycin, STR	=	128	0	256	0	MIC
19	Tetracycline, TET	=	16	8	32	1	MIC
19	Vancomycin, VAN	=	4	1	4	1	MIC
20	Ampicillin , AMP	=	2		2	1	MIC
20	Chloramphenicol, CHL	=	8	4	16	1	MIC
20	Ciprofloxacin , CIP	=	1		2	1	MIC
20	Erythromycin, ERY	=	1	1	4	1	MIC
20	Gentamicin, GEN	=	8	4	16	1	MIC
20	Linezolid, LZD	=	2	1	4	1	MIC
20	Quinu-dalfo-pristin, Q-D	=	4	2	8	1	MIC
20	Streptomycin, STR	=	64	0	256	0	MIC
20	Tetracycline, TET	=	32	8	32	1	MIC
20	Vancomycin, VAN	=	4	1	4	1	MIC
21	Chloramphenicol, CHL	=	4	4	16	1	MIC
21	Ciprofloxacin , CIP	=	0.5		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	Quinu-dalfo-pristin, Q-D	=	4	2	8	1	MIC
21	Tetracycline, TET	=	16	8	32	1	MIC
21	Vancomycin, VAN	=	2	1	4	1	MIC
22	Ampicillin , AMP	=	1		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	Streptomycin, STR	=	64	0	256	0	MIC
22	Tetracycline, TET	=	32	8	32	1	MIC
22	Vancomycin, VAN	=	4	1	4	1	MIC
23	Chloramphenicol, CHL	=	8	4	16	1	MIC
23	Ciprofloxacin , CIP	=	1		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

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
25	Ampicillin , AMP	<=	1		2	1	MIC
25	Chloramphenicol, CHL	=	8	4	16	1	MIC
25	Ciprofloxacin , CIP	=	1		2	1	MIC
25	Erythromycin, ERY	=	2	1	4	1	MIC
25	Gentamicin, GEN	=	16	4	16	1	MIC
25	Linezolid, LZD	=	2	1	4	1	MIC
25	Quinu-dalfo-pristin, Q-D	=	8	2	8	1	MIC
25	Streptomycin, STR	=	128	0	256	0	MIC
25	Tetracycline, TET	=	32	8	32	1	MIC
25	Vancomycin, VAN	=	4	1	4	1	MIC
29	Ampicillin , AMP	=	0.5		2	1	MIC
29	Chloramphenicol, CHL	=	4	4	16	1	MIC
29	Ciprofloxacin , CIP	=	0.25		2	1	MIC
29	Erythromycin, ERY	=	2	1	4	1	MIC
29	Gentamicin, GEN	=	4	4	16	1	MIC
29	Tetracycline, TET	=	32	8	32	1	MIC
29	Vancomycin, VAN	=	1	1	4	1	MIC
33	Ampicillin , AMP	=	1		2	1	MIC
33	Chloramphenicol, CHL	=	8	4	16	1	MIC
33	Erythromycin, ERY	=	1	1	4	1	MIC
33	Gentamicin, GEN	=	4	4	16	1	MIC
33	Linezolid, LZD	=	1	1	4	1	MIC
33	Streptomycin, STR	=	64	0	256	0	MIC
33	Tetracycline, TET	=	16	8	32	1	MIC
33	Vancomycin, VAN	=	2	1	4	1	MIC
34	Ampicillin , AMP	=	1		2	1	MIC
34	Chloramphenicol, CHL	=	8	4	16	1	MIC
34	Ciprofloxacin , CIP	=	0.5		2	1	MIC
34	Erythromycin, ERY	=	2	1	4	1	MIC
34	Gentamicin, GEN	=	16	4	16	1	MIC
34	Linezolid, LZD	=	2	1	4	1	MIC
34	Quinu-dalfo-pristin, Q-D	>	4	2	8	1	MIC
34	Streptomycin, STR	=	128	0	256	0	MIC
34	Tetracycline, TET	=	16	8	32	1	MIC
34	Vancomycin, VAN	=	2	1	4	1	MIC
36	Ampicillin , AMP	=	1		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	Streptomycin, STR	=	64	0	256	0	MIC
36	Tetracycline, TET	=	32	8	32	1	MIC
36	Vancomycin, VAN	>=	1	1	4	1	MIC
37	Ampicillin , AMP	=	1		2	1	AGA
37	Chloramphenicol, CHL	=	8	4	16	1	AGA
37	Ciprofloxacin , CIP	=	1		2	1	AGA
37	Erythromycin, ERY	=	1	1	4	1	AGA
37	Gentamicin, GEN	=	8	4	16	1	AGA
37	Streptomycin, STR	=	16	0	256	0	AGA
37	Tetracycline, TET	=	16	8	32	1	AGA
37	Vancomycin, VAN	=	2	1	4	1	AGA
39	Ampicillin , AMP	=	0.5		2	1	MIC
39	Chloramphenicol, CHL	=	8	4	16	1	MIC
39	Erythromycin, ERY	=	2	1	4	1	MIC
39	Gentamicin, GEN	=	8	4	16	1	MIC
39	Linezolid, LZD	=	2	1	4	1	MIC
39	Tetracycline, TET	=	16	8	32	1	MIC
39	Vancomycin, VAN	=	2	1	4	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
40	Chloramphenicol, CHL	=	8	4	16	1	MIC
40	Ciprofloxacin , CIP	=	0.25		2	1	MIC
40	Erythromycin, ERY	=	1	1	4	1	MIC
40	Gentamicin, GEN	=	16	4	16	1	MIC
40	Linezolid, LZD	=	2	1	4	1	MIC
40	Tetracycline, TET	=	32	8	32	1	MIC
40	Vancomycin, VAN	=	2	1	4	1	MIC
41	Ampicillin , AMP	=	1		2	1	MIC
41	Chloramphenicol, CHL	=	8	4	16	1	MIC
41	Ciprofloxacin , CIP	=	1		2	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	Quinu-dalfo-pristin, Q-D	=	4	2	8	1	MIC
41	Streptomycin, STR	<=	1000	0	256	0	MIC
41	Tetracycline, TET	=	8	8	32	1	MIC
41	Vancomycin, VAN	=	2	1	4	1	MIC
42	Ampicillin , AMP	<=	2		2	1	MIC
42	Chloramphenicol, CHL	=	16	4	16	1	MIC
42	Ciprofloxacin , CIP	=	1		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	Quinu-dalfo-pristin, Q-D	=	8	2	8	1	MIC
42	Streptomycin, STR	<=	128	0	256	0	MIC
42	Tetracycline, TET	=	32	8	32	1	MIC
42	Vancomycin, VAN	=	2	1	4	1	MIC
58	Ampicillin , AMP	=	1		2	1	MIC
58	Chloramphenicol, CHL	=	4	4	16	1	MIC
58	Ciprofloxacin , CIP	=	1		2	1	MIC
58	Erythromycin, ERY	=	2	1	4	1	MIC
58	Gentamicin, GEN	=	16	4	16	1	MIC
58	Linezolid, LZD	=	2	1	4	1	MIC
58	Quinu-dalfo-pristin, Q-D	=	8	2	8	1	MIC
58	Streptomycin, STR	<=	512	0	256	0	MIC
58	Tetracycline, TET	=	16	8	32	1	MIC
58	Vancomycin, VAN	=	4	1	4	1	MIC

Test results from *Staphylococcus aureus* reference strains

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method	ATCC 29213	ATCC 25923
1	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
1	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
1	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
1	Erythromycin, ERY	=	0.5		1	1	MIC	x	
1	Florfenicol, FFN	=	4	2	8	1	MIC	x	
1	Gentamicin, GEN	<=	0.25		1	1	MIC	x	
1	Penicillin, PEN	=	0.5		2	1	MIC	x	
1	Streptomycin, STR	=	8	0	256	0	MIC	x	
1	Tetracycline, TET	<=	0.5		1	1	MIC	x	
1	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
2	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
2	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
2	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
2	Erythromycin, ERY	=	0.5		1	1	MIC	x	
2	Gentamicin, GEN	<=	1		1	1	MIC	x	
2	Penicillin, PEN	=	0.25		2	1	MIC	x	
2	Streptomycin, STR	<=	4	0	256	0	MIC	x	
2	Tetracycline, TET	<=	0.5		1	1	MIC	x	
2	Trimethoprim, TMP	<=	2	1	4	1	MIC	x	
4	Cefoxitin, FOX	=	29.92	23	29	0	ROS		x
4	Chloramphenicol, CHL	=	22.95	23	30	0	ROS		x
4	Ciprofloxacin, CIP	=	25.52	21	29	1	ROS		x
4	Erythromycin, ERY	=	26.22	26	33	1	ROS		x
4	Florfenicol, FFN	=	25.03	0	256	0	ROS		x
4	Gentamicin, GEN	=	25.21	25	32	1	ROS		x
4	Sulfisoxazole, FIS	=	25.31	26	34	0	ROS		x
4	Tetracycline, TET	=	27.59	23	33	1	ROS		x
4	Trimethoprim, TMP	=	21.15	19	25	1	ROS		x
6	Cefoxitin, FOX	=	2	1	4	1	MIC	x	
6	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
6	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
6	Erythromycin, ERY	=	0.5		1	1	MIC	x	
6	Gentamicin, GEN	<	1		1	1	MIC	x	
6	Penicillin, PEN	=	0.5		2	1	MIC	x	
6	Streptomycin, STR	=	8	0	256	0	MIC	x	
6	Tetracycline, TET	=	1		1	1	MIC	x	
6	Trimethoprim, TMP	<	2	1	4	1	MIC	x	
9	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
9	Chloramphenicol, CHL	=	4	2	16	1	MIC	x	
9	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
9	Erythromycin, ERY	=	0.5		1	1	MIC	x	
9	Florfenicol, FFN	=	4	2	8	1	MIC	x	
9	Gentamicin, GEN	=	1		1	1	MIC	x	
9	Penicillin, PEN	=	0.5		2	1	MIC	x	
9	Streptomycin, STR	=	4	0	256	0	MIC	x	
9	Sulfisoxazole, FIS	=	64	32	128	1	MIC	x	
9	Tetracycline, TET	=	0.5		1	1	MIC	x	
9	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
11	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
11	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
11	Erythromycin, ERY	=	0.5		1	1	MIC	x	
11	Gentamicin, GEN	<=	0.5		1	1	MIC	x	
11	Penicillin, PEN	=	1		2	1	MIC	x	
11	Tetracycline, TET	<=	0.5		1	1	MIC	x	
11	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
12	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
12	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
12	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
12	Erythromycin, ERY	=	0.5		1	1	MIC	x	
12	Florfenicol, FFN	<=	4	2	8	1	MIC	x	
12	Gentamicin, GEN	<=	0.5		1	1	MIC	x	
12	Penicillin, PEN	=	0.25		2	1	MIC	x	
12	Streptomycin, STR	=	8	0	256	0	MIC	x	
12	Tetracycline, TET	<=	0.5		1	1	MIC	x	
12	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
13	Cefoxitin, FOX	=	28	23	29	1	DD		x
13	Ciprofloxacin, CIP	=	29	22	30	1	DD		x
13	Erythromycin, ERY	=	28	22	30	1	DD		x
13	Gentamicin, GEN	=	26	19	27	1	DD		x
13	Penicillin, PEN	=	37	26	37	1	DD		x
13	Sulfisoxazole, FIS	=	24	24	30	1	DD		x
13	Tetracycline, TET	=	28	24	34	1	DD		x

