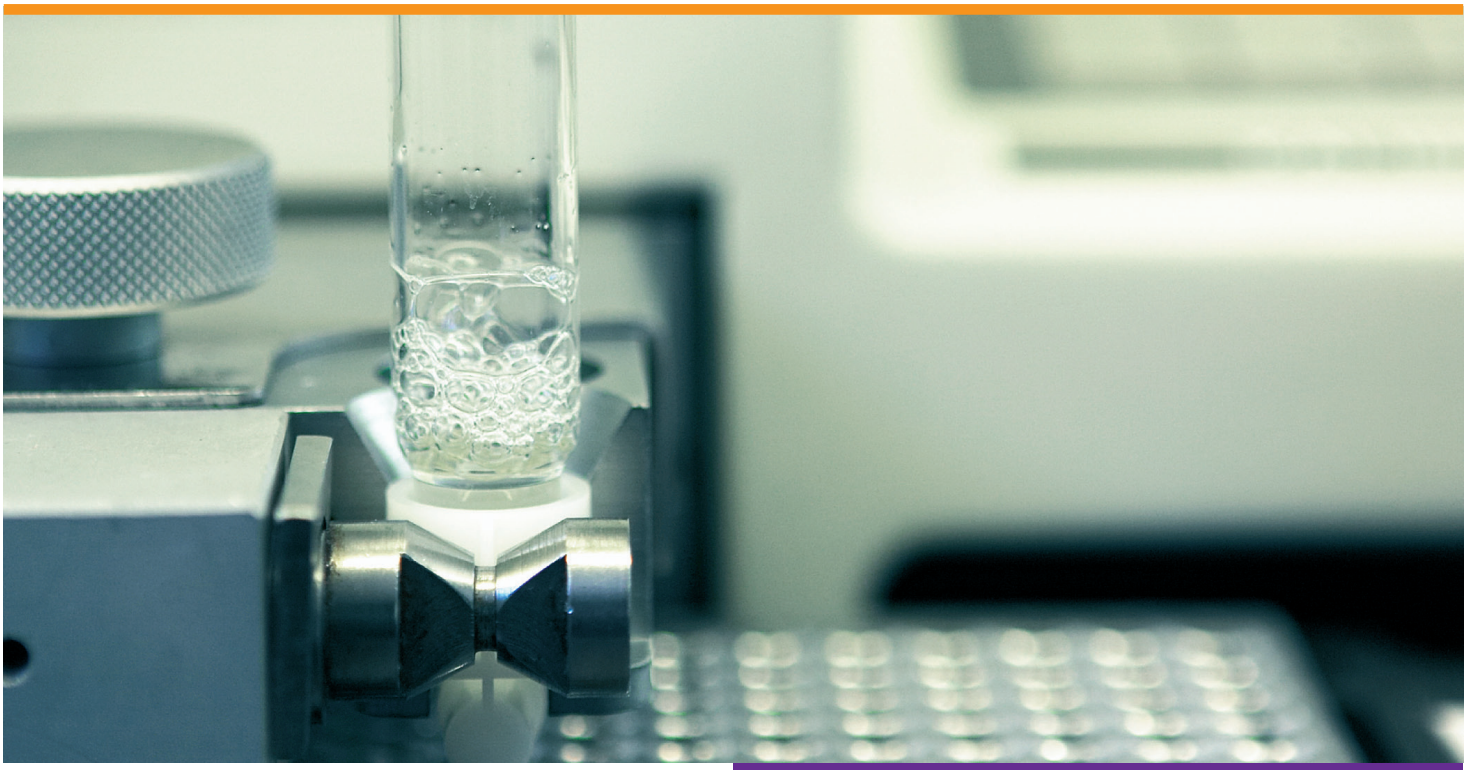
The 3rd CRL Proficiency Testing *Salmonella* and *Campylobacter* 2007



DTU Food
National Food Institute

Community Reference Laboratory Antimicrobial Resistance

Community Reference Laboratory – Antimicrobial Resistance

THE 3RD CRL PROFICIENCY TESTING
SALMONELLA AND *CAMPYLOBACTER* – 2007

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1. Introduction

In this report, results are summarised from the third proficiency test trial conducted by the National Food Institute (DTU Food) as the Community Reference Laboratory (CRL) for antimicrobial resistance. This proficiency test focuses on *Salmonella* and *Campylobacter* and is the second External Quality Assurance System (EQAS) conducted for these microorganisms (the first was EQAS 2006).

The objective of the EQAS is to monitor the quality of the antimicrobial susceptibility data produced and to point out areas or laboratories, for which guidance or assistance would be required as means of producing reliable susceptibility data. The goal is having all laboratories perform susceptibility testing with less than 7% incorrect interpretations.

The technical advisory group for the CRL EQAS scheme consists of competent representatives from all NRL's, who meet once a year at the CRL- workshop.

The data in this report are presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only to the CRL and the EU Commission. All conclusions are public.

2. Materials and methods

2.1 Participants

A pre-notification (App. 1) of the CRL EQAS on susceptibility testing of *Salmonella* and *Campylobacter* was distributed on the 4th of September 2007 by e-mail to the 33 national reference laboratories (NRL) in the CRL-network (including Norway). The pre-notification was sent to NRL's in all EU countries except Malta, where no contact has been established. All 33 laboratories responded. One laboratory declined to participate, as susceptibility testing no longer is a particular interest of the laboratory. The laboratory in question was not appointed NRL by the member state, but was asked by the CRL to join the network. A second laboratory declined to participate as they have neither *Salmonella* nor *Campylobacter* as their field of responsibility.

Appendix 2 shows that 26 of the 31 participating NRL's were appointed by the individual member states. Four NRL's have not been appointed, but have – along with Norway – been

enrolled on equal terms as the designated NRL's, based on their participation in an EU funded concerned action (FAIR5-QLK2-2002-01146), the ARBAO II project (Antibiotic Resistance in Bacteria of Animal Origin). The laboratory in Norway has been charged a fee for the participation in the EQAS, whereas the NRL's from EU member states participate free of charge.

Figure 1 shows that 26 countries participated of which four countries uploaded only the *Salmonella* results, and 22 countries tested both *Salmonella* and *Campylobacter*.

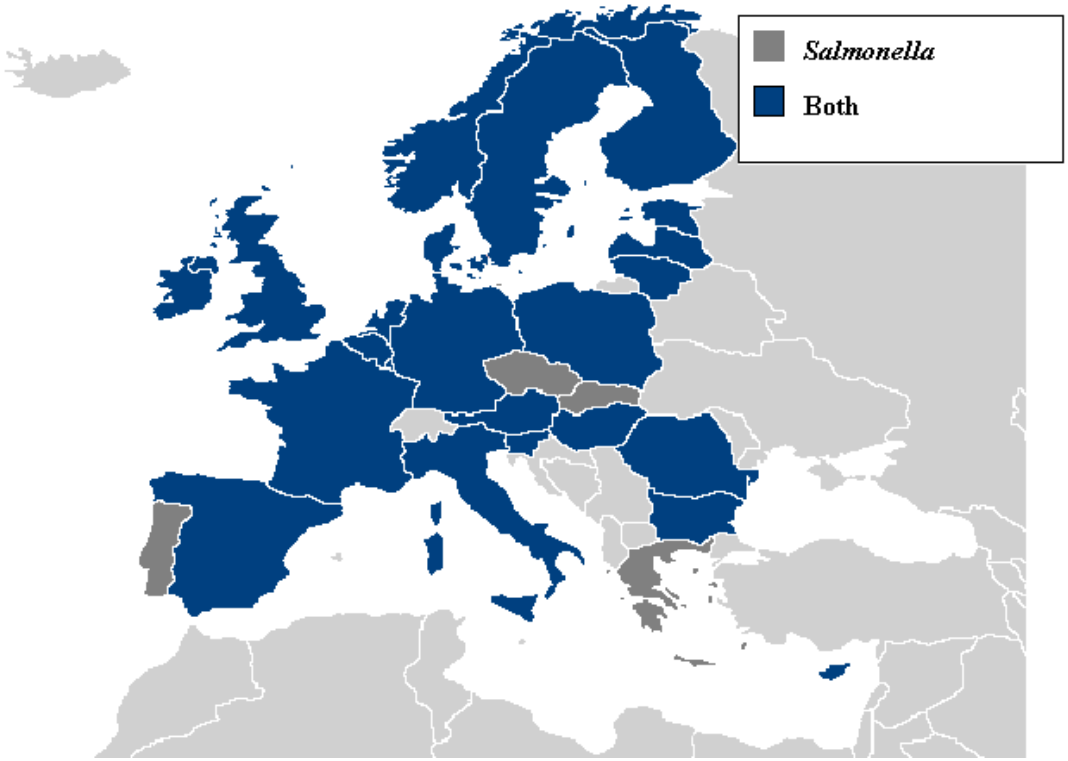

Figure 1: Participating countries that perform antimicrobial susceptibility testing of *Salmonella* or both *Salmonella* and *Campylobacter*

2.2 Strains

Eight strains of *Salmonella* and eight strains of *Campylobacter* were selected for this trial among isolates from the strain collection at DTU Food. Individual sets of the *Salmonella* strains were inoculated as agar stab cultures and the *Campylobacter* strains were lyophilised by Czech Collection of Micro-organisms (CCM); The Czech Republic. The verification of the test strains before shipping indicated a problem with the viability of the lyophilised *Campylobacter* test strain C2.4. This test strain was therefore shipped as a charcoal swab. As a consequence, it

has been decided that for future EQAS's in the CRL-network, all *Campylobacter* test strains will be shipped as charcoal swabs.

The shipment of strains also included the lyophilised international reference strains for susceptibility testing; *E. coli* CCM 3954 (ATCC 25922) and *Campylobacter jejuni* CCM 6214 (ATCC 33560) purchased at CCM. This was relevant only for the NRL's which had not been provided with these reference strains in prior EQAS's conducted by DTU Food.

Antimicrobial susceptibility testing (AST) on the *Salmonella* and *Campylobacter* strains was performed at DTU Food and verified by the US Food and Drug Administration (FDA) prior to distribution. The obtained MIC values serve as reference for the test strains (App. 3a and 3b). However, results from the following antimicrobials were not verified by FDA: cefotaxime; cefotaxime + clavulanic acid; ceftazidime; ceftazidime + clavulanic acid; imipenem; imipenem + EDTA; and trimethoprim for *Salmonella*, and streptomycin and chloramphenicol for *Campylobacter*.

2.3 Antimicrobials

The antimicrobials used in the EQAS are listed in the protocol (App. 4b) and have been included mainly according to the recommendations in the EFSA monitoring programme. A few additional antimicrobials have been added as indicated in the protocol.