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method	ATCC 29213	ATCC 25923
14	Cefoxitin, FOX	=	32	23	29	0	DD		x
14	Chloramphenicol, CHL	=	24	16	26	1	DD		x
14	Ciprofloxacin, CIP	=	28	22	30	1	DD		x
14	Erythromycin, ERY	=	30	22	30	1	DD		x
14	Gentamicin, GEN	=	30	19	27	0	DD		x
14	Penicillin, PEN	=	40	26	37	0	DD		x
14	Tetracycline, TET	=	31	24	34	1	DD		x
15	Cefoxitin, FOX	=	29	23	29	1	DD		x
15	Chloramphenicol, CHL	=	23	16	26	1	DD		x
15	Erythromycin, ERY	=	30	22	30	1	DD		x
15	Gentamicin, GEN	=	27	19	27	1	DD		x
15	Penicillin, PEN	=	37	26	37	1	DD		x
15	Tetracycline, TET	=	31	24	34	1	DD		x
17	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
17	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
17	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
17	Erythromycin, ERY	=	0.5		1	1	MIC	x	
17	Florfenicol, FFN	=	4	2	8	1	MIC	x	
17	Gentamicin, GEN	<=	1		1	1	MIC	x	
17	Penicillin, PEN	=	0.5		2	1	MIC	x	
17	Streptomycin, STR	=	8	0	256	0	MIC	x	
17	Sulfisoxazole, FIS	<=	64	32	128	1	MIC	x	
17	Tetracycline, TET	<=	0.5		1	1	MIC	x	
17	Trimethoprim, TMP	<=	2	1	4	1	MIC	x	
18	Cefoxitin, FOX	=	26	23	29	1	DD		x
18	Chloramphenicol, CHL	=	23	16	26	1	DD		x
18	Ciprofloxacin, CIP	=	24	22	30	1	DD		x
18	Erythromycin, ERY	=	25	22	30	1	DD		x
18	Florfenicol, FFN	=	25	0	256	0	DD		x
18	Gentamicin, GEN	=	22	19	27	1	DD		x
18	Penicillin, PEN	=	32	26	37	1	DD		x
18	Streptomycin, STR	=	15	14	22	1	DD		x
18	Sulfisoxazole, FIS	=	24	24	30	1	DD		x
18	Tetracycline, TET	=	27	24	34	1	DD		x
18	Trimethoprim, TMP	=	21	19	26	1	DD		x
19	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
19	Chloramphenicol, CHL	=	4	2	16	1	MIC	x	
19	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
19	Erythromycin, ERY	=	0.5		1	1	MIC	x	
19	Gentamicin, GEN	=	1		1	1	MIC	x	
19	Penicillin, PEN	=	0.25		2	1	MIC	x	
19	Streptomycin, STR	=	4	0	256	0	MIC	x	
19	Sulfisoxazole, FIS	=	64	32	128	1	MIC	x	
19	Tetracycline, TET	=	0.5		1	1	MIC	x	
19	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
20	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
20	Chloramphenicol, CHL	=	16	2	16	1	MIC	x	
20	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
20	Erythromycin, ERY	=	1		1	1	MIC	x	
20	Gentamicin, GEN	<=	1		1	1	MIC	x	
20	Penicillin, PEN	=	2		2	1	MIC	x	
20	Streptomycin, STR	=	8	0	256	0	MIC	x	
20	Sulfisoxazole, FIS	=	128	32	128	1	MIC	x	
20	Tetracycline, TET	=	1		1	1	MIC	x	
20	Trimethoprim, TMP	<=	2	1	4	1	MIC	x	
21	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
21	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
21	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
21	Erythromycin, ERY	=	0.5		1	1	MIC	x	
21	Gentamicin, GEN	=	1		1	1	MIC	x	
21	Penicillin, PEN	=	0.25		2	1	MIC	x	
21	Sulfisoxazole, FIS	=	64	32	128	1	MIC	x	
21	Tetracycline, TET	=	0.5		1	1	MIC	x	
21	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
22	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
22	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
22	Erythromycin, ERY	=	0.5		1	1	MIC	x	
22	Gentamicin, GEN	=	1		1	1	MIC	x	
22	Penicillin, PEN	=	0.5		2	1	MIC	x	
22	Streptomycin, STR	<	4	0	256	0	MIC	x	
22	Sulfisoxazole, FIS	=	128	32	128	1	MIC	x	
22	Tetracycline, TET	=	1		1	1	MIC	x	
22	Trimethoprim, TMP	<	2	1	4	1	MIC	x	

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method	ATCC 29213	ATCC 25923
23	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
23	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
23	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
23	Erythromycin, ERY	=	0.5		1	1	MIC	x	
23	Gentamicin, GEN	<	1		1	1	MIC	x	
23	Penicillin, PEN	=	0.25		2	1	MIC	x	
23	Streptomycin, STR	<	4	0	256	0	MIC	x	
23	Sulfisoxazole, FIS	<	64	32	128	1	MIC	x	
23	Tetracycline, TET	<	0.5		1	1	MIC	x	
23	Trimethoprim, TMP	<	2	1	4	1	MIC	x	
25	Erythromycin, ERY	=	0.5		1	1	MIC	x	
25	Penicillin, PEN	=	1		2	1	MIC	x	
25	Streptomycin, STR	=	4	0	256	0	MIC	x	
25	Tetracycline, TET	=	0.5		1	1	MIC	x	
26	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
26	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
26	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
26	Erythromycin, ERY	=	0.5		1	1	MIC	x	
26	Florfenicol, FFN	=	4	2	8	1	MIC	x	
26	Gentamicin, GEN	=	0.5		1	1	MIC	x	
26	Penicillin, PEN	=	0.5		2	1	MIC	x	
26	Streptomycin, STR	=	8	0	256	0	MIC	x	
26	Sulfisoxazole, FIS	=	64	32	128	1	MIC	x	
26	Tetracycline, TET	=	1		1	1	MIC	x	
26	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
29	Chloramphenicol, CHL	=	4	2	16	1	MIC	x	
29	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
29	Erythromycin, ERY	=	0.5		1	1	MIC	x	
29	Gentamicin, GEN	=	0.5		1	1	MIC	x	
29	Penicillin, PEN	=	0.25		2	1	MIC	x	
29	Tetracycline, TET	=	0.5		1	1	MIC	x	
29	Trimethoprim, TMP	=	2	1	4	1	MIC	x	
30	Cefoxitin, FOX	<=	6	1	4	1	MIC	x	
30	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
30	Ciprofloxacin, CIP	<=	1			1	MIC	x	
30	Erythromycin, ERY	<=	0.25		1	1	MIC	x	
30	Gentamicin, GEN	<=	2		1	1	MIC	x	
30	Penicillin, PEN	=	0.25		2	1	MIC	x	
30	Streptomycin, STR	<=	1000	0	256	0	MIC	x	
30	Tetracycline, TET	<=	2		1	1	MIC	x	
31	Cefoxitin, FOX	<=	4	1	4	1	MIC	x	
31	Chloramphenicol, CHL	<=	16	2	16	1	MIC	x	
31	Ciprofloxacin, CIP	<=	0.12			1	MIC	x	
31	Erythromycin, ERY	<=	0.5		1	1	MIC	x	
31	Florfenicol, FFN	<=	8	2	8	1	MIC	x	
31	Gentamicin, GEN	<=	2		1	1	MIC	x	
31	Penicillin, PEN	=	0.5		2	1	MIC	x	
31	Streptomycin, STR	<=	16	0	256	0	MIC	x	
31	Sulfisoxazole, FIS	<=	128	32	128	1	MIC	x	
31	Tetracycline, TET	<=	1		1	1	MIC	x	
31	Trimethoprim, TMP	<=	2	1	4	1	MIC	x	
33	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
33	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
33	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
33	Erythromycin, ERY	=	0.5		1	1	MIC	x	
33	Gentamicin, GEN	=	1		1	1	MIC	x	
33	Penicillin, PEN	=	0.25		2	1	MIC	x	
33	Tetracycline, TET	<=	0.5		1	1	MIC	x	
33	Trimethoprim, TMP	=	4	1	4	1	MIC	x	
34	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
34	Chloramphenicol, CHL	=	16	2	16	1	MIC	x	
34	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
34	Erythromycin, ERY	=	0.5		1	1	MIC	x	
34	Gentamicin, GEN	<=	1		1	1	MIC	x	
34	Penicillin, PEN	=	1		2	1	MIC	x	
34	Streptomycin, STR	=	8	0	256	0	MIC	x	
34	Sulfisoxazole, FIS	=	128	32	128	1	MIC	x	
34	Tetracycline, TET	=	1		1	1	MIC	x	
34	Trimethoprim, TMP	<=	2	1	4	1	MIC	x	
36	Cefoxitin, FOX	=	25	1	4	0	MIC	x	
36	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
36	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
36	Erythromycin, ERY	=	0.5		1	1	MIC	x	
36	Gentamicin, GEN	<=	0.5		1	1	MIC	x	
36	Penicillin, PEN	=	0.5		2	1	MIC	x	
36	Tetracycline, TET	<=	0.5		1	1	MIC	x	
36	Trimethoprim, TMP	=	4	1	4	1	MIC	x	

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method	ATCC 29213	ATCC 25923
37	Cefoxitin, FOX	=	4	1	4	1	AGA	x	
37	Chloramphenicol, CHL	=	4	2	16	1	AGA	x	
37	Ciprofloxacin, CIP	=	0.25			1	AGA	x	
37	Erythromycin, ERY	=	0.25		1	1	AGA	x	
37	Gentamicin, GEN	=	0.5		1	1	AGA	x	
37	Penicillin, PEN	=	0.25		2	1	AGA	x	
37	Streptomycin, STR	=	4	0	256	0	AGA	x	
37	Tetracycline, TET	=	0.5		1	1	AGA	x	
37	Trimethoprim, TMP	=	1	1	4	1	AGA	x	
39	Chloramphenicol, CHL	=	2	2	16	1	MIC	x	
39	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
39	Erythromycin, ERY	=	1		1	1	MIC	x	
39	Gentamicin, GEN	=	1		1	1	MIC	x	
39	Penicillin, PEN	=	2		2	1	MIC	x	
39	Tetracycline, TET	=	1		1	1	MIC	x	
39	Trimethoprim, TMP	=	1	1	4	1	MIC	x	
40	Cefoxitin, FOX	=	2	1	4	1	MIC	x	
40	Chloramphenicol, CHL	=	4	2	16	1	MIC	x	
40	Ciprofloxacin, CIP	=	0.25			1	MIC	x	
40	Erythromycin, ERY	=	0.25		1	1	MIC	x	
40	Gentamicin, GEN	=	1		1	1	MIC	x	
40	Penicillin, PEN	=	2		2	1	MIC	x	
40	Sulfisoxazole, FIS	=	128	32	128	1	MIC	x	
40	Tetracycline, TET	=	0.5		1	1	MIC	x	
40	Trimethoprim, TMP	=	4	1	4	1	MIC	x	
42	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
42	Ciprofloxacin, CIP	<=	0.25			1	MIC	x	
42	Erythromycin, ERY	=	0.5		1	1	MIC	x	
42	Gentamicin, GEN	<=	1		1	1	MIC	x	
42	Penicillin, PEN	=	0.5		2	1	MIC	x	
42	Streptomycin, STR	=	8	0	256	0	MIC	x	
42	Sulfisoxazole, FIS	<=	64	32	128	1	MIC	x	
42	Tetracycline, TET	<=	0.5		1	1	MIC	x	
42	Trimethoprim, TMP	<=	2	1	4	1	MIC	x	
56	Cefoxitin, FOX	=	30	23	29	0	DD		x
56	Chloramphenicol, CHL	=	23	16	26	1	DD		x
56	Ciprofloxacin, CIP	=	26	22	30	1	DD		x
56	Erythromycin, ERY	=	27	22	30	1	DD		x
56	Florfenicol, FFN	=	24	0	256	0	DD		x
56	Gentamicin, GEN	=	26	19	27	1	DD		x
56	Penicillin, PEN	=	34	26	37	1	DD		x
56	Streptomycin, STR	=	18	14	22	1	DD		x
56	Sulfisoxazole, FIS	=	24	24	30	1	DD		x
56	Tetracycline, TET	=	28	24	34	1	DD		x
56	Trimethoprim, TMP	=	20	19	26	1	DD		x
57	Cefoxitin, FOX	=	29	23	29	1	DD		x
57	Chloramphenicol, CHL	=	26	16	26	1	DD		x
57	Ciprofloxacin, CIP	=	29	22	30	1	DD		x
57	Erythromycin, ERY	=	30	22	30	1	DD		x
57	Florfenicol, FFN	=	27	0	256	0	DD		x
57	Gentamicin, GEN	=	27	19	27	1	DD		x
57	Penicillin, PEN	=	33	26	37	1	DD		x
57	Streptomycin, STR	=	22	14	22	1	DD		x
57	Sulfisoxazole, FIS	=	30	24	30	1	DD		x
57	Tetracycline, TET	=	27	24	34	1	DD		x
57	Trimethoprim, TMP	=	26	19	26	1	DD		x
58	Cefoxitin, FOX	=	4	1	4	1	MIC	x	
58	Chloramphenicol, CHL	=	8	2	16	1	MIC	x	
58	Ciprofloxacin, CIP	=	0.5			1	MIC	x	
58	Erythromycin, ERY	=	0.5		1	1	MIC	x	
58	Gentamicin, GEN	<=	1		1	1	MIC	x	
58	Penicillin, PEN	=	0.25		2	1	MIC	x	
58	Streptomycin, STR	=	8	0	256	0	MIC	x	
58	Tetracycline, TET	<=	0.5		1	1	MIC	x	
58	Trimethoprim, TMP	=	4	1	4	1	MIC	x	