The selection of antimicrobials used in the trial for *Salmonella* was: amoxicillin + clavulanic acid; ampicillin; cefotaxime; cefotaxime + clavulanic acid; cefoxitin; ceftazidime; ceftazidime + clavulanic acid; ceftiofur; chloramphenicol; ciprofloxacin; gentamicin; imipenem; imipenem + EDTA; nalidixic acid; streptomycin; sulfonamides (sulphamethoxazole); tetracycline; trimethoprim and trimethoprim + sulfonamides.

MIC determination of the *Salmonella* test strains was performed using Sensititre systems from Trek diagnostics Ltd with the exception of cefotaxime + clavulanic acid, cefoxitin, ceftazidime + clavulanic acid, imipenem, imipenem + EDTA; and trimethoprim + sulfonamides. These exceptions were tested using E-test from AB-Biodisk. The method guidelines used were according to the Clinical and Laboratory Standards Institute (CLSI) document M07-A7 (2006) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically" (Approved Standard - Seventh Edition), document M100-S17 (2007) "Performance Standards

for Antimicrobial Susceptibility Testing" (Seventeenth Informational Supplement) and document M31-A2 (2002) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated from Animals" (Approved Standard - Second Edition).

For *Campylobacter* the following antimicrobials were included: chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin, and tetracycline. MIC determination was performed using Sensititre systems from Trek diagnostics Ltd according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) document M45-A (2006) "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria" (Approved Guideline).

2.4 Distribution

The test strains and a welcome letter (App. 4a) were enclosed in double pack containers (class UN 6.2) and shipped ultimo October 2007 to the selected laboratories as dangerous goods UN3373 according to the International Air Transport Association (IATA) regulations. Prior to shipping, each laboratory was informed about the parcels and the air way bill (AWB) number for tracking of the parcel. A number of the participants (countries in which Fedex does not offer delivery of dangerous goods) were asked to pick up the parcel at the airport nearest to their institute. Import permit was necessary for shipping the parcel to Norway, Latvia and Romania.

2.5 Procedure

By email, the laboratories were provided with protocols and information regarding the handling of the test strains and reference strains (App. 4b, c, d). Additionally, an evaluation form and a questionnaire (App. 4e, f) were attached to the email. The participants were instructed to subculture the strains according to the description in the protocol prior to performing the antimicrobial susceptibility test. Furthermore, they were requested to save and maintain the ATCC reference strain(s) for future proficiency tests.

It is the aim that MIC methods only should be used when performing AST for the CRL EQAS's and for the monitoring conducted by the Commission. Consequently, it was decided by the participants at the CRL-workshop in May 2007 that the NRL's should work towards harmonising to MIC methods for these AST analyses. Additionally, it was agreed upon that all NRL's work towards covering the antimicrobial panel and cut-off values recommended by the CRL. For this EQAS, the participants were instructed to use as many as possible of the antimicrobials listed, using the method routinely used in the laboratory.

The cut off values recommended by EFSA should be used (listed in the protocol). All cut off values used in the interpretation of the *Campylobacter* MIC results have been developed by EUCAST (www.eucast.org). This is also the case for most cut off values with regard to *Salmonella*, exceptions are streptomycin and sulphonamides where values from DTU Food and CLSI, respectively, were used according to the description in the protocol (App. 4b).

Participants using disk diffusion and E-test were recommended to interpret the results according to their individual routine, categorising the test strains into the terms resistant and sensitive. The breakpoints used were submitted to the web based database, from which the relevant breakpoints (disk diffusion for *Salmonella*) are listed in Appendix 5.

It should be noted that for AST of *Campylobacter* only MIC methods are recommendable, i.e. broth or agar dilution methods. The CRL do not recommend the use of neither disk diffusion nor E-test for AST of *Campylobacter*. In addition, when reporting monitoring data to EFSA these have to be submitted as MIC-results.

The laboratories were instructed to upload the obtained MIC values or zone-diameter in millimetres and the susceptibility categories (resistant or sensitive) to an electronic record sheet in the CRL web based database through a secured individual login and passwords. Alternatively, the record sheets from the protocol could be sent by fax to DTU Food. The website was open for entry in the period from the 2nd of November 2007 to the 31st of January 2008.

Detection of ESBL-producing test strains should be performed and interpreted according to recommendations in the protocol: when an isolate is found resistant to one cephalosporin, the isolate should be regarded resistant to all cephalosporins.

Results from the reference strains should also be entered into the database. The results could be either the zone diameter in millimetres or the MIC value for the reference strains *E. coli* (ATCC 25922) and *C. jejuni* (ATCC 33560). The results should individually be in agreement with the quality control ranges according to the relevant guideline of the following: the CLSI documents M31-A2 (2002) / M100-S17 (2007) / M45-A (2006); The Sensititre System, Trek Diagnostic; or E-tests, AB-Biodisk (App. 7).

After submitting the data the laboratories were instructed to retrieve the instantly generated, individual evaluation report from the secured web site. The evaluation report evaluated the submitted results, reporting all deviations from the expected. Deviations were categorised as 'incorrect'.

The returned evaluation forms and questionnaires were later collected and summarised (App. 8, 9).

3. Results

The participants were asked to report results, including MIC values or disk diffusion diameters as well as the categorisation as either resistant or sensitive. Only the categorisation was evaluated, whereas the MIC value and disk diffusion was background information.

Some participants included 'intermediate' as a category due to the fact that this was their daily routine. The protocol refers to the EFSA monitoring programme and the use of epidemiological cut off values as regards the categorisation of susceptibility. Moreover, it is not possible to upload 'intermediate' as a result in the database. 'Intermediate' results have therefore not been evaluated.

During the first evaluation of the results it was observed that the percentage of correct results of the combination of the *Campylobacter* C2.1 and streptomycin was very low (14%). This issue was evaluated by the organisers and the technical advisory group at the annual workshop, and consequently it was decided to leave the results from this test strain and antimicrobial out of the EQAS-evaluation.

3.1 Methods used by EQAS-participants

In the *Salmonella* trials, 19 laboratories used MIC determination, one used E-test, and ten laboratories used disk diffusion (however, some laboratories supplement one method with the other). The majority of laboratories (n=21) used MIC determination for the *Campylobacter* (microbroth or agar dilution). Two NRL's reported the use of E-test (#4 and #15), whereas two laboratories (#5 and #23) used disk diffusion.

The categorisation – not the specific results – of *Campylobacter* is evaluated in this report when *Campylobacter* AST has been performed by disk diffusion or by E-test.

3.2 Deviations by strain and antimicrobial

Figure 2 shows the total percentage of deviations from the expected results of AST performed by participating laboratories. For the *Salmonella* strains, 96.7% of the AST's were interpreted correctly. For the *Campylobacter* strains, 94.2% of AST's were correct. Compared to the CRL EQAS 2006 this is an improvement for the *Salmonella* AST, whereas the level of performance with regard to *Campylobacter* is similar this year. The number of participants in EQAS 2007 was the same as in EQAS 2006, except the *Salmonella* EQAS, where one more laboratory participated in 2007.

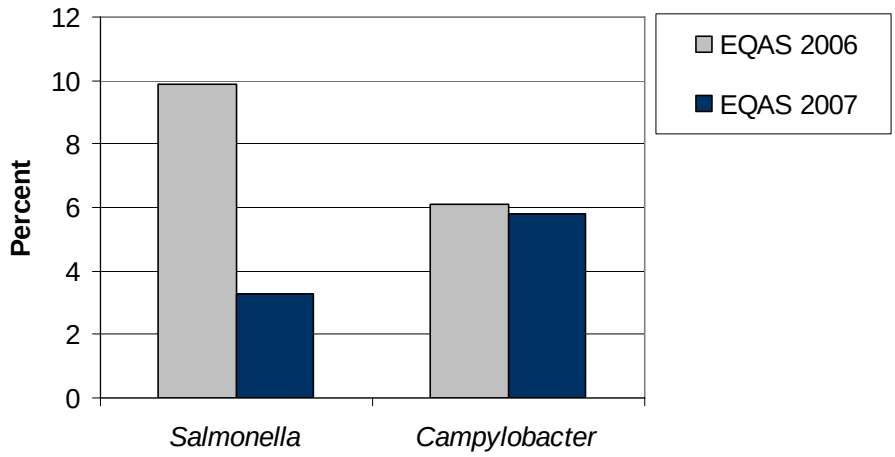

Figure 2: A comparison between EQAS 2006 and EQAS 2007 showing the percent of deviations in total for antimicrobial susceptibility testing performed by participating laboratories

Figure 3 shows the total percentage of deviations from the expected results of AST performed by MIC-methods as opposed to disk diffusion or E-test. For both the *Salmonella* and the *Campylobacter* strains the deviation percent is more than twice as high when performed by diffusion methods compared to MIC-methods.

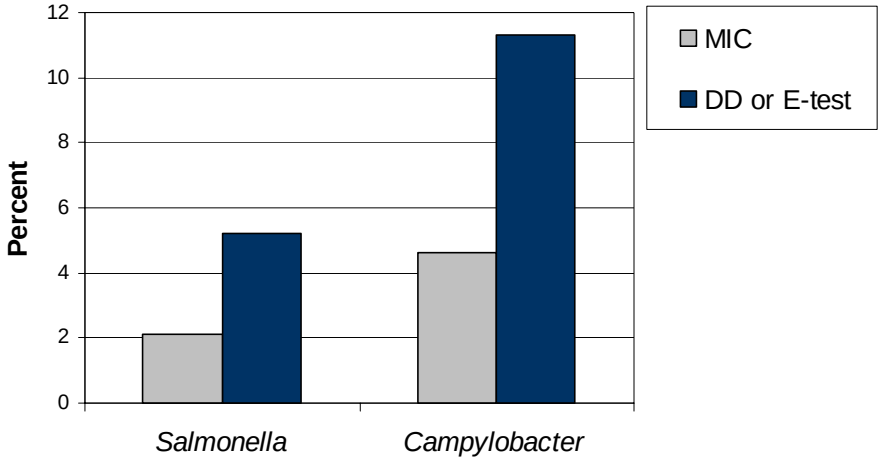

Figure 3: The percent of deviations in total for EQAS 2007 for AST's is shown comparing the results when using MIC-methods as opposed to disk diffusion or E-test.

The number of AST's performed and the percentage of correct results for the individual *Salmonella* and *Campylobacter* strains in the EQAS, are listed in Table 1. There is a large variation between strains of the same species, from 92.3-99.7% for *Salmonella* and from 84.4-99.3% for *Campylobacter*.

EQAS 2007 - *Salmonella*			EQAS 2007 - *Campylobacter*		
Test strain	AST in total	% correct	Test strain	AST in total	% correct
S-2.1	336	95.2	C-2.1 (*C. jejuni*)	126	97.6
S-2.2	339	**92.3**	C-2.2 (*C. coli*)	152	99.3
S-2.3	346	96.0	C-2.3 (*C. jejuni*)	154	97.4
S-2.4	347	97.1	C-2.4 (*C. coli*)	160	96.9
S-2.5	344	95.3	C-2.5 (*C. coli*)	147	**84.4**
S-2.6	333	99.7	C-2.6 (*C. jejuni*)	97	**89.7**
S-2.7	347	98.8	C-2.7 (*C. jejuni*)	146	**91.8**
S-2.8	342	99.1	C-2.8 (*C. jejuni*)	146	95.2

Table 1: The number of AST performed and the percentage of correct results for each strain of *Salmonella* and *Campylobacter*

For *Salmonella* strains, much difficulty was observed for strain S2.2 (92.3% correct). This strain was also included in EQAS 2006, where the percentage of correct results was considerably lower: 85.3%. Strain S2.2 is resistant to the following antimicrobials: amoxicillin + clavulanic acid; ampicillin; cefotaxime; cefotaxime + clavulanic acid; ceftazidime; ceftiofur; ciprofloxacin; nalidixic acid and tetracycline.

For *Campylobacter*, strain C2.5, C2.6 and strain C2.7 caused problems for the participants. In particular the percentage of correct results was low regarding strain C2.5 (84.4% correct) which is resistant to erythromycin and tetracycline.

In Table 2, the percentage of correct AST per antimicrobial by species is shown. When testing *Salmonella* it seems that two antimicrobial have considerably lower percentages than the others. For both amoxicillin + clavulanic acid and ciprofloxacin the levels of correct results based on the susceptibility categorisation are low (81.5% and 90.0%). In the 2006 EQAS, amoxicillin + clavulanic acid also posed a problem (70.0% correct) as did ciprofloxacin (79.8% correct). Thus, in both cases we have observed an increase in performance.

For *Campylobacter* one antimicrobial stands out as different in deviation percent compared to the other antimicrobials on the list. This is tetracycline for which the percentage was 87.2% correct. This antimicrobial also caused some problems in the 2006 EQAS with 90.1% correct.

EQAS 2007 Antimicrobial	% correct	
	Salmonella	*Campylobacter*
Amoxicillin cl., AUG	**81.5**	-
Ampicillin, AMP	99.2	-
Cefotaxime, CTX	96.7	-
Ceftazidime, CAZ	93.7	-
Ceftiofur, XNL	98.3	-
Chloramphenicol, CHL	97.8	98.1
Ciprofloxacin, CIP	**90.0**	93.3
Erythromycin, ERY	-	96.1
Gentamicin, GEN	98.3	95.4
Nalidixic acid, NAL	99.6	97.0
Streptomycin, STR	95.3	94,3
Sulphonamides, SMX	99.5	-
TMP+SMX, SXT	98.3	-
Tetracycline, TET	98.7	**87.2**
Trimethoprim, TMP	99.1	-

Table 2: Percentage of correct antimicrobial susceptibility tests per antimicrobial by microorganism. Marked in grey are antimicrobials recommended in the EFSA zoonosis monitoring manual.

The laboratories were asked to detect the ESBL producing *Salmonella* strains (S2.2 and S2.8) according to the description in the protocol, in which it is described that ESBL producing strains that are resistant to one cephalosporin should be interpreted resistant to all cephalosporins regardless the value detected from the results. Of the 30 laboratories which tested *Salmonella*, 29 uploaded results from ESBL-testing.

The test strain S2.2 was a 'true ESBL' with a CTX M-9-gene, whereas the S2.8 was an AmpC with a CMY-2-gene. As presented in Tables 3 and 4, it seems that the laboratories quite confidently detected and confirmed the 'true ESBL' (96%) but had some difficulties confirming the AmpC-isolate (83%).

There is a differences in the number of cephalosporins used by the laboratories in their routine for testing for ESBL-production, ranging between the five compounds which are included in this proficiency test: cefotaxime, ceftazidime, ceftiofur, cefotaxime + clavulanic acid and ceftazidime + clavulanic acid. The first three are used for initial screening whereas the last two are used for confirmatory test (the double disk test).

The sole use of cefotaxime or ceftazidime posed a problem for both ESBL-producing strains, where an average of 63% of the laboratories did not find the expected resistance. Especially strain S2.2 with the CTX M-9-gene only showed resistance to either of the two drugs in one of five tests.

The combination of the antimicrobials cefotaxime and ceftazidime also posed some problems. Only one of the deviating results was caused by 'sensitive' categorizations to both antimicrobials. The remaining five deviating results were from participants of which one participant found ceftazidime to be resistant and cefotaxime to be sensitive, and four participants as expected categorized cefotaxime as resistant, but failed to follow the guideline that describes the fact that if one cephalosporin is resistant, all cephalosporins should be regarded resistant.

The use of the combination of the antimicrobials cefotaxime and ceftiofur was very successful in detecting ESBL-producing strain (100%) as was the combination of all three of cefotaxime, ceftazidime and ceftiofur (in total: 92%). When using the combination of the three mentioned antibiotics, one participant failed to follow the guideline regarding cephalosporins, and categorized ceftazidime according to the cut off value as sensitive.

Two participants used only ceftiofur, and both obtained the expected result that the test strains S2.2 and S2.8 were both resistant to this antimicrobial.

In addition to the test strains that were ESBL-producing, three laboratories reported resistance towards cephalosporins for either S2.3 or S2.4. None of these three performed confirmatory tests on these strains and did not report the strains as ESBL-producing or AmpC.

	Strain S2.2 (CTX M-9)		Strain S2.8 (CMY-2)	
	ESBL/AmpC NOT indicated	ESBL/AmpC indicated	ESBL/AmpC NOT indicated	ESBL/AmpC indicated
CTX, CAZ, XNL	1 of 6 (17%)	5 of 6 (83%)	0	7 of 7 (100%)
CTX, CAZ	5 of 10 (50%)	5 of 10 (50%)	1 of 9 (11%)	8 of 9 (89%)
CTX, XNL	0	6 of 6 (100%)	0	6 of 6 (100%)
CAZ	2 of 2 (100%)	0	0	1 of 1 (100%)
CTX	2 of 3 (67%)	1 of 3 (33%)	1 of 2 (50%)	1 of 2 (50%)
XNL	0	2 of 2 (100%)	0	2 of 2 (100%)

Table 3: Number and percentages of laboratories which correctly and incorrectly detected the two ESBL producing *Salmonella* strains. Shaded squares are expected results.

	Strain S2.2 (CTX M-9)		Strain S2.8 (CMY-2)	
	NOT confirmed	Confirmed	NOT confirmed	Confirmed
CTX/Cl:CTX	1 of 23 (4%)	22 of 23 (96%)	12 of 14 (86%)	2 of 14 (14%)
CAZ/Cl:CAZ	12 of 20 (60%)	8 of 20 (40%)	12 of 14 (86%)	2 of 14 (14%)
FOX	16 of 17 (94%)	1 of 17 (6%)	1 of 17 (6%)	16 of 17 (94%)
Confirmed ESBL in the database	1 of 23 (4%)	22 of 23 (96%)	15 of 15 (100%)	0
Confirmed AmpC in the database	16 of 16 (100%)	0	3 of 18 (17%)	15 of 18 (83%)

Table 4: Number and percentages of laboratories which correctly and incorrectly confirmed the two ESBL producing *Salmonella* strains. Shaded squares are expected results.

3.3 Deviations by laboratory

Figure 4 and 6 illustrate the percentage of deviations and the severity hereof for each participating laboratory. The laboratories are ranked according to their performance determined by the percentage of deviating results with regard to all uploaded results. Obtained results only including tests with antimicrobials recommended by EFSA are additionally indicated. In Figure 5 and 7 the total amount of deviations in percentages is illustrated by number of laboratories.

3.3.1 *Salmonella* trial

Five of the laboratories obtained a result of 100% correctly tested *Salmonella* strains. The maximum percentage of deviations was 13.6% in laboratory #32.

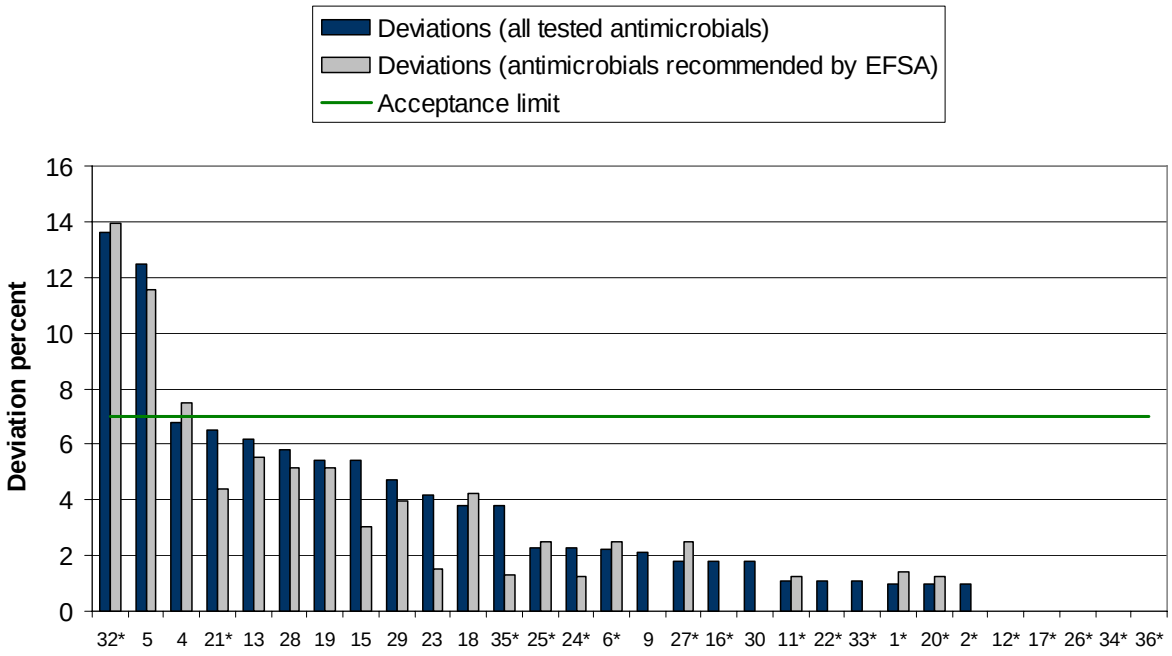

Figure 4: Individual participants' deviations in percent of their total number of *Salmonella* AST's. An asterisk indicates that the laboratory has performed AST using microbroth dilution or agar dilution

The vast majority of the laboratories have a deviation percentage below 7. Two laboratories can be categorized as outliers with levels of deviation at 12.5% and 13.6%. A significant difference ($p<0.01$) is obtained when comparing results obtained by the use of disk diffusion and by a MIC method.