Test results from 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			1	MIC
1	Ceftiofur, XNL	<=	0.5		1	1	MIC
1	Chloramphenicol, CHL	=	4	2	8	1	MIC
1	Ciprofloxacin, CIP	<=	0.015			1	MIC
1	Gentamicin, GEN	=	1		1	1	MIC
1	Nalidixic acid, NAL	<=	4	1	4	1	MIC
1	Streptomycin, STR	<=	8	4	16	1	MIC
1	Tetracycline, TET	<=	2		2	1	MIC
1	Trimethoprim, TMP	<=	1		2	1	MIC
2	Ampicillin, AMP	=	4	2	8	1	MIC
2	Cefotaxime, CTX	<=	0.06			1	MIC
2	Ceftazidime, CAZ	=	0.25			1	MIC
2	Chloramphenicol, CHL	=	8	2	8	1	MIC
2	Ciprofloxacin, CIP	=	0.016			1	MIC
2	Gentamicin, GEN	=	0.5		1	1	MIC
2	Nalidixic acid, NAL	<=	2	1	4	1	MIC
2	Streptomycin, STR	<=	4	4	16	1	MIC
2	Tetracycline, TET	<=	1		2	1	MIC
2	Trimethoprim, TMP	=	0.5		2	1	MIC
4	Ampicillin, AMP	=	8	2	8	1	MIC
4	Cefotaxime, CTX	=	0.06			1	MIC
4	Ceftazidime, CAZ	=	0.25			1	MIC
4	Chloramphenicol, CHL	=	4	2	8	1	MIC
4	Ciprofloxacin, CIP	=	0.015			1	MIC
4	Gentamicin, GEN	=	4		1	0	MIC
4	Imipenem, IMI	=	0.5			0	MIC
4	Nalidixic acid, NAL	=	4	1	4	1	MIC
4	Streptomycin, STR	=	8	4	16	1	MIC
4	Tetracycline, TET	=	1		2	1	MIC
4	Trimethoprim, TMP	=	1		2	1	MIC
6	Ampicillin, AMP	=	2	2	8	1	MIC
6	Cefotaxime, CTX	<	0.06			1	MIC
6	Ceftazidime, CAZ	<	0.25			1	MIC
6	Chloramphenicol, CHL	=	2	2	8	1	MIC
6	Ciprofloxacin, CIP	<	0.008			1	MIC
6	Gentamicin, GEN	=	0.5		1	1	MIC
6	Nalidixic acid, NAL	<	4	1	4	1	MIC
6	Streptomycin, STR	=	8	4	16	1	MIC
6	Tetracycline, TET	<	1		2	1	MIC
6	Trimethoprim, TMP	=	0.5		2	1	MIC
9	Ampicillin, AMP	=	4	2	8	1	MIC
9	Cefotaxime, CTX	=	0.12			1	MIC
9	Cefoxitin, FOX	=	4	2	8	1	MIC
9	Ceftazidime, CAZ	=	0.25			1	MIC
9	Ceftiofur, XNL	=	0.25		1	1	MIC
9	Chloramphenicol, CHL	=	4	2	8	1	MIC
9	Ciprofloxacin, CIP	=	0.008			1	MIC
9	Gentamicin, GEN	=	0.5		1	1	MIC
9	Imipenem, IMI	=	0.12			1	MIC
9	Nalidixic acid, NAL	=	4	1	4	1	MIC
9	Streptomycin, STR	=	8	4	16	1	MIC
9	Sulfisoxazole, FIS	=	16	8	32	1	MIC
9	Tetracycline, TET	=	1		2	1	MIC
9	Trimethoprim, TMP	=	1		2	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
11	Ampicillin, AMP	=	4	2	8	1	MIC
11	Cefotaxime, CTX	=	0.06			1	MIC
11	Ceftazidime, CAZ	=	0.5			1	MIC
11	Chloramphenicol, CHL	=	4	2	8	1	MIC
11	Ciprofloxacin, CIP	=	0.03			0	MIC
11	Gentamicin, GEN	=	1		1	1	MIC
11	Nalidixic acid, NAL	=	2	1	4	1	MIC
11	Streptomycin, STR	=	4	4	16	1	MIC
11	Sulfisoxazole, FIS	=	16	8	32	1	MIC
11	Tetracycline, TET	<=	1		2	1	MIC
11	Trimethoprim, TMP	=	0.5		2	1	MIC
12	Ampicillin, AMP	=	2	2	8	1	MIC
12	Cefotaxime, CTX	=	0.12			1	MIC
12	Cefoxitin, FOX	=	4	2	8	1	MIC
12	Ceftazidime, CAZ	=	0.5			1	MIC
12	Chloramphenicol, CHL	=	4	2	8	1	MIC
12	Ciprofloxacin, CIP	=	0.03			0	MIC
12	Gentamicin, GEN	=	2		1	0	MIC
12	Nalidixic acid, NAL	=	2	1	4	1	MIC
12	Streptomycin, STR	=	16	4	16	1	MIC
12	Tetracycline, TET	<=	1		2	1	MIC
12	Trimethoprim, TMP	=	0.5		2	1	MIC
14	Cefotaxime, CTX	=	36	29	35	0	DD
14	Cefoxitin, FOX	=	28	23	29	1	DD
14	Ceftazidime, CAZ	=	32	25	32	1	DD
14	Ceftiofur, XNL	=	31	26	31	1	DD
14	Ciprofloxacin, CIP	=	37	30	40	1	DD
14	Gentamicin, GEN	=	26	19	26	1	DD
14	Nalidixic acid, NAL	=	28	22	28	1	DD
14	Tetracycline, TET	=	26	18	25	0	DD
15	Cefotaxime, CTX	=	35	29	35	1	DD
15	Cefoxitin, FOX	=	28	23	29	1	DD
15	Ceftazidime, CAZ	=	32	25	32	1	DD
15	Ceftiofur, XNL	=	31	26	31	1	DD
15	Chloramphenicol, CHL	=	26	21	27	1	DD
15	Gentamicin, GEN	=	26	19	26	1	DD
15	Nalidixic acid, NAL	=	25	22	28	1	DD
15	Streptomycin, STR	=	19	12	20	1	DD
15	Tetracycline, TET	=	25	18	25	1	DD
15	Trimethoprim, TMP	=	25	21	28	1	DD
16	Ampicillin, AMP	=	4	2	8	1	MIC
16	Cefotaxime, CTX	=	0.12			1	MIC
16	Ceftazidime, CAZ	=	0.25			1	MIC
16	Chloramphenicol, CHL	=	8	2	8	1	MIC
16	Ciprofloxacin, CIP	=	0.015			1	MIC
16	Gentamicin, GEN	=	0.5		1	1	MIC
16	Nalidixic acid, NAL	=	2	1	4	1	MIC
16	Streptomycin, STR	=	8	4	16	1	MIC
16	Sulfisoxazole, FIS	=	32	8	32	1	MIC
16	Tetracycline, TET	<=	1		2	1	MIC
16	Trimethoprim, TMP	=	2		2	1	MIC
17	Ampicillin, AMP	=	2	2	8	1	MIC
17	Cefotaxime, CTX	<=	0.06			1	MIC
17	Cefoxitin, FOX	=	4	2	8	1	MIC
17	Ceftazidime, CAZ	<=	0.25			1	MIC
17	Chloramphenicol, CHL	=	4	2	8	1	MIC
17	Ciprofloxacin, CIP	=	0.015			1	MIC
17	Gentamicin, GEN	=	0.5		1	1	MIC
17	Nalidixic acid, NAL	<=	4	1	4	1	MIC
17	Streptomycin, STR	=	4	4	16	1	MIC
17	Sulfisoxazole, FIS	=	32	8	32	1	MIC
17	Tetracycline, TET	<=	1		2	1	MIC
17	Trimethoprim, TMP	=	1		2	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
18	Ampicillin, AMP	=	4	2	8	1	MIC
18	Cefotaxime, CTX	=	0.06			1	MIC
18	Ceftazidime, CAZ	<=	0.25			1	MIC
18	Chloramphenicol, CHL	=	2	2	8	1	MIC
18	Ciprofloxacin, CIP	=	0.008			1	MIC
18	Gentamicin, GEN	=	0.5		1	1	MIC
18	Nalidixic acid, NAL	<=	4	1	4	1	MIC
18	Streptomycin, STR	=	4	4	16	1	MIC
18	Sulfisoxazole, FIS	=	32	8	32	1	MIC
18	Tetracycline, TET	<=	1		2	1	MIC
18	Trimethoprim, TMP	=	1		2	1	MIC
19	Ampicillin, AMP	=	4	2	8	1	MIC
19	Cefotaxime, CTX	=	0.06			1	MIC
19	Ceftazidime, CAZ	=	0.25			1	MIC
19	Chloramphenicol, CHL	=	4	2	8	1	MIC
19	Ciprofloxacin, CIP	=	0.015			1	MIC
19	Gentamicin, GEN	=	0.5		1	1	MIC
19	Nalidixic acid, NAL	=	4	1	4	1	MIC
19	Streptomycin, STR	=	4	4	16	1	MIC
19	Sulfisoxazole, FIS	=	32	8	32	1	MIC
19	Tetracycline, TET	=	1		2	1	MIC
19	Trimethoprim, TMP	=	0.5		2	1	MIC
20	Ampicillin, AMP	=	4	2	8	1	MIC
20	Cefotaxime, CTX	<=	0.06			1	MIC
20	Ceftazidime, CAZ	<=	0.25			1	MIC
20	Chloramphenicol, CHL	=	4	2	8	1	MIC
20	Ciprofloxacin, CIP	<=	0.008			1	MIC
20	Gentamicin, GEN	=	0.5		1	1	MIC
20	Nalidixic acid, NAL	<=	4	1	4	1	MIC
20	Streptomycin, STR	=	4	4	16	1	MIC
20	Sulfisoxazole, FIS	=	32	8	32	1	MIC
20	Tetracycline, TET	=	2		2	1	MIC
20	Trimethoprim, TMP	<=	0.5		2	1	MIC
21	Ampicillin, AMP	=	2	2	8	1	MIC
21	Cefotaxime, CTX	=	0.12			1	MIC
21	Ceftazidime, CAZ	=	0.5			1	MIC
21	Chloramphenicol, CHL	=	2	2	8	1	MIC
21	Ciprofloxacin, CIP	=	0.015			1	MIC
21	Gentamicin, GEN	=	0.5		1	1	MIC
21	Nalidixic acid, NAL	=	4	1	4	1	MIC
21	Sulfisoxazole, FIS	=	8	8	32	1	MIC
21	Tetracycline, TET	=	1		2	1	MIC
21	Trimethoprim, TMP	=	1		2	1	MIC
22	Ampicillin, AMP	=	8	2	8	1	MIC
22	Cefotaxime, CTX	<	0.06			1	MIC
22	Ceftazidime, CAZ	<	0.25			1	MIC
22	Chloramphenicol, CHL	=	4	2	8	1	MIC
22	Ciprofloxacin, CIP	=	0.015			1	MIC
22	Gentamicin, GEN	=	0.5		1	1	MIC
22	Nalidixic acid, NAL	<	4	1	4	1	MIC
22	Streptomycin, STR	=	4	4	16	1	MIC
22	Sulfisoxazole, FIS	=	32	8	32	1	MIC
22	Tetracycline, TET	=	2		2	1	MIC
22	Trimethoprim, TMP	=	1		2	1	MIC
23	Ampicillin, AMP	=	1	2	8	0	MIC
23	Cefotaxime, CTX	=	0.06			1	MIC
23	Cefoxitin, FOX	<	4	2	8	1	MIC
23	Ceftazidime, CAZ	<	0.25			1	MIC
23	Chloramphenicol, CHL	=	4	2	8	1	MIC
23	Ciprofloxacin, CIP	<	0.008			1	MIC
23	Gentamicin, GEN	=	0.5		1	1	MIC
23	Nalidixic acid, NAL	=	4	1	4	1	MIC
23	Streptomycin, STR	=	4	4	16	1	MIC
23	Sulfisoxazole, FIS	=	32	8	32	1	MIC
23	Tetracycline, TET	<	1		2	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
25	Ampicillin, AMP	=	4	2	8	1	MIC
25	Cefotaxime, CTX	<=	0.06			1	MIC
25	Ceftazidime, CAZ	<=	0.25			1	MIC
25	Chloramphenicol, CHL	=	4	2	8	1	MIC
25	Ciprofloxacin, CIP	=	0.015			1	MIC
25	Gentamicin, GEN	=	1		1	1	MIC
25	Nalidixic acid, NAL	<=	4	1	4	1	MIC
25	Streptomycin, STR	=	8	4	16	1	MIC
25	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
25	Tetracycline, TET	<=	1		2	1	MIC
25	Trimethoprim, TMP	<=	0.5		2	1	MIC
26	Ampicillin, AMP	=	4	2	8	1	MIC
26	Cefotaxime, CTX	=	0.12			1	MIC
26	Ceftazidime, CAZ	<=	0.25			1	MIC
26	Chloramphenicol, CHL	=	4	2	8	1	MIC
26	Ciprofloxacin, CIP	=	0.015			1	MIC
26	Gentamicin, GEN	=	0.5		1	1	MIC
26	Nalidixic acid, NAL	<=	4	1	4	1	MIC
26	Streptomycin, STR	=	8	4	16	1	MIC
26	Tetracycline, TET	<=	1		2	1	MIC
26	Trimethoprim, TMP	<=	0.5		2	1	MIC
29	Ampicillin, AMP	=	8	2	8	1	MIC
29	Cefotaxime, CTX	=	0.12			1	MIC
29	Ceftazidime, CAZ	=	0.5			1	MIC
29	Chloramphenicol, CHL	=	8	2	8	1	MIC
29	Ciprofloxacin, CIP	=	0.016			1	MIC
29	Gentamicin, GEN	=	0.5		1	1	MIC
29	Nalidixic acid, NAL	=	4	1	4	1	MIC
29	Streptomycin, STR	=	4	4	16	1	MIC
29	Tetracycline, TET	=	2		2	1	MIC
29	Trimethoprim, TMP	=	1		2	1	MIC
30	Ampicillin, AMP	=	4	2	8	1	MIC
30	Cefotaxime, CTX	<=	0.06			1	MIC
30	Cefoxitin, FOX	<=	4	2	8	1	MIC
30	Ceftazidime, CAZ	<=	0.25			1	MIC
30	Chloramphenicol, CHL	=	4	2	8	1	MIC
30	Ciprofloxacin, CIP	<=	0.008			1	MIC
30	Gentamicin, GEN	=	1		1	1	MIC
30	Imipenem, IMI	<=	0.5			1	MIC
30	Nalidixic acid, NAL	<=	4	1	4	1	MIC
30	Streptomycin, STR	=	4	4	16	1	MIC
30	Sulfisoxazole, FIS	=	32	8	32	1	MIC
30	Tetracycline, TET	<=	1		2	1	MIC
30	Trimethoprim, TMP	<=	0.5		2	1	MIC
32	Ampicillin, AMP	=	4	2	8	1	MIC
32	Cefotaxime, CTX	<=	0.06			1	MIC
32	Ceftazidime, CAZ	<=	0.25			1	MIC
32	Chloramphenicol, CHL	=	4	2	8	1	MIC
32	Ciprofloxacin, CIP	<=	0.008			1	MIC
32	Gentamicin, GEN	=	0.5		1	1	MIC
32	Nalidixic acid, NAL	<=	4	1	4	1	MIC
32	Streptomycin, STR	=	8	4	16	1	MIC
32	Sulfisoxazole, FIS	=	16	8	32	1	MIC
32	Tetracycline, TET	<=	1		2	1	MIC
32	Trimethoprim, TMP	<=	0.5		2	1	MIC
33	Ampicillin, AMP	=	4	2	8	1	MIC
33	Cefotaxime, CTX	=	0.12			1	MIC
33	Cefoxitin, FOX	=	4	2	8	1	MIC
33	Chloramphenicol, CHL	=	4	2	8	1	MIC
33	Ciprofloxacin, CIP	=	0.06			0	MIC
33	Gentamicin, GEN	=	0.5		1	1	MIC
33	Nalidixic acid, NAL	=	4	1	4	1	MIC
33	Streptomycin, STR	=	4	4	16	1	MIC
33	Sulfisoxazole, FIS	=	32	8	32	1	MIC
33	Tetracycline, TET	=	2		2	1	MIC
33	Trimethoprim, TMP	=	1		2	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
34	Ampicillin, AMP	=	4	2	8	1	MIC
34	Cefotaxime, CTX	=	0.12			1	MIC
34	Ceftazidime, CAZ	<=	0.25			1	MIC
34	Chloramphenicol, CHL	=	4	2	8	1	MIC
34	Ciprofloxacin, CIP	=	0.015			1	MIC
34	Gentamicin, GEN	=	1		1	1	MIC
34	Nalidixic acid, NAL	<=	4	1	4	1	MIC
34	Streptomycin, STR	=	4	4	16	1	MIC
34	Sulfisoxazole, FIS	=	32	8	32	1	MIC
34	Tetracycline, TET	<=	1		2	1	MIC
34	Trimethoprim, TMP	=	1		2	1	MIC
36	Ampicillin, AMP	=	4	2	8	1	MIC
36	Cefotaxime, CTX	=	0.12			1	MIC
36	Ceftazidime, CAZ	=	1			0	MIC
36	Chloramphenicol, CHL	=	4	2	8	1	MIC
36	Ciprofloxacin, CIP	=	0.03			0	MIC
36	Gentamicin, GEN	=	0.5		1	1	MIC
36	Nalidixic acid, NAL	=	4	1	4	1	MIC
36	Streptomycin, STR	=	4	4	16	1	MIC
36	Sulfisoxazole, FIS	=	32	8	32	1	MIC
36	Tetracycline, TET	=	2		2	1	MIC
36	Trimethoprim, TMP	=	1		2	1	MIC
37	Ampicillin, AMP	=	4	2	8	1	AGA
37	Cefotaxime, CTX	<=	0.06			1	AGA
37	Chloramphenicol, CHL	=	4	2	8	1	AGA
37	Ciprofloxacin, CIP	<=	0.008			1	AGA
37	Gentamicin, GEN	=	0.5		1	1	AGA
37	Nalidixic acid, NAL	=	4	1	4	1	AGA
37	Streptomycin, STR	=	4	4	16	1	AGA
37	Tetracycline, TET	=	2		2	1	AGA
37	Trimethoprim, TMP	=	0.5		2	1	AGA
39	Ampicillin, AMP	=	4	2	8	1	MIC
39	Cefotaxime, CTX	=	0.12			1	MIC
39	Ceftazidime, CAZ	=	0.5			1	MIC
39	Chloramphenicol, CHL	=	2	2	8	1	MIC
39	Ciprofloxacin, CIP	=	0.016			1	MIC
39	Gentamicin, GEN	=	0.5		1	1	MIC
39	Nalidixic acid, NAL	=	4	1	4	1	MIC
39	Streptomycin, STR	=	16	4	16	1	MIC
39	Tetracycline, TET	=	2		2	1	MIC
39	Trimethoprim, TMP	=	2		2	1	MIC
40	Ampicillin, AMP	=	8	2	8	1	MIC
40	Cefotaxime, CTX	=	0.25			0	MIC
40	Cefoxitin, FOX	=	8	2	8	1	MIC
40	Ceftazidime, CAZ	=	0.5			1	MIC
40	Ceftiofur, XNL	=	1		1	1	MIC
40	Chloramphenicol, CHL	=	16	2	8	0	MIC
40	Ciprofloxacin, CIP	=	0.03			0	MIC
40	Gentamicin, GEN	=	2		1	0	MIC
40	Nalidixic acid, NAL	=	16	1	4	0	MIC
40	Streptomycin, STR	=	8	4	16	1	MIC
40	Sulfisoxazole, FIS	=	256	8	32	0	MIC
40	Tetracycline, TET	=	8		2	0	MIC
40	Trimethoprim, TMP	=	2		2	1	MIC
42	Ampicillin, AMP	=	8	2	8	1	MIC
42	Cefotaxime, CTX	=	0.12			1	MIC
42	Cefoxitin, FOX	<=	4	2	8	1	MIC
42	Ceftazidime, CAZ	<=	0.25			1	MIC
42	Chloramphenicol, CHL	=	8	2	8	1	MIC
42	Ciprofloxacin, CIP	=	0.015			1	MIC
42	Gentamicin, GEN	=	1		1	1	MIC
42	Imipenem, IMI	<=	0.5			1	MIC
42	Nalidixic acid, NAL	<=	4	1	4	1	MIC
42	Streptomycin, STR	=	4	4	16	1	MIC
42	Sulfisoxazole, FIS	=	32	8	32	1	MIC
42	Tetracycline, TET	<=	1		2	1	MIC
42	Trimethoprim, TMP	=	1		2	1	MIC