Figure 5 illustrates that the majority of laboratories has less than 7% deviation, whereas two laboratories (#5, #32) are outliers with levels of deviations between 12 and 14%.

Seven percent is the acceptable amount of deviations determined by the CRL. This level of performance is met by 28 of the 30 participating laboratories. The future focus will be on the two laboratories with the highest percentage of deviation which will be offered the possibility to re-test additional *Salmonella* strains and receive individual guidance.

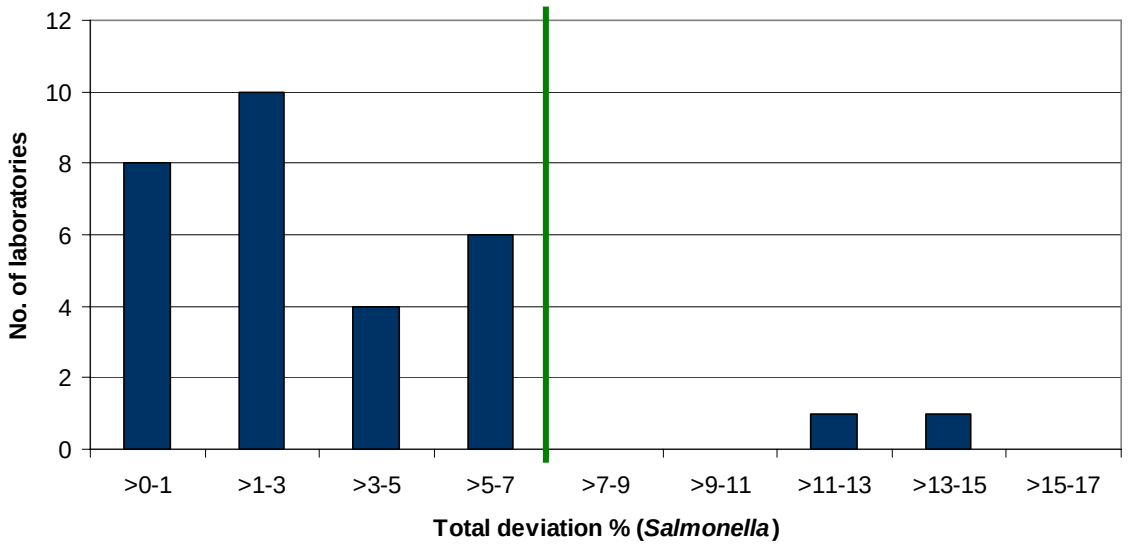

Figure 5: The number of laboratories listed in intervals of percent of total deviations. The green line marks the acceptance limit set by the CRL

3.3.2 *Campylobacter* trial

In the *Campylobacter* trial most laboratories performed well. Applying the earlier mentioned acceptance threshold, 19 of 25 participating laboratories performed acceptably, with five

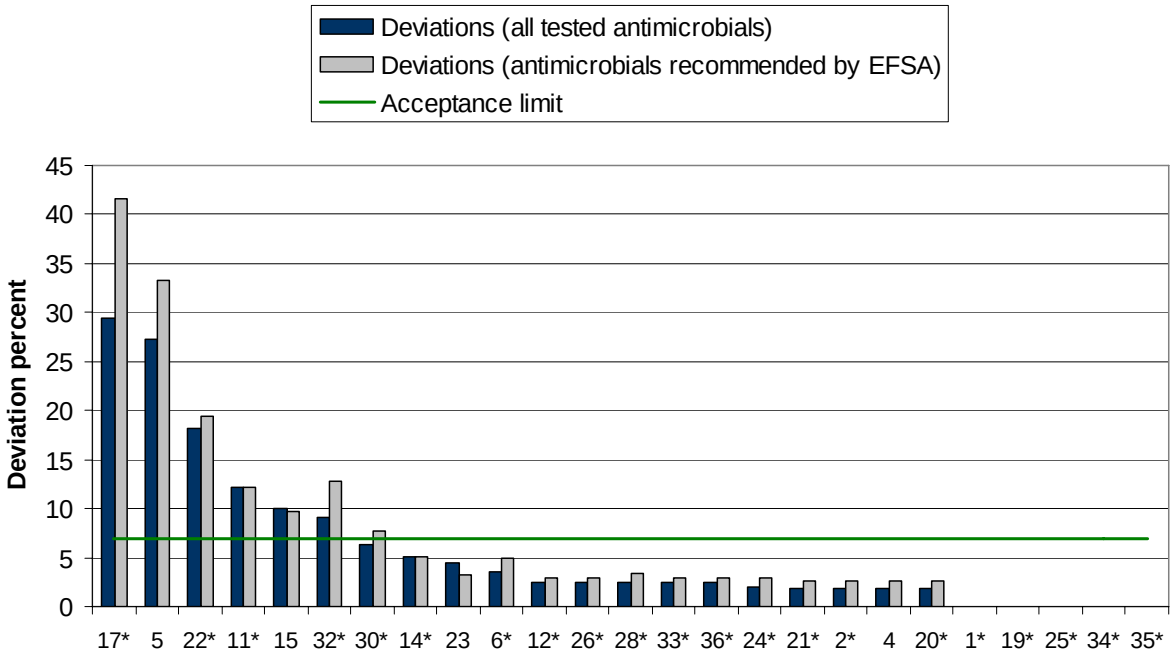

Figure 6: Individual participants' deviations in percent of their total number of *Campylobacter* AST's. An asterisk indicates that the laboratory has performed AST using microbroth dilution or agar dilution

laboratories having no deviations at all. Two laboratories (#5 and #17) had very high levels of deviation (27.3% and 29.4%, respectively). The remaining four laboratories obtained levels of deviation between 9% and 18% (Figure 6).

When clustering the laboratories in intervals of total amount of deviations in percentages (Figure 7), three laboratories (#5, #17 and #22) seem to have a considerably higher level of deviations (from 18 % up to 29%) than the majority of the participating laboratories. These three laboratories can be considered as outliers.

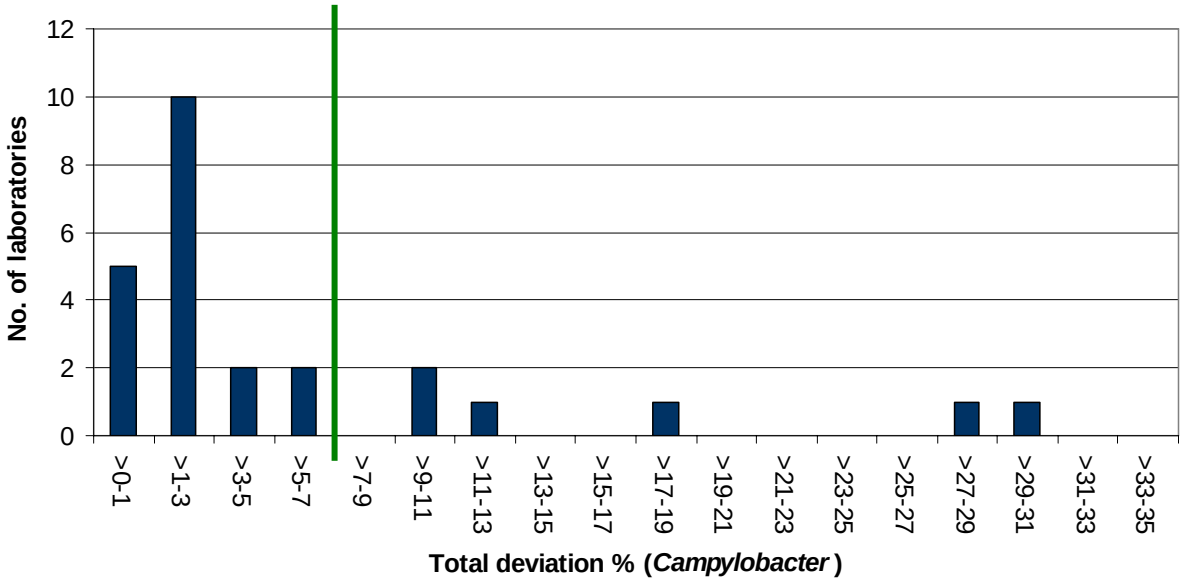

Figure 7: The number of laboratories listed in intervals of percent of total deviations

Of the laboratories with deviation levels higher than 7%, one laboratory used disk diffusion (#5) and one performed E-test (#15). Laboratory #15 obtained results on the test strains with a 10% deviation. The deviating results were all categorized as sensitive, whereas the expected categorization was resistant.

The laboratories #11, #17, #22 and #32 all used microbroth for susceptibility testing *Campylobacter*, and also had deviation levels higher than 7%. Laboratories #11, #15 and #32 all had slightly higher levels of deviation than 7 (12.2%, 10.0% and 9.1%, respectively).

Laboratory #17 had the highest level of deviation (29.4%). Ciprofloxacin may be out of range for the reference strain, it is measured at a range above the QC limit. Additionally, it is seen for all the deviating test results that the MIC value is higher or even considerably higher than the expected MIC value (App. 11b). This indicates that a methodical error causes the deviating results, why the laboratory is encouraged to evaluate the method in detail.

In contrast, laboratory #22 (18.2% deviation) had all deviating test results being considerably lower MIC values than the expected. There seems to be no correlation to the results from the reference strain in which the two deviating results were above the QC limit. The laboratory is encouraged to evaluate the method in detail.

Laboratory #11 (12.2% deviation) had deviations in a range of antimicrobials. Testing the reference strain *C. jejuni* ATCC 33560 caused two deviations, one above and one below the QC range, which does not as such indicate a methodical error. However, the MIC values for the test strains are below the expected value for five of the six deviations, which could indicate that assessment of the method would be beneficial to the laboratory.

The future focus will be on the three laboratories with the highest deviation percentage (#5, #17, #22) which will be offered the possibility to re-test additional *Campylobacter* strains. In addition, laboratory #11 has been offered a re-test of *Campylobacter*, due to fact that the first evaluation of the results including the combination of the *Campylobacter* C2.1 and streptomycin placed this laboratory in the outlier-group.