Lab no	Antib	Operator	Value	Low limit	High limit	Mark	Method
57	Ampicillin, AMP	=	21	16	22	1	DD
57	Cefotaxime, CTX	=	32	29	35	1	DD
57	Cefoxitin, FOX	=	28	23	29	1	DD
57	Ceftazidime, CAZ	=	26	25	32	1	DD
57	Ceftiofur, XNL	=	23	26	31	0	DD
57	Chloramphenicol, CHL	=	32	21	27	0	DD
57	Ciprofloxacin, CIP	=	25	30	40	0	DD
57	Gentamicin, GEN	=	23	19	26	1	DD
57	Nalidixic acid, NAL	=	27	22	28	1	DD
57	Streptomycin, STR	=	20	12	20	1	DD
57	Sulfisoxazole, FIS	=	24	15	23	0	DD
57	Tetracycline, TET	=	23	18	25	1	DD
57	Trimethoprim, TMP	=	27	21	28	1	DD
58	Ampicillin, AMP	=	8	2	8	1	MIC
58	Cefotaxime, CTX	<=	0.06			1	MIC
58	Cefoxitin, FOX	=	4	2	8	1	MIC
58	Ceftazidime, CAZ	<=	0.25			1	MIC
58	Ceftiofur, XNL	=	0.5		1	1	MIC
58	Chloramphenicol, CHL	=	4	2	8	1	MIC
58	Ciprofloxacin, CIP	=	0.015			1	MIC
58	Gentamicin, GEN	=	1		1	1	MIC
58	Imipenem, IMI	<=	0.5			1	MIC
58	Nalidixic acid, NAL	<=	4	1	4	1	MIC
58	Streptomycin, STR	=	8	4	16	1	MIC
58	Sulfisoxazole, FIS	=	32	8	32	1	MIC
58	Tetracycline, TET	<=	1		2	1	MIC
58	Trimethoprim, TMP	<=	0.5		2	1	MIC

Appendix 7a - Summary of obtained results for the enterococci trial

Strain	Antimicrobial	Expected result	% R	% S	Number expected results	Number deviating results
EURL ENT 6.1	Ampicillin , AMP	S	4	96	24	1
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	S	24	76	22	7
	Gentamicin, GEN	S	4	96	25	1
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	S	6	94	16	1
	Streptomycin, STR	S	8	92	24	2
	Tetracycline, TET	S	3	97	28	1
	Vancomycin, VAN	S	3	97	28	1
EURL ENT 6.2	Ampicillin , AMP	S	16	84	21	4
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	S	7	93	27	2
	Gentamicin, GEN	S	4	96	25	1
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	S	0	100	17	0
	Streptomycin, STR	S	12	88	23	3
	Tetracycline, TET	R	100	0	29	0
	Vancomycin, VAN	R	100	0	29	0
EURL ENT 6.3	Ampicillin , AMP	S	4	96	24	1
	Chloramphenicol, CHL	R	82	18	23	5
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	R	100	0	29	0
	Gentamicin, GEN	R	100	0	29	0
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	-	100	0	0	0
	Streptomycin, STR	R	97	3	28	1
	Tetracycline, TET	R	100	0	29	0
	Vancomycin, VAN	S	7	93	27	2
EURL ENT 6.4	Ampicillin , AMP	S	4	96	24	1
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	S	3	97	28	1
	Gentamicin, GEN	S	8	92	24	2
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	-	100	0	0	0
	Streptomycin, STR	S	10	90	26	3
	Tetracycline, TET	S	3	97	28	1
	Vancomycin, VAN	S	7	93	27	2
EURL ENT 6.5	Ampicillin , AMP	S	4	96	24	1
	Chloramphenicol, CHL	R	100	0	29	0
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	S	3	97	28	1
	Gentamicin, GEN	S	8	92	24	2
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	-	100	0	0	0
	Streptomycin, STR	R	83	17	24	5
	Tetracycline, TET	R	100	0	29	0
	Vancomycin, VAN	S	3	97	28	1

Strain	Antimicrobial	Expected result	% R	% S	Number expected results	Number deviating results
EURL ENT 6.6	Ampicillin , AMP	S	4	96	24	1
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	R	100	0	29	0
	Gentamicin, GEN	S	4	96	25	1
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	-	100	0	0	0
	Streptomycin, STR	R	97	3	28	1
	Tetracycline, TET	R	100	0	29	0
	Vancomycin, VAN	S	3	97	28	1
EURL ENT 6.7	Ampicillin , AMP	S	0	100	25	0
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	S	3	97	28	1
	Gentamicin, GEN	S	8	92	24	2
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	-	100	0	0	0
	Streptomycin, STR	S	10	90	26	3
	Tetracycline, TET	R	97	3	28	1
	Vancomycin, VAN	S	3	97	28	1
EURL ENT 6.8	Ampicillin , AMP	S	0	100	25	0
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin , CIP	S	4	96	23	1
	Erythromycin, ERY	R	100	0	29	0
	Gentamicin, GEN	S	8	92	24	2
	Linezolid, LZD	S	4	96	24	1
	Quinu-dalfo-pristin, Q-D	-	100	0	0	0
	Streptomycin, STR	S	14	86	24	4
	Tetracycline, TET	R	100	0	29	0
	Vancomycin, VAN	S	3	97	28	1

Appendix 7b - Summary of obtained results for the staphylococci trial

Strain	Antimicrobial	Expected result	% R	% S	Number expected results	Number deviating results
EURL ST 6.1	Cefoxitin, FOX	R	100	0	29	0
	Chloramphenicol, CHL	S	0	100	32	0
	Erythromycin, ERY	R	100	0	34	0
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	S	3	97	32	1
	Penicillin, PEN	R	100	0	33	0
	Streptomycin, STR	S	8	92	24	2
	Sulfamethoxazole, SMX	S	4	96	24	1
	Tetracycline, TET	R	100	0	34	0
	Trimethoprim, TMP	S	0	100	29	0
EURL ST 6.2	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Erythromycin, ERY	S	0	100	34	0
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	S	3	97	32	1
	Penicillin, PEN	R	100	0	33	0
	Streptomycin, STR	S	12	88	23	3
	Sulfamethoxazole, SMX	S	16	84	21	4
	Tetracycline, TET	S	0	100	34	0
Trimethoprim, TMP	S	21	79	23	6	
EURL ST 6.3	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	3	97	31	1
	Erythromycin, ERY	S	0	100	34	0
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	S	3	97	32	1
	Penicillin, PEN	R	100	0	32	0
	Streptomycin, STR	S	4	96	25	1
	Sulfamethoxazole, SMX	S	12	88	23	3
	Tetracycline, TET	S	0	100	34	0
Trimethoprim, TMP	S	14	86	25	4	
EURL ST 6.4	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Erythromycin, ERY	S	0	100	34	0
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	S	3	97	32	1
	Penicillin, PEN	R	100	0	33	0
	Streptomycin, STR	S	4	96	25	1
	Sulfamethoxazole, SMX	S	0	100	24	0
	Tetracycline, TET	R	97	3	33	1
Trimethoprim, TMP	S	0	100	29	0	
EURL ST 6.5	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Erythromycin, ERY	S	0	100	34	0
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	S	3	97	32	1
	Penicillin, PEN	R	100	0	33	0
	Streptomycin, STR	R	100	0	26	0
	Sulfamethoxazole, SMX	S	8	92	24	2
	Tetracycline, TET	R	97	3	33	1
Trimethoprim, TMP	S	0	100	29	0	

EURL ST 6.6	Cefoxitin, FOX	R	97	3	28	1
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Erythromycin, ERY	S	3	97	33	1
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	S	0	100	33	0
	Penicillin, PEN	R	97	3	32	1
	Streptomycin, STR	R	100	0	26	0
	Sulfamethoxazole, SMX	S	0	100	26	0
	Tetracycline, TET	R	97	3	33	1
	Trimethoprim, TMP	R	100	0	29	0
EURL ST 6.7	Cefoxitin, FOX	R	100	0	29	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Erythromycin, ERY	S	3	97	33	1
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	S	3	97	32	1
	Penicillin, PEN	R	100	0	33	0
	Streptomycin, STR	S	12	88	23	3
	Sulfamethoxazole, SMX	S	0	100	25	0
	Tetracycline, TET	R	100	0	34	0
	Trimethoprim, TMP	S	3	97	28	1
EURL ST 6.8	Cefoxitin, FOX	R	100	0	29	0
	Chloramphenicol, CHL	S	0	100	32	0
	Erythromycin, ERY	S	0	100	34	0
	Florfenicol, FFN	S	0	100	12	0
	Gentamicin, GEN	R	97	3	32	1
	Penicillin, PEN	R	100	0	33	0
	Streptomycin, STR	R	100	0	26	0
	Sulfamethoxazole, SMX	R	88	12	22	3
	Tetracycline, TET	R	100	0	34	0
	Trimethoprim, TMP	S	0	100	29	0

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

Strain	Antimicrobial	Expected result	% R	% S	Number expected results	Number deviating results
EURL EC 6.1	Ampicillin, AMP	R	100	0	31	0
	Cefotaxime, CTX	R	100	0	33	0
	Ceftazidime, CAZ	R	100	0	31	0
	Ceftiofur, XNL	R	86	14	6	1
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Florfenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	3	97	32	1
	Nalidixic acid, NAL	S	0	100	33	0
	Streptomycin, STR	S	13	87	27	4
	Sulfamethoxazole, SMX	S	3	97	29	1
	Tetracycline, TET	S	3	97	32	1
	Trimethoprim, TMP	S	0	100	31	0
	EURL EC 6.2	Ampicillin, AMP	S	10	90	28
Cefotaxime, CTX		S	3	97	32	1
Ceftazidime, CAZ		S	3	97	29	1
Ceftiofur, XNL		S	0	100	8	0
Chloramphenicol, CHL		S	0	100	31	0
Ciprofloxacin, CIP		R	94	6	30	2
Florfenicol, FFN		S	0	100	26	0
Gentamicin, GEN		S	3	97	32	1
Nalidixic acid, NAL		R	100	0	33	0
Streptomycin, STR		S	6	94	30	2
Sulfamethoxazole, SMX		S	7	93	28	2
Tetracycline, TET		R	100	0	33	0
Trimethoprim, TMP		S	0	100	31	0
EURL EC 6.3		Ampicillin, AMP	R	100	0	31
	Cefotaxime, CTX	S	3	97	32	1
	Ceftazidime, CAZ	S	3	97	29	1
	Ceftiofur, XNL	S	0	100	7	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	3	97	31	1
	Florfenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	3	97	32	1
	Nalidixic acid, NAL	S	0	100	33	0
	Streptomycin, STR	R	100	0	32	0
	Sulfamethoxazole, SMX	R	100	0	30	0
	Tetracycline, TET	R	100	0	33	0
	Trimethoprim, TMP	R	100	0	31	0

Strain	Antimicrobial	Expected result	% R	% S	Number expected results	Number deviating results
EURL EC 6.4	Ampicillin, AMP	R	100	0	31	0
	Cefotaxime, CTX	R	100	0	33	0
	Ceftazidime, CAZ	R	97	3	30	1
	Ceftiofur, XNL	R	100	0	7	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Florfenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	0	100	33	0
	Nalidixic acid, NAL	S	0	100	33	0
	Streptomycin, STR	S	3	97	31	1
	Sulfamethoxazole, SMX	S	0	100	30	0
	Tetracycline, TET	S	0	100	33	0
	Trimethoprim, TMP	S	0	100	30	0
	EURL EC 6.5	Ampicillin, AMP	S	0	100	31
Cefotaxime, CTX		S	0	100	33	0
Ceftazidime, CAZ		S	3	97	29	1
Ceftiofur, XNL		S	0	100	7	0
Chloramphenicol, CHL		S	0	100	32	0
Ciprofloxacin, CIP		R	100	0	32	0
Florfenicol, FFN		S	0	100	26	0
Gentamicin, GEN		S	3	97	32	1
Nalidixic acid, NAL		R	100	0	33	0
Streptomycin, STR		R	97	3	31	1
Sulfamethoxazole, SMX		R	100	0	30	0
Tetracycline, TET		S	0	100	33	0
Trimethoprim, TMP		S	0	100	31	0
EURL EC 6.6		Ampicillin, AMP	S	3	97	30
	Cefotaxime, CTX	S	0	100	33	0
	Ceftazidime, CAZ	S	3	97	29	1
	Ceftiofur, XNL	S	0	100	7	0
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	0	100	32	0
	Florfenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	3	97	32	1
	Nalidixic acid, NAL	S	0	100	33	0
	Streptomycin, STR	S	6	94	30	2
	Sulfamethoxazole, SMX	S	3	97	29	1
	Tetracycline, TET	S	3	97	32	1
	Trimethoprim, TMP	S	0	100	31	0
	EURL EC 6.7	Ampicillin, AMP	R	100	0	31
Cefotaxime, CTX		R	100	0	33	0
Ceftazidime, CAZ		R	86	14	25	4
Ceftiofur, XNL		R	100	0	7	0
Chloramphenicol, CHL		S	0	100	32	0
Ciprofloxacin, CIP		R	100	0	32	0
Florfenicol, FFN		S	0	100	26	0
Gentamicin, GEN		R	97	3	32	1
Nalidixic acid, NAL		R	100	0	33	0
Sulfamethoxazole, SMX		R	100	0	30	0
Tetracycline, TET		R	100	0	33	0
Trimethoprim, TMP		R	100	0	31	0