3.4 Deviations by reference strains

In this section, deviations are defined as results from tests on the reference strain that exceed the quality control (QC) interval limits (App. 7). Values from the participants' testing of the QC strains are listed in Appendix 6a and 6b, along with Tables 5, 6 and 7 which summarize results from the laboratories' quality control.

Table 5 presents the proportion of laboratories that obtained values out of range for the *E. coli* reference strain (ATCC 25922), when performing disk diffusion. All laboratories participating in the *Salmonella* EQAS performed QC testing of the reference strain, of which ten laboratories tested the reference strain using the disk diffusion method. For the individual antimicrobials the highest number of laboratories with deviation results is two, and in total ten of the 15 tested

antimicrobials caused values outside the recommended QC interval. Overall, the highest level of deviation was 5 mm less than the end point of the QC interval and 7 mm greater. No deviations were recorded for cefpodoxime, ciprofloxacin, gentamicin, nalidixic acid and trimethoprim. For six antimicrobials only one laboratory had obtained a value outside the QC interval, and two laboratories had deviating results for both ampicillin, cefotaxime, chloramphenicol and imipenem.

EQAS 2007 Antimicrobial	**Disk diffusion *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in mm zones (min/max)	
		Below lower QC limit	Above upper QC limit
Amoxicillin cl., AUG	1/9 (11%)	-	2
Ampicillin, AMP	2/8 (25%)	2	1
Cefotaxime, CTX	2/11 (18%)	5	2
Cefoxitin, FOX	1/3 (33%)	-	3
Cefpodoxime, POD	0/2 (0%)	-	-
Ceftazidime, CAZ	1/8 (12.5%)	-	2
Ceftiofur, XNL	1/4 (25%)	-	1
Chloramphenicol, CHL	2/10 (20%)	-	3
Ciprofloxacin, CIP	0/9 (0%)	-	-
Gentamicin, GEN	0/10 (0%)	-	-
Imipenem, IMI	2/2 (100%)	-	6
Nalidixic acid, NAL	0/10 (0%)	-	-
Sulphonamides, SMX	1/9 (11%)	-	7
Tetracycline, TET	1/10 (10%)	-	1
Trimethoprim, TMP	0/7 (0%)	-	-

Table 5: Obtained values for reference testing of *E. coli* ATCC 25922 by disk diffusion.

Using MIC determination towards the reference strain *E. coli* ATCC 25922 resulted in considerably fewer deviations. Twenty laboratories submitted MIC data (including one laboratory which performed E-test). No mistakes were seen for 12 antimicrobials, but for four of the 16 tested antimicrobials deviating results were obtained. Only for ciprofloxacin a high deviation percentage was detected (22%). For this antimicrobial, four out of 18 laboratories had obtained results up to two MIC steps higher than the recommended QC interval.

Quality control was also performed using MIC determination against the *C. jejuni* reference strain ATCC 33560 with participation of 21 laboratories (including two laboratories which used E-test). One laboratory which used a different incubation than recommended by CLSI (#14) and a laboratory which uploaded disk diffusion results (#23) were excluded in this summary (App. 6b). Two laboratories did not perform QC testing of the *Campylobacter* reference strain,

one of which was laboratory #5 which used disk diffusion and therefore has no reference values, and the second was laboratory #35 which used microbroth dilution.

EQAS 2007 Antimicrobial	**MIC determination *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
Amoxicillin cl., AUG	0/4 (0%)	-	-
Ampicillin, AMP	0/19 (0%)	-	-
Cefotaxime, CTX	0/16 (0%)	-	-
Cefoxitin, FOX	0/3 (0%)	-	-
Cefpodoxime, POD	0/1 (0%)	-	-
Ceftazidime, CAZ	0/9 (0%)	-	-
Ceftiofur, XNL	0/9 (0%)	-	-
Chloramphenicol, CHL	1/18 (6%)	-	1 step
Ciprofloxacin, CIP	**4/18 (22%)**	-	2 steps
Gentamicin, GEN	0/19 (0%)	-	-
Imipenem, IMI	0/0 (0%)	-	-
Nalidixic acid, NAL	0/19 (0%)	-	-
Streptomycin, STR	0/17 (0%)	-	-
Sulphonamides, SMX	1/16 (6%)	-	5 steps
Tetracycline, TET	1/19 (5%)	-	1 step
Trimethoprim, TMP	0/19 (0%)	-	-

Table 6: Obtained values for reference testing of *E. coli* ATCC 25922 by MIC determination (including E-test)

Table 7 presents the proportion of the laboratories with results from the QC strain below or above the QC interval. No mistakes were seen for two antimicrobials, but for four of the relevant six antimicrobials deviating results were obtained. Ciprofloxacin had a high deviation percentage (24%), as also in EQAS 2006 (29%). Compared to EQAS 2006, the results for erythromycin have improved considerably from 39% to 14% this year. No laboratories differ from the others with higher numbers of deviations.

EQAS 2007 Antimicrobial	**MIC determination *C. jejuni* ATCC 33560**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
Chloramphenicol, CHL	0/10 (0%)	-	-
Ciprofloxacin, CIP	**5/21 (24%)**	-	1 step
Doxycycline, DOX	-	-	-
Erythromycin, ERY	3/21 (14%)	-	1 step
Gentamicin, GEN	2/13 (15%)	1 step	2 steps
Meropenem, MERO	-	-	-
Nalidixic acid, NAL	0/19 (0%)	-	-
Tetracycline, TET	1/19 (5%)	-	1 step

Table 7: Obtained values for reference testing of *C. jejuni* ATCC 33560 using MIC determination (incl. E-test)

4. Discussion

4.1 *Salmonella* trial

Overall, the percentage of correct susceptibility test results of *Salmonella* was 96.7%. The majority of participants (28) obtained satisfactory results according to the level of acceptance set by the CRL (<7% deviation). A significant difference ($p<0.01$) was obtained when comparing results obtained by the use of disk diffusion and by a MIC method.

Compared to the performance in EQAS 2006 (90.1% correct results) it would therefore seem that the quality of the results has improved. Also, the levels of deviations have diminished compared to EQAS 2006, where only two laboratories had less than 4% deviation, this group is now 20 laboratories (the 4%-limit was chosen according to the distribution of deviation percentages in EQAS 2006).

Two laboratories had a deviation level higher than 7% (#5, #32), and both were detected as outliers with deviation levels at 12.5% and 13.6% deviation, respectively.

Laboratory #5 used disc diffusion and had 12.5% deviations. They tested the reference strain *E. coli* ATCC 25922 for ten antimicrobials, which were all within the QC ranges (App. 6a). The breakpoints used for the categorization are shared with the majority of the other laboratories which also use disk diffusion (App. 5). The obtained results and the deviation report should be evaluated as means of looking into what may be the reason for the deviations. It seems that a methodical deviation may be the reason for the deviating results, since the obtained zone diameters in general are quite high.

Laboratory #32, which had 13.6% deviations, used MIC determination. For the reference strain *E. coli* ATCC 25922 two of the 12 tested antimicrobials were out of range; chloramphenicol was one MIC step above the QC limit and sulphisoxazole was more than five MIC steps above the QC limit (App. 6a). However, this does not seem to be the obvious reason for the deviations on the test strains, since only two of the 11 deviations are on chloramphenicol and none are seen for sulphonamides. The deviating test results all had high or very high MIC's compared to the expected value. This indicates that there may be a methodical deviation since also the values for the reference strain in general were at the top of the QC range. Searching for an indication of the problems causing the deviations should therefore include an evaluation of the methodology used (e.g. inoculum concentration and/or volume).

In general, amoxicillin + clavulanic acid and ciprofloxacin caused unsatisfactory results when testing *Salmonella*.

A number of participants performing MIC seem to have problems with the reference strain towards ciprofloxacin, as a third of these participants have obtained a result outside the QC range.

Twelve of the 30 participating laboratories had deviating results for ciprofloxacin. Most of these laboratories performed the test by disk diffusion and, in general, the deviations cause more isolates than expected to be categorized as sensitive, which could be due to the breakpoints used. None of the laboratories performing disk diffusion had deviating results for ciprofloxacin when testing the reference strain.

For amoxicillin + clavulanic acid (AUG) two strains seem to have caused problems (App. 10a). The expected MIC values for both these two test strains are right above the cut off value which appears to have caused problems to the majority of participants. The test results from *E. coli* ATCC 33560 towards AUG were in good agreement with the QC intervals.

The second *Salmonella* test strain (S2.2) that also caused some problems for the participants (92.3% correct results) was the 'true ESBL'-isolate which caused problems due to fact that a number of participants failed to follow the CLSI guidelines as advised. Additionally, AUG and CIP posed problems for this strain also. Nevertheless, the results from this internal control strain improved considerably from EQAS 2006 to EQAS 2007 with the level of correct results increasing from 85.3% to 92.3%.

The results for the reference strains in this year's EQAS were considerably better in comparison to the results EQAS 2006, where the results for AUG, SMX and TET were all deviating with 40% or close to 40%. Additionally, in this year's EQAS the total number of deviation results for when testing the *E. coli* reference strain by disk diffusion was 14, of which 9 belong to laboratory #15.

The highest level of deviations in EQAS 2006 was 30% (#29) which has improved considerably in performance in this year's EQAS (4.7% deviations). In general, a follow-up on the laboratories which were outliers in the *Salmonella* trial in EQAS 2006 (#5, #19, #27 and #29) shows that the deviation levels have improved considerably. The laboratories #5, #19, #27

and #29 all had more than 20% deviations in EQAS 2006, and improved the deviation level to 12.5%, 5.4%, 1.8% and 4.7% respectively.

The isolate S2.4 was a *S.* Corvallis which, when tested and verified, showed low-resistance to ciprofloxacin with the MIC-value 0.5µg/mL. The isolate was sensitive to nalidixic acid due to the fact that it contained the plasmid mediated quinolone resistance gene, qnrS. However, it seems that the isolate lost the plasmid before it was tested by the NRL's. The CRL have performed a re-test of the strain and could not retrieve the ciprofloxacin resistance but obtained a new expected MIC value for ciprofloxacin (0.03µg/mL) which leads to a categorization as sensitive.

ESBL-producing *Salmonella* test strains

ESBL-producing microorganisms are an emerging problem worldwide, and it should be of a high priority for the NRL's to be able to detect these problem strains. The detection of ESBL producing test strains has therefore been included as an optional test in this EQAS.

Two of the *Salmonella* test strains were ESBL producing (S2.2 and S2.8), and the participants were asked to interpret their results according to the clinical guidelines from CLSI, in which it is described that an ESBL-producing strain that is resistant to one cephalosporin should be interpreted resistant to all cephalosporins. Of the 30 laboratories which tested *Salmonella*, 29 uploaded results from ESBL-testing, 96% of which could confirm that S2.2 was a 'true ESBL', and 83% of which could confirm that S2.8 was an AmpC-isolate.

The CTX-M9-gene is known to be difficult to detect, and it is therefore not surprising to observe the results from this EQAS indicating that the sole use of one cephalosporin was not effective in detecting ESBL production when using either cefotaxime or ceftazidime, since an average of 63% of the laboratories did not find the expected resistance. In contrast, the sole use of ceftiofur (two laboratories) resulted in 100% detection.

Combining the use of the antimicrobials cefotaxime and ceftazidime (as recommended by CLSI) would have been 90% effective if the CLSI guideline had been used for the interpretation. One of ten participants found both CTX and CAZ sensitive.

Using the combination of the antimicrobials cefotaxime and ceftiofur as well as the combination of all three of cefotaxime, ceftazidime and ceftiofur would have shown 100% efficacy in detecting the ESBL-producing strain if the CLSI guideline regarding cephalosporins had been used.

It is noteworthy that even though ceftazidime did not show much effect in detecting ESBL-production in the two isolates in this EQAS, this antimicrobial would be more effective when other genes are the matter of attention.

It seems that it caused more difficulty to confirm the AmpC-isolate (characterised by being resistant to FOX) than the 'true ESBL'. One good reason for this difference could be the registration of the ESBL- and AmpC-results in the database, which was found not to be optimal. This will therefore be evaluated and optimized for future EQAS's.

4.2 *Campylobacter* trial

The amount of deviations was somewhat higher in the *Campylobacter* susceptibility results (94.2% correct results) compared to the *Salmonella* results. Between the laboratories the performance varied from no deviations at all to 29.4% deviations, with 19 laboratories performing satisfactorily according to the acceptance ranges established by the CRL.

Due to the fact that the forwarded lyophilised *Campylobacter* test strains were difficult to reconstitute, the participating laboratories could not upload as many results as otherwise possible. As a consequence the CRL will ship the test strains as charcoal swabs for following EQAS's on *Campylobacter*.

Three laboratories (#5, # 17 and #22) were outliers and did not perform as well as the other laboratories. Additionally, laboratories #11, #15 and #32 had deviation percentages above the goal set by the CRL.

Laboratories #5 and #23 used the methodology based on disk diffusion and had 27.3% and 4.4% of deviating susceptibility tests, respectively. The CLSI guidelines (M45-A) state that appearance of any zone of inhibition would require MIC determination for accurate categorization of susceptibility. Also, diffusion tests are not internationally recognised for susceptibility testing of *Campylobacter* as there are no international breakpoints or quality

control intervals available. The results obtained by disk diffusion will therefore not be discussed in further details.

For both tetracycline more deviations were seen than for the other antimicrobials (Table 2). The QC results for this agent give no indication that there should be irregularities, since almost all results were within range.

Tetracycline especially caused problems with regard to test strain C2.5 (App. 10b) which seventeen laboratories found to be sensitive to tetracycline, whereas the reference value was 'resistant'. With the exception of these deviations tetracycline has an acceptably low deviation percentage (4%).

Two additional strains have high deviation levels (C2.6 and C2.7) (Table 1). In total, the number of deviations are 23 among the seven laboratories, of which two laboratories have five (lab #5) and seven (lab #22), respectively. It does not seem that a general reason can explain these deviations.

The overall performance of 94.2% correct results, is similar to last year's EQAS (93.9%). It should be taken into consideration that a number of laboratories have taken the opportunity to change their routine methods which may have affected the obtained results.

A follow-up on the laboratories which were outliers in the *Campylobacter* trial in EQAS 2006 (#14, #16, #22, #26, #28 and #29) shows deviation levels which have improved considerably with regard to three of the laboratories which also uploaded results in EQAS 2007 (#16 and #29 did not upload results). All three laboratories #14, #26 and #28 had deviation levels below 6% in EQAS 2007 (improved from 14-40% deviations in 2006 (corrected data)). One laboratory (#22) is an outlier with 14% deviation in 2006 (corrected data), and 18% in 2007.

The follow-up of both the *Salmonella* and *Campylobacter* EQAS's included retests being offered the relevant participants, and additionally, a training course was carried out in March 2008 for selected laboratories.

5. Conclusion

The goal of the CRL EQAS is having all participating NRL's perform susceptibility testing of *Salmonella* and *Campylobacter* with a deviation percent less than 7. This seems within reach

for *Salmonella*, whereas the performance of susceptibility testing of *Campylobacter* for some laboratories appears to need attention as means of improving the quality of the results.

The NRLs' performance seem to have improved for *Salmonella* AST's this EQAS (96.7%) compared to the results obtained at the EQAS 2006 (90.1%), whereas it seems that the performance with regard to *Campylobacter* AST is comparable to the 2006-result (93.9% in 2006 and 94.2% in 2007).

The laboratories which had high deviation percentages should follow the recommendations mentioned in order to work towards obtaining results in better agreement with the expected in the next proficiency test. The laboratories which did not perform according to the acceptance limit set by the CRL will be expected to participate in a discussion regarding investigation of the reasons behind the unsatisfactory performance. Additionally, a re-test will be carried out as well as a training course for selected laboratories (next training course planned to be hosted in 2009).

Harmonising breakpoints, antimicrobials and ranges of these, are issues that are important to focus at in the future. Also, attention should be directed towards the problem of detecting ESBL producing strains.

In general, the laboratories seemed content about the proficiency test (App. 8). The comments and issues raised will be taken into consideration, and at the annual workshop this year's EQAS's will be addressed.

National Food Institute DTU
Technical University of Denmark

CRL-AR EQAS pre-notification

EQAS 2007 FOR *SALMONELLA* AND *CAMPYLOBACTER*

The CRL are pleased to announce the launch of another EQAS. The EQAS provides the opportunity for proficiency testing, which is considered an important tool for the production of reliable laboratory results of consistently good quality.

This EQAS offers antimicrobial susceptibility testing of eight *Salmonella* isolates and eight *Campylobacter* isolates. Additionally, new participants will be offered the following QC strains: *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214).

This EQAS is specifically for NRL's on antimicrobial resistance. Thus, you do not need to sign up to be a participant. All who receive this pre-notification are automatically regarded as participants.

Participation is free of charge for all NRL's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY
Please remember to provide the coordinator with documents or other information that can ease the parcel's way through customs (eg. specific text that should be written on the invoice). As means of avoiding passing the deadline we ask you to send us this information already at this stage. For your information, the contents of the parcel is "Biological Substance Category B": Eight *Salmonella* strains, eight *Campylobacter*, and for new participants also the QC strains mentioned above. The strains are expected to arrive at your laboratory in October 2007.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The isolates will be shipped in October 2007. The protocol will be provided by e-mail.

Returning of results: Results must be returned to the National Food Institute, by December 14th 2007. When you enter your results via a password-protected website, an evaluation report of your results will be generated immediately.

EQAS report: When the EQAS is concluded, the data will be collected in an overall report in which it is possible to see all participants' results in comparison. In the report the laboratories will be coded, thus ensuring full anonymity; only the National Food Institute and the EU Commission will be given access to un-coded results.

Next EQAS: The next CRL EQAS that we will have is on antimicrobial susceptibility testing of *E. coli*, staphylococci and enterococci which will be carried out in June 2008.

Any comments regarding the EQAS, please contact me by e-mail (rsh@food.dtu.dk) or by fax (+45 7234 6001).

Sincerely,

Rene S. Hendriksen
EQAS-Coordinator

Participant list

Campy	Salm	Institute	Country
X	X	The National Food Institute	Denmark
X	X	Austrian Agency for Health and Food Safety	Austria
X	X	Institute of Public Health	Belgium
X	X	National Center of Infectious and Parasitic Diseases	Bulgaria
X	X	Veterinary Services	Cyprus
-	X	State Veterinary Institute Praha	Czech Republic
X	X	Estonian Veterinary and Food Laboratory	Estonia
X	X	Finnish Food Safety Authority EVIRA	Finland
-	X	AFSSA LERQAP Maisons Alfort	France
X	-	AFSSA Ploufragan - LERAP	France
X	X	AFSSA Lyon	France
-	X	AFSSA Fougères LERMVD	France
X	X	Federal Institute for Risk Assessment	Germany
-	X	Veterinary Laboratory of Chalkis	Greece
X	X	Central Agricultural Office, Veterinary Diagnostical Directorate	Hungary
X	X	Central Veterinary Research Laboratory	Ireland
X	X	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	National Diagnostic Centre of Food and VeterinaryService	Latvia
X	X	National Veterinary Laboratory	Lithuania
X	X	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	National Veterinary Research Institute	Poland
-	X	Instituto Nacional de Saude (INSA)	Portugal
X	X	National Institute of Research-Development for Microbiology and Immunology “Cantacuzino”	Romania
X	X	State Veterinary and Food Institute (SVFI)	Slovakia
-	X	National Veterinary Institute	Slovenia
X	X	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	X	National Veterinary Institute, SVA	Sweden
X	X	The Veterinary Laboratory Agency	United Kingdom
X	X	Complutense University of Madrid	Spain
X	X	Veterinærinstituttet	Norway

Designated NRL by the compentent authority of the member state
Laboratories enroled by the CRL
Not a Member State of the EU

Salmonella test strains and reference values (MIC)

Strain no.	AMP/AMX	AUG	CAZ	CAZ/CL	CHL	CIP	CTX	CTX/CL	ESBL gene	FX	GEN	IP/IPE	NAL	SMX	STR	SXT	TET	TMP	XNL
S-2.1	>32	16/8	1	>4	8	1	0,25	0,25	-	4	16	<1,0 / <0,4	>64	>1024	64	0,125	4	<4	2
S-2.2	>32	8/4	1,0	>4	4	0,25	128	<=0,016	CTX M-9	4	1	<1,0 / <0,4	>64	64	8	0,125	>32	<4	>8
S-2.3	>32	8/4	0,5	0,125	>64	0,03	0,125	0,32	-	2	32	<1,0 / <0,4	4	>1024	>64	>32	>32	>32	1
S-2.4	1	2/1	0,5	0,125	4	0,03	0,064	0,32	-	2	1	<1,0 / <0,4	8	>1024	>64	0,125	>32	<4	0,5
S-2.5	>32	4/2	0,5	<0,5	8	0,5	0,125	0,064	-	4	>32	<1,0 / <0,4	>64	>1024	32	>32	>32	>32	<0,5
S-2.6	2	<2/1	0,25	0,125	4	<0,03	0,125	0,032	-	4	<1	<1,0 / <0,4	<4	<64	8	0,125	<2	<4	1
S-2.7	<1	<2/1	0,5	0,125	>64	<0,03	0,125	0,032	-	2	<1	<1,0 / <0,4	<4	<64	64	0,064	32	<4	1
S-2.8	>32	>32/16	16	>4	>64	<0,03	16	>1,0	AmpC CMY-2	32	<1	<1,0 / <0,4	<4	>1024	>64	0,25	>32	<4	>8

Strain no.	AMP/AMX	AUG	CAZ	CAZ/CL	CHL	CIP	CTX	CTX/CL	ESBL gene	FX	GEN	IP/IPE	NAL	SMX	STR	SXT	TET	TMP	XNL
S-2.1	R	R	S	MIC ratio <8	S	R	S	MIC ratio <8	none ESBL	none ampC	R	none Metallo beta lactamase	R	R	R	S	S	S	S
S-2.2	R	R	R	MIC ratio <8	S	R	R	MIC ratio =>8	ESBL = CTX M-9	none ampC	S	none Metallo beta lactamase	R	S	S	S	R	S	R
S-2.3	R	R	S	MIC ratio <8	R	S	S	MIC ratio <8	none ESBL	none ampC	R	none Metallo beta lactamase	S	R	R	R	R	R	S
S-2.4	S	S	S	MIC ratio <8	S	S	S	MIC ratio <8	none ESBL	none ampC	S	none Metallo beta lactamase	S	R	R	S	R	S	S
S-2.5	R	S	S	MIC ratio <8	S	R	S	MIC ratio <8	none ESBL	none ampC	R	none Metallo beta lactamase	R	R	S	R	R	R	S
S-2.6	S	S	S	MIC ratio <8	S	S	S	MIC ratio <8	none ESBL	none ampC	S	none Metallo beta lactamase	S	S	S	S	S	S	S
S-2.7	S	S	S	MIC ratio <8	R	S	S	MIC ratio <8	none ESBL	none ampC	S	none Metallo beta lactamase	S	S	R	S	R	S	S
S-2.8	R	R	R	MIC ratio <8	R	S	R	Not Determinable	none ESBL	AmpC = CMY-2	S	none Metallo beta lactamase	S	R	R	S	R	S	R

Resistant

ESBL/AmpC

Campylobacter test strains and reference values (MIC)

Strain no.	Species	CHL	CIP	ERY	GEN	NAL	STR	TET
C-2.1	C. jejuni	S	S	S	S	S	S	S
C-2.2	C. coli	S	S	S	S	S	R	S
C-2.3	C. jejuni	S	R	S	S	R	S	S
C-2.4	C. coli	S	S	S	S	S	R	R
C-2.5	C. coli	S	S	R	S	S	S	R
C-2.6	C. jejuni	S	R	R	R	R	R	R
C-2.7	C. jejuni	S	R	S	S	R	S	R
C-2.8	C. jejuni	S	R	R	S	R	S	R

Strain no.	Species	CHL	CIP	ERY	GEN	NAL	STR	TET
C-2.1	C. jejuni	<=2	0,125	<=0,5	0,25	4	<=2	<=0,25
C-2.2	C. coli	2	0,125	<=0,5	0,5	4	>16	0,5
C-2.3	C. jejuni	2	>4	<=0,5	0,25	>64	<=2	<=0,25
C-2.4	C. coli	4	0,25	2	0,5	16	16	>16
C-2.5	C. coli	4	0,125	>32	0,5	8	2	4
C-2.6	C. jejuni	4	>4	>32	>16	>64	>16	>16
C-2.7	C. jejuni	2	>4	<=0,5	<=0,125	>64	2	>16
C-2.8	C. jejuni	8	>4	>32	0,25	>64	<=2	>16

Resistant

CRL-AR Inter-laboratory Proficiency Test 2007

- *Salmonella* and *Campylobacter*

Lab no.: >Lab no.<
>Name<
>Institute<
>Country<

Copenhagen, *October 2007*

Dear Participant,

Please find enclosed the bacterial strains for the CRL AR EQAS 2007.
The following documents are also relevant and have been sent to you electronically:

- Protocol for *Salmonella* and *Campylobacter*
- Instructions for Opening and Reviving Lyophilised Cultures
- Subculture and Maintenance of Quality Strains
- Evaluation form
- Questionnaire

We would like you to examine all strains that we send to you by performing antimicrobial susceptibility testing. In the protocol you will find detailed description of how to test the strains. Additionally, you will find a description of how to enter your results into the interactive web database. For entering data you need this username and password.

Your username:

Your password:

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

After receipt the strains should be stored dark and at 4°C for stabs, and dark and cool for freeze-dried strains.

The results should be returned to us no later than *December 14th, 2007*.

Please acknowledge receipt of parcel immediately on arrival (by email to rsh@food.dtu.dk). For further information, please do not hesitate to contact us.

Yours sincerely,

Rene S. Hendriksen
EQAS-Coordinator

EU Community Reference Laboratory, Antimicrobial Resistance, Bülowsvej 27, DK-1790, Copenhagen V, Denmark
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National Food Institute

PROTOCOL

For susceptibility testing of *Salmonella* and *Campylobacter*

1 INTRODUCTION

One of the tasks as the EU Community Reference Laboratory for Antimicrobial Resistance is to organise and conduct an External Quality Assurance System (EQAS) on susceptibility testing of *Salmonella* and *Campylobacter*. The *Salmonella* and *Campylobacter* EQAS 2007 will include susceptibility testing of eight *Salmonella* and eight *Campylobacter* strains together with susceptibility testing of the reference strains *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214).

For new participants of the EQAS who have not already received the mentioned reference strains, these are included in the parcel. The reference strains will not be included in the years to come. The reference strains are original certified cultures and are free of charge. Please take proper care of the strains. Handle and maintain them as suggested in the enclosed manual. Please use them for future internal quality control for susceptibility testing in your laboratory.

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of susceptibility testing of pathogens originating from food and animal sources, especially *Salmonella* and *Campylobacter*. Furthermore, to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported by different laboratories on *Salmonella* and *Campylobacter* and to harmonise the breakpoints used within the EU.

3 OUTLINE OF THE EQAS 2007

3.1 Shipping, receipt and storage of strains

In October 2007 all EU appointed National Reference Laboratories will receive a parcel from The National Food Institute containing eight *Salmonella* and eight *Campylobacter* strains. Reference

strains will be included for participants who have not previously received these. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains among the selected material.

The reference strains and seven of the eight *Campylobacter* strains are shipped lyophilised, one *Campylobacter* is shipped as a charcoal swab and the *Salmonella* test strains are stab cultures. On arrival, the stab cultures and the charcoal swab must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of lyophilised *E. coli* reference strains and *Campylobacter* is presented below.

3.2 Suggested procedure for reconstitution of lyophilised strains

Please see the document 'Instructions for opening and reviving lyophilised cultures' for additional information.

a) Open the ampoule. Take some of the material and dissolve it in 0,5 ml appropriate broth. Leave it for 10 minutes. Inoculate the solution on a non selective agar plate (*E. coli*) or on a blood agar plate (*Campylobacter*) using either a 10 µl loop or a cotton swab. Incubate at 35ºC in ambient air for 16-18 h (*E. coli*) or microaerophilic for 24-48 h at 37ºC or 42ºC (*Campylobacter*).

b) Incubate the remaining culture/broth in the vial/ampoule as mentioned above (seal the vial/ampoule with parafilm if necessary). After incubation re-inoculate the culture using either a 10 µl loop or a cotton swab on none selective agar or blood agar as described above and incubate.

If you do not succeed with a) or b) shake the vial/ampoule and empty it directly onto an agar plate. Add 100 µl 0,9% saline to the plate, and spread the culture properly with a triangle or 'hockey stick'. Incubate as mentioned above.

3.3 Susceptibility testing

The strains should be susceptibility tested towards as many as possible of the following antimicrobials by the methods routinely used in the laboratory. For MIC please use the cut off values listed in tables 3.3.1 and 3.3.2. In this EQAS, epidemiological MIC cut off values are used for MIC determination which allow only two categories of characterisation – resistant or sensitive.

Participants using disk diffusion are recommended to interpret the results according to the individually daily routinely used breakpoints categorising them into the terms resistant and sensitive. Interpretations in concordance with the expected value will be categorised as 'correct', whereas interpretation that deviates from the expected interpretation will be categorised as 'incorrect'.

The cut off values used in the interpretation of the MIC results are developed by EUCAST (www.eucast.org).

As to the breakpoints that you routinely use in your laboratories to determine the susceptibility category we ask you to fill in the breakpoints used in the database (see test form below). Also, with regard to MIC range and/or disc concentration we ask you to fill in these pieces of information in the enclosed questionnaire.

3.3.1 *Salmonella*

Testing of gentamicin and streptomycin may be of value for monitoring. Please, do not take into account in this study, that the CLSI guidelines state that for aminoglycosides *Salmonella* should not be reported as susceptible.

When testing cephalosporins, please follow the guidelines according to CLSI M100-S16 table 2A; that when an isolate is found resistant to one cephalosporin, the isolate is regarded resistant to all cephalosporins.

Also, when following EUCAST epidemiological cut-off values, *Salmonella* resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin. When using disc diffusion and CLSI clinical breakpoints this connection between nalidixic acid and ciprofloxacin is not taken into account. Thus, the result in this situation with regard to ciprofloxacin will deviate from the expected result in this EQAS.

Antimicrobials for *Salmonella*	MIC (µg/mL) **R is >**
Amoxicillin + clavulanic acid (AUG)***	4
Ampicillin (AMP)	4
Cefotaxime (CTX)	0,5
Ceftazidime (CAZ)****	2
Ceftiofur (XNL)****	2
Chloramphenicol CHL)	16
Ciprofloxacin (CIP)	0.06
Gentamicin (GEN)	2
Nalidixic acid (NAL)	16
Streptomycin (STR)*	32
Sulphonamides (SMX)**	256
Tetracycline (TET)	8
Trimethoprim (TMP)	2
Trimethoprim-sulfamethoxazole (SXT)***	2

* ARBAO ** CLSI *** Not part of the EFSA monitoring programme (tentative EUCAST cut off values)

**** Not part of the EFSA monitoring programme (used for confirmatory tests for ESBL production)

ESBL production

It is optional to continue with the following tests regarding ESBL production:

All strains categorized resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) could be confirmed by confirmatory tests for ESBL production.

The confirmatory tests for ESBL production require testing with a pure antimicrobial (CTX and CAZ) vs. a test with the same antimicrobial combined with a β-lactamase inhibitor (clavulanic acid). Synergy is defined as a 3 dilution steps difference between the two compounds in at least one of the two cases (MIC ratio ≥ 8, E-test 3 dilution steps) or an increase in zone diameter ≥ 5 mm (CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

Confirmatory tests for Metallo beta lactamase require comparison between imipenem (IMI) and IMI/EDTA, synergy is in this test defined as a MIC ratio ≥ 8 or E-test 3 dilution steps difference (CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

Additionally, AmpC detection can be performed by testing the microorganism to cefoxitin (FOX), resistance to FOX could indicate AmpC. Verification of AmpC requires PCR or sequencing.

3.3.2 *Campylobacter*

Antimicrobials for *Campylobacter*	MIC (µg/mL) **R is >** *C. jejuni*	MIC (µg/mL) **R is >** *C. coli*
Erythromycin	4	16
Ciprofloxacin	1	1
Tetracycline	2	2
Streptomycin	2	4
Gentamicin	1	2
Chloramphenicol*	16	16
Nalicixic acid*	16	32

*Not part of the EFSA monitoring programme

4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the enclosed test form. Please enter your results into the interactive web database. Please read the detailed description below before entering the web database. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print an evaluation report of your results. Please submit results by latest December, 14th, 2007.

If you do not have access to the Internet or if you experience difficulties entering the data, please return results by e-mail, fax or mail to the National Food Institute.

All results will summarized in a report which will be made available to all participants. Individual results will be anonymous and will only be known to the CRL and the European Commission.

If you have any questions, please do not hesitate to contact the EQAS Coordinator:

Rene Hendriksen

The National Food Institute

Technical University of Denmark

27 Bülowsvej, DK-1790 Copenhagen V

Denmark

Tel: +45 7234 6288

Fax: +45 7234 6001

E-mail: rsh@food.dtu.dk

5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read this passage before entering the web page. Before you go ahead, you need your test form by your side together with your breakpoint values.

You are able to browse back and forth by using the forward and back keys or click on the CRL logo.

You enter the EU CRL-AR EQAS 2007 start web page (http://thor.dfvf.dk/crl) then write your username and password in low cases and press enter. Your username and password is the same as in the previous EQAS's arranged by The National Food Institute. If you have problems with the login please contact us.

Click on either "*Salmonella* test results" or "*Campylobacter* test results" depending on your results. The below description is aimed at *Salmonella* entry but are the exact the same as for *Campylobacter* entry.

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

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

Fill in what kind of method you have used for the susceptibility testing of *Salmonella* and the brand of discs, tablets, MIC trays etc.

Fill in the breakpoints that are routinely used at your laboratory to determine the susceptibility category. Remember to use the operator keys in order to show – equal to, less than, less or equal to, greater than or greater or equal to.

You will find one more box to fill in on this page when testing *Campylobacter*.

Fill in the actual incubation condition used for susceptibility testing of *Campylobacter* – 36°C/48h or 42°C/24h.

Click on "save and go to next page”

In the data entry pages for each *Salmonella* and *Campylobacter* strain, you enter the obtained value and the interpretation as R or S.

If relevant for the microorganism, you also have the option to type in results for the ESBL tests.

If you have not used an antimicrobial or have not performed ESBL tests, please leave the field empty.

Click on "save and go to next page"

When uploading data on the reference strains please enter the zonediameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to, etc.

Click on "Save and go to next page"

This page is a menu, from where you can review the input pages, approve your input and finally see and print the evaluated results:

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

Approve your input. Be sure that you have filled in all the results before approval, as YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database, but allows you to see the evaluated results.

See the evaluated results. You can print each page. *You may have to choose a smaller text* size to print the whole screen on one piece of paper. In the Internet Explorer (or the Internet program you may have), you click on "view", "text size" and e.g. "smallest".

6 TEST FORM

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

Comments:

National Food Institute

TEST FORM

Survey for routinely applied breakpoints for antimicrobial susceptibility testing of *Salmonella*

Antimicrobial	Interpretation, Zonediam (mm) or MIC-value (µg/ml)				
	<, ≤	Sensitive	Intermediate	>, ≥	Resistant
Ampicillin, AMP					
Amoxicillin + clavulanic acid, AUG					
Cefotaxime, CTX					
Ceftazidime, CAZ					
Ceftiofur, XNL					
Chloramphenicol, CHL					
Ciprofloxacin, CIP					
Gentamicin, GEN					
Nalidixic acid, NAL					
Streptomycin, STR					
Sulfonamides, SMX					
Tetracycline, TET					
Trimethoprim, TMP					
Trimethoprim + sulfamethoxazole, SXT					

Regarding method used for antimicrobial susceptibility testing of *Salmonella* in this EQAS:

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution (tubes)
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

National Food Institute

TEST FORM

Survey for routinely applied breakpoints for antimicrobial susceptibility testing of *Campylobacter*

Antimicrobial	Interpretation, Zonediam (mm) or MIC-value (µg/ml)				
	<, ≤	Sensitive	Intermediate	>, ≥	Resistant
Chloramphenicol					
Ciprofloxacin					
Erythromycin					
Gentamicin					
Nalidixic Acid					
Streptomycin					
Tetracycline					

Regarding method used for antimicrobial susceptibility testing of *Campylobacter* in this EQAS:

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution (tubes)
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.1	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.2	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.3	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.4	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.5	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.6	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.7	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 2.8	Ampicillin, AMP			
	Amoxicillin + clavulanic acid, AUG			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. ≥ 5 mm or (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8		

Comments:

National Food Institute

TEST FORM

Susceptibility testing of *E. coli* referencestrain ATCC 25922

Strain	Antimicrobial	Zonediameter (mm) or MIC-value (µg/ml)
E. coli ATCC 25922	Amoxicillin + clavulanic acid	
	Ampicillin	
	Cefotaxime	
	Ceftazidime	
	Cefpodoxime	
	Ceftiofur	
	Chloramphenicol	
	Ciprofloxacin	
	Florphenicol	
	Gentamicin	
	Nalidixic Acid	
	Streptomycin	
	Sulphonamides	
	Tetracycline	
	Trimethoprim	
	Trimethoprim + Sulphonamides	

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation	
		Zonediam (mm) or MIC-value (µg/ml)	S / R
Campylobacter CRL C. 2.1 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 2.2 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 2.3 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 2.4 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation	
		Zonediam (mm) or MIC-value (µg/ml)	S / R
Campylobacter CRL C. 2.5 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 2.6 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 2.7 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 2.8 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

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TEST FORM

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	Zonediameter (mm) or MIC-value (µg/ml)	
		36 °C/48 hours	42 °C/24 hours
C. jejuni ATCC 33560	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Nalidixic Acid		
	Tetracycline		

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INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Manual from Czech Collection of Microorganisms (CCM)
Masaryk University
Tvrdého 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!

National Food Institute

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-S17, January 2007 (Performance Standards for Antimicrobial Susceptibility Testing)

M07-A6, January 2003 (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.

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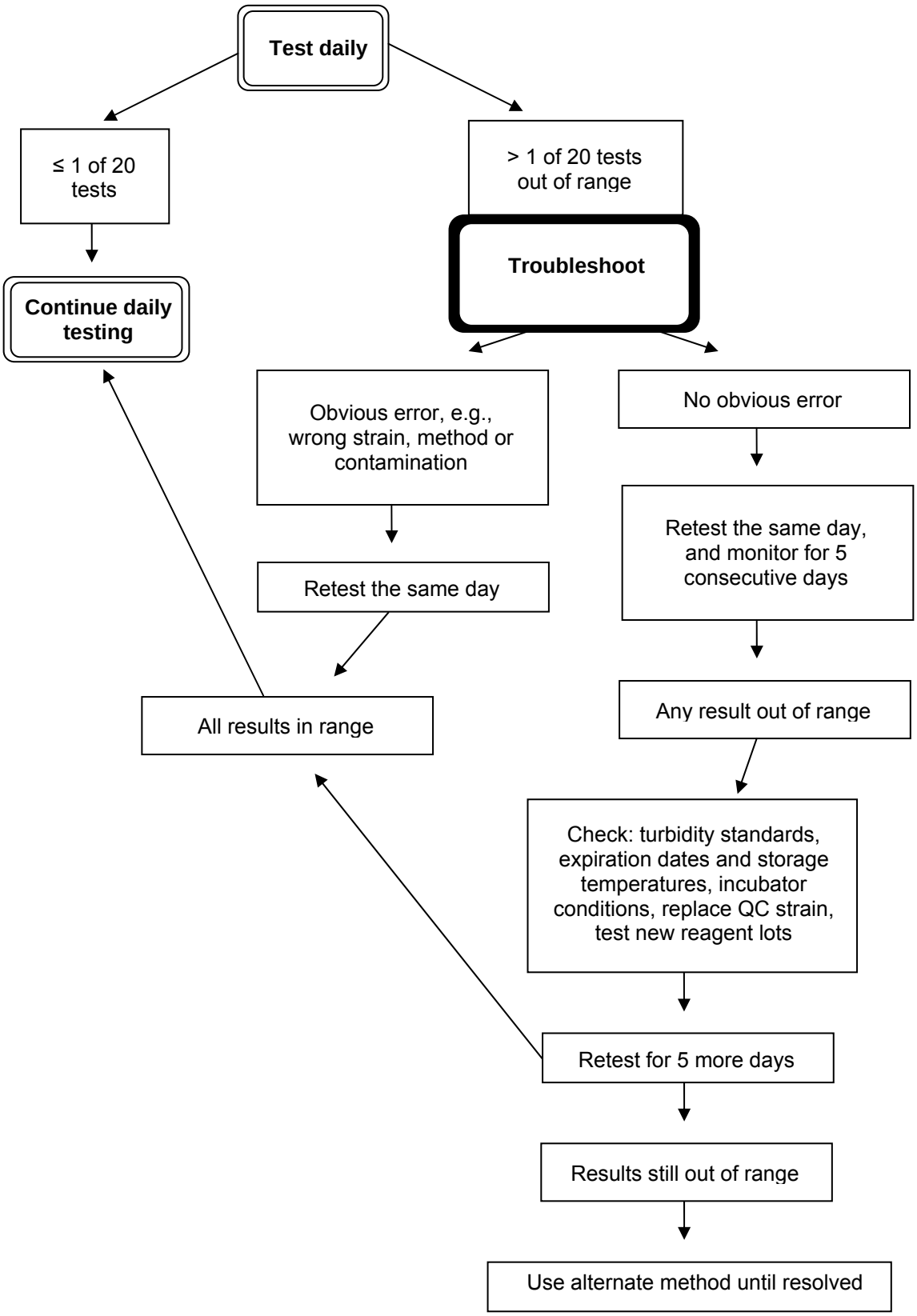