Strain	Antimicrobial	Expected result	% R	% S	Number expected results	Number deviating results
EURL EC 6.8	Ampicillin, AMP	R	100	0	31	0
	Cefotaxime, CTX	R	97	3	32	1
	Ceftazidime, CAZ	R	100	0	31	0
	Ceftiofur, XNL	R	86	14	6	1
	Chloramphenicol, CHL	S	0	100	32	0
	Ciprofloxacin, CIP	S	3	97	31	1
	Florfenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	3	97	32	1
	Nalidixic acid, NAL	S	0	100	33	0
	Streptomycin, STR	S	9	91	29	3
	Sulfamethoxazole, SMX	S	3	97	29	1
	Tetracycline, TET	S	0	100	33	0
	Trimethoprim, TMP	R	100	0	31	0

Appendix 8a - Deviations from expected results in the enterococci trial

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic	Count	Method
LAB. 001	EURL ENT 6.3	Chloramphenicol, CHL	S	32	R	64	1	MIC
LAB. 011	EURL ENT 6.5	Streptomycin, STR	S	512	R	2048	1	MIC
LAB. 016	EURL ENT 6.1	Erythromycin, ERY	R	8	S	2	1	MIC
LAB. 017	EURL ENT 6.1	Erythromycin, ERY	R	8	S	2	1	MIC
	EURL ENT 6.3	Chloramphenicol, CHL	S	32	R	64	1	
LAB. 020	EURL ENT 6.2	Ampicillin , AMP	R	8	S	4	1	MIC
	EURL ENT 6.5	Streptomycin, STR	S	512	R	2048	1	
LAB. 021	EURL ENT 6.5	Streptomycin, STR	S		R	2048	1	MIC
LAB. 022	EURL ENT 6.2	Ampicillin , AMP	R	8	S	4	1	MIC
LAB. 025	EURL ENT 6.1	Erythromycin, ERY	R	8	S	2	1	MIC
	EURL ENT 6.2	Ampicillin , AMP	R	8	S	4	1	
LAB. 026	EURL ENT 6.3	Streptomycin, STR	S	10	R	>2048	1	DD
	EURL ENT 6.5	Streptomycin, STR	S	10	R	2048	1	
LAB. 029	EURL ENT 6.3	Vancomycin, VAN	R	128	S	<=1	1	MIC
	EURL ENT 6.4	Vancomycin, VAN	R	128	S	4	1	
LAB. 033	EURL ENT 6.7	Tetracycline, TET	S	>64	R	>32	1	MIC
LAB. 034	EURL ENT 6.1	Erythromycin, ERY	R	>8	S	2	1	MIC
	EURL ENT 6.2	Erythromycin, ERY	R	8	S	1	1	
	EURL ENT 6.6	Streptomycin, STR	S	128	R	>2048	1	
	EURL ENT 6.8	Streptomycin, STR	R	1024	S	128	1	
LAB. 036	EURL ENT 6.1	Erythromycin, ERY	R	16	S	2	1	MIC
LAB. 037	EURL ENT 6.3	Chloramphenicol, CHL	S	32	R	64	1	MIC
	EURL ENT 6.5	Streptomycin, STR	S	512	R	2048	1	
LAB. 039	EURL ENT 6.3	Chloramphenicol, CHL	S	32	R	64	1	MIC
LAB. 040	EURL ENT 6.1	Quinu-dalfo-pristin, Q-D	R	8	S	4	1	MIC
	EURL ENT 6.1	Streptomycin, STR	R	512	S	<=64	1	
	EURL ENT 6.2	Streptomycin, STR	R	512	S	<=64	1	
	EURL ENT 6.4	Gentamicin, GEN	R	128	S	<=16	1	
	EURL ENT 6.4	Streptomycin, STR	R	512	S	128	1	
	EURL ENT 6.5	Gentamicin, GEN	R	128	S	<=16	1	
	EURL ENT 6.7	Gentamicin, GEN	R	128	S	<=16	1	
	EURL ENT 6.7	Streptomycin, STR	R	1024	S	128	1	
	EURL ENT 6.8	Gentamicin, GEN	R	128	S	<=16	1	
	EURL ENT 6.8	Streptomycin, STR	R	1024	S	128	1	
LAB. 041	EURL ENT 6.2	Streptomycin, STR	R	>128	S	<=64	1	MIC
	EURL ENT 6.3	Chloramphenicol, CHL	S	32	R	64	1	
LAB. 046	EURL ENT 6.1	Erythromycin, ERY	R	8	S	2	1	MIC

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic	Count	Method
LAB. 054	EURL ENT 6.1	Ampicillin , AMP	R	14	S	<=2	1	DD
	EURL ENT 6.1	Ciprofloxacin , CIP	R	12	S	<=0.5	1	
	EURL ENT 6.1	Erythromycin, ERY	R	8	S	2	1	
	EURL ENT 6.1	Gentamicin, GEN	R	0	S	<=16	1	
	EURL ENT 6.1	Linezolid, LZD	R	18	S	2	1	
	EURL ENT 6.1	Streptomycin, STR	R	0	S	<=64	1	
	EURL ENT 6.1	Tetracycline, TET	R	13	S	<=1	1	
	EURL ENT 6.1	Vancomycin, VAN	R	13	S	<=1	1	
	EURL ENT 6.2	Ampicillin , AMP	R	14	S	4	1	
	EURL ENT 6.2	Chloramphenicol, CHL	R	12	S	4	1	
	EURL ENT 6.2	Ciprofloxacin , CIP	R	10	S	<=0.5	1	
	EURL ENT 6.2	Erythromycin, ERY	R	9	S	1	1	
	EURL ENT 6.2	Gentamicin, GEN	R	9	S	<=16	1	
	EURL ENT 6.2	Linezolid, LZD	R	16	S	2	1	
	EURL ENT 6.2	Streptomycin, STR	R	0	S	<=64	1	
	EURL ENT 6.3	Ampicillin , AMP	R	13	S	<=2	1	
	EURL ENT 6.3	Ciprofloxacin , CIP	R	12	S	1	1	
	EURL ENT 6.3	Linezolid, LZD	R	19	S	2	1	
	EURL ENT 6.3	Vancomycin, VAN	R	11	S	<=1	1	
	EURL ENT 6.4	Ampicillin , AMP	R	16	S	<=2	1	
	EURL ENT 6.4	Chloramphenicol, CHL	R	0	S	8	1	
	EURL ENT 6.4	Ciprofloxacin , CIP	R	9	S	1	1	
	EURL ENT 6.4	Erythromycin, ERY	R	8	S	<=0.5	1	
	EURL ENT 6.4	Gentamicin, GEN	R	0	S	<=16	1	
	EURL ENT 6.4	Linezolid, LZD	R	17	S	2	1	
	EURL ENT 6.4	Streptomycin, STR	R	0	S	128	1	
	EURL ENT 6.4	Tetracycline, TET	R	12	S	<=1	1	
	EURL ENT 6.4	Vancomycin, VAN	R	10	S	4	1	
	EURL ENT 6.5	Ciprofloxacin , CIP	R	0	S	1	1	
	EURL ENT 6.5	Erythromycin, ERY	R	12	S	<=0.5	1	
	EURL ENT 6.5	Gentamicin, GEN	R	0	S	<=16	1	
	EURL ENT 6.5	Linezolid, LZD	R	19	S	2	1	
	EURL ENT 6.5	Vancomycin, VAN	R	12	S	<=1	1	
	EURL ENT 6.6	Ampicillin , AMP	R	14	S	<=2	1	
	EURL ENT 6.6	Chloramphenicol, CHL	R	12	S	8	1	
	EURL ENT 6.6	Ciprofloxacin , CIP	R	10	S	1	1	
	EURL ENT 6.6	Gentamicin, GEN	R	0	S	<=16	1	
	EURL ENT 6.6	Linezolid, LZD	R	20	S	1	1	
	EURL ENT 6.6	Vancomycin, VAN	R	9	S	<=1	1	
	EURL ENT 6.7	Chloramphenicol, CHL	R	0	S	8	1	
	EURL ENT 6.7	Ciprofloxacin , CIP	R	10	S	1	1	
	EURL ENT 6.7	Erythromycin, ERY	R	12	S	<=0.5	1	
	EURL ENT 6.7	Gentamicin, GEN	R	0	S	<=16	1	
	EURL ENT 6.7	Linezolid, LZD	R	20	S	2	1	
	EURL ENT 6.7	Streptomycin, STR	R	0	S	128	1	
	EURL ENT 6.7	Vancomycin, VAN	R	10	S	<=1	1	
	EURL ENT 6.8	Chloramphenicol, CHL	R	0	S	8	1	
EURL ENT 6.8	Ciprofloxacin , CIP	R	12	S	1	1		
EURL ENT 6.8	Gentamicin, GEN	R	0	S	<=16	1		
EURL ENT 6.8	Linezolid, LZD	R	19	S	2	1		
EURL ENT 6.8	Streptomycin, STR	R	0	S	128	1		
EURL ENT 6.8	Vancomycin, VAN	R	11	S	<=1	1		
LAB. 057	EURL ENT 6.4	Streptomycin, STR	R	9	S	128	1	DD
	EURL ENT 6.7	Streptomycin, STR	R	10	S	128	1	
	EURL ENT 6.8	Streptomycin, STR	R	11	S	128	1	
LAB. 058	EURL ENT 6.5	Ampicillin , AMP	R	>8	S	<=2	1	MIC

Appendix 8b - Deviations from expected results in the staphylococci trial

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic	Count	Method
LAB. 004	EURL ST 6.6	Cefoxitin, FOX	S	26.95	R	16	1	MIC
LAB. 006	EURL ST 6.3	Trimethoprim, TMP	R	<=4	S	1	1	MIC
	EURL ST 6.7	Erythromycin, ERY	R	>8	S	0.5	1	
	EURL ST 6.7	Streptomycin, STR	R	<=32	S	<=4	1	
LAB. 013	EURL ST 6.4	Tetracycline, TET	S	30	R	16	1	DD
	EURL ST 6.8	Sulfamethoxazole, SMX	S	16	R	256	1	
LAB. 017	EURL ST 6.2	Trimethoprim, TMP	R	4	S	2	1	MIC
	EURL ST 6.3	Trimethoprim, TMP	R	4	S	1	1	
LAB. 018	EURL ST 6.8	Sulfamethoxazole, SMX	S	14	R	256	1	DD
LAB. 020	EURL ST 6.2	Sulfamethoxazole, SMX	R	>512	S	64	1	MIC
	EURL ST 6.2	Trimethoprim, TMP	R	4	S	2	1	
	EURL ST 6.3	Sulfamethoxazole, SMX	R	>512	S	<=32	1	
	EURL ST 6.3	Trimethoprim, TMP	R	4	S	1	1	
	EURL ST 6.5	Sulfamethoxazole, SMX	R	512	S	<=32	1	
	EURL ST 6.7	Trimethoprim, TMP	R	4	S	1	1	
LAB. 026	EURL ST 6.6	Tetracycline, TET	S	>32	R	>32	1	MIC
LAB. 034	EURL ST 6.2	Streptomycin, STR	R	32	S	<=4	1	MIC
	EURL ST 6.2	Trimethoprim, TMP	R	4	S	2	1	
LAB. 036	EURL ST 6.2	Trimethoprim, TMP	R	4	S	2	1	MIC
	EURL ST 6.3	Trimethoprim, TMP	R	4	S	1	1	
LAB. 039	EURL ST 6.2	Trimethoprim, TMP	R	4	S	2	1	MIC
LAB. 040	EURL ST 6.1	Streptomycin, STR	R	32	S	8	1	MIC
	EURL ST 6.1	Sulfamethoxazole, SMX	R	256	S	<=32	1	
	EURL ST 6.6	Erythromycin, ERY	R	4	S	0.5	1	
	EURL ST 6.7	Streptomycin, STR	R	32	S	<=4	1	
LAB. 042	EURL ST 6.2	Sulfamethoxazole, SMX	R	>512	S	64	1	MIC
	EURL ST 6.5	Sulfamethoxazole, SMX	R	512	S	<=32	1	
LAB. 054	EURL ST 6.1	Gentamicin, GEN	R	11	S	<=0.25	1	DD
	EURL ST 6.1	Streptomycin, STR	R	9	S	8	1	
	EURL ST 6.2	Gentamicin, GEN	R	9	S	<=0.25	1	
	EURL ST 6.2	Streptomycin, STR	R	8	S	<=4	1	
	EURL ST 6.3	Ciprofloxacin, CIP	R	14	S	0.5	1	
	EURL ST 6.3	Gentamicin, GEN	R	10	S	0.5	1	
	EURL ST 6.3	Streptomycin, STR	R	8	S	8	1	
	EURL ST 6.4	Gentamicin, GEN	R	10	S	<=0.25	1	
	EURL ST 6.4	Streptomycin, STR	R	9	S	8	1	
	EURL ST 6.5	Gentamicin, GEN	R	10	S	0.5	1	
	EURL ST 6.7	Gentamicin, GEN	R	12	S	0.5	1	
	EURL ST 6.7	Streptomycin, STR	R	0	S	<=4	1	
LAB. 056	EURL ST 6.2	Sulfamethoxazole, SMX	R	6	S	64	1	DD
	EURL ST 6.3	Sulfamethoxazole, SMX	R	6	S	<=32	1	
LAB. 057	EURL ST 6.6	Penicillin, PEN	S	13	R	8	1	DD
	EURL ST 6.8	Gentamicin, GEN	S	13	R	>16	1	
	EURL ST 6.8	Sulfamethoxazole, SMX	S	15	R	256	1	
LAB. 058	EURL ST 6.2	Streptomycin, STR	R	32	S	<=4	1	MIC
	EURL ST 6.2	Sulfamethoxazole, SMX	R	512	S	64	1	
	EURL ST 6.2	Trimethoprim, TMP	R	4	S	2	1	
	EURL ST 6.3	Sulfamethoxazole, SMX	R	512	S	<=32	1	
	EURL ST 6.5	Tetracycline, TET	S	1	R	32	1	

Appendix 8c- Deviations from expected results in the *E. coli* trial

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected Mic	Count	Method
LAB. 004	EURL EC 6.1	Streptomycin, STR	R	16	S	<=8	1	MIC
LAB. 019	EURL EC 6.2	Ciprofloxacin, CIP	S	0.5	R	0.25	1	MIC
LAB. 020	EURL EC 6.2	Sulfamethoxazole, SMX	R	128	S	<=64	1	MIC
LAB. 026	EURL EC 6.7	Ceftazidime, CAZ	S	0.5	R	1	1	MIC
LAB. 029	EURL EC 6.1	Ceftiofur, XNL	S	20mm	R	8	1	MIC
	EURL EC 6.2	Ampicillin, AMP	R	32	S	4	1	
	EURL EC 6.8	Ceftiofur, XNL	S	20mm	R	8	1	
LAB. 032	EURL EC 6.7	Ceftazidime, CAZ	S	<=0.5	R	1	1	MIC
LAB. 033	EURL EC 6.1	Streptomycin, STR	R	16	S	<=8	1	MIC
LAB. 039	EURL EC 6.1	Streptomycin, STR	R	16	S	<=8	1	MIC
	EURL EC 6.1	Sulfamethoxazole, SMX	R	>1024	S	<=64	1	
	EURL EC 6.2	Ampicillin, AMP	R	32	S	4	1	
	EURL EC 6.2	Cefotaxime, CTX	R	0.5	S	<=0.12	1	
	EURL EC 6.2	Ceftazidime, CAZ	R	4	S	0.125	1	
	EURL EC 6.2	Streptomycin, STR	R	16	S	<=8	1	
	EURL EC 6.2	Sulfamethoxazole, SMX	R	>1024	S	<=64	1	
	EURL EC 6.3	Cefotaxime, CTX	R	2	S	<=0.12	1	
	EURL EC 6.3	Ceftazidime, CAZ	R	16	S	0.125	1	
	EURL EC 6.3	Ciprofloxacin, CIP	R	0.12	S	<=0.015	1	
	EURL EC 6.5	Ceftazidime, CAZ	R	2	S	0.06	1	
	EURL EC 6.6	Ceftazidime, CAZ	R	8	S	0.25	1	
	EURL EC 6.6	Sulfamethoxazole, SMX	R	>1024	S	<=64	1	
EURL EC 6.8	Sulfamethoxazole, SMX	R	1024	S	<=64	1		
LAB. 040	EURL EC 6.6	Streptomycin, STR	R	32	S	<=8	1	MIC
	EURL EC 6.7	Ceftazidime, CAZ	S	0.5	R	1	1	
	EURL EC 6.8	Ciprofloxacin, CIP	R	0.12	S	0.03	1	
	EURL EC 6.8	Streptomycin, STR	R	32	S	<=8	1	
LAB. 054	EURL EC 6.1	Gentamicin, GEN	R	12	S	1	1	DD
	EURL EC 6.1	Streptomycin, STR	R	9	S	<=8	1	
	EURL EC 6.1	Tetracycline, TET	R	14	S	<=2	1	
	EURL EC 6.2	Ampicillin, AMP	R	12	S	4	1	
	EURL EC 6.2	Gentamicin, GEN	R	12	S	1	1	
	EURL EC 6.2	Streptomycin, STR	R	8	S	<=8	1	
	EURL EC 6.3	Gentamicin, GEN	R	12	S	<=0.5	1	
	EURL EC 6.4	Streptomycin, STR	R	10	S	<=8	1	
	EURL EC 6.5	Gentamicin, GEN	R	12	S	1	1	
	EURL EC 6.6	Ampicillin, AMP	R	12	S	4	1	
	EURL EC 6.6	Gentamicin, GEN	R	11	S	<=0.5	1	
	EURL EC 6.6	Streptomycin, STR	R	10	S	<=8	1	
	EURL EC 6.6	Tetracycline, TET	R	14	S	<=2	1	
	EURL EC 6.8	Gentamicin, GEN	R	12	S	1	1	
EURL EC 6.8	Streptomycin, STR	R	11	S	<=8	1		
LAB. 057	EURL EC 6.2	Ciprofloxacin, CIP	S	29	R	0.25	1	DD
	EURL EC 6.4	Ceftazidime, CAZ	S	24	R	2	1	
	EURL EC 6.5	Streptomycin, STR	S	13	R	>128	1	
	EURL EC 6.7	Ceftazidime, CAZ	S	25	R	1	1	
	EURL EC 6.7	Gentamicin, GEN	S	14	R	>16	1	
	EURL EC 6.8	Cefotaxime, CTX	S	19	R	>4	1	
LAB. 058	EURL EC 6.8	Streptomycin, STR	R	16	S	<=8	1	MIC

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

ISBN: 978-87-92763-79-2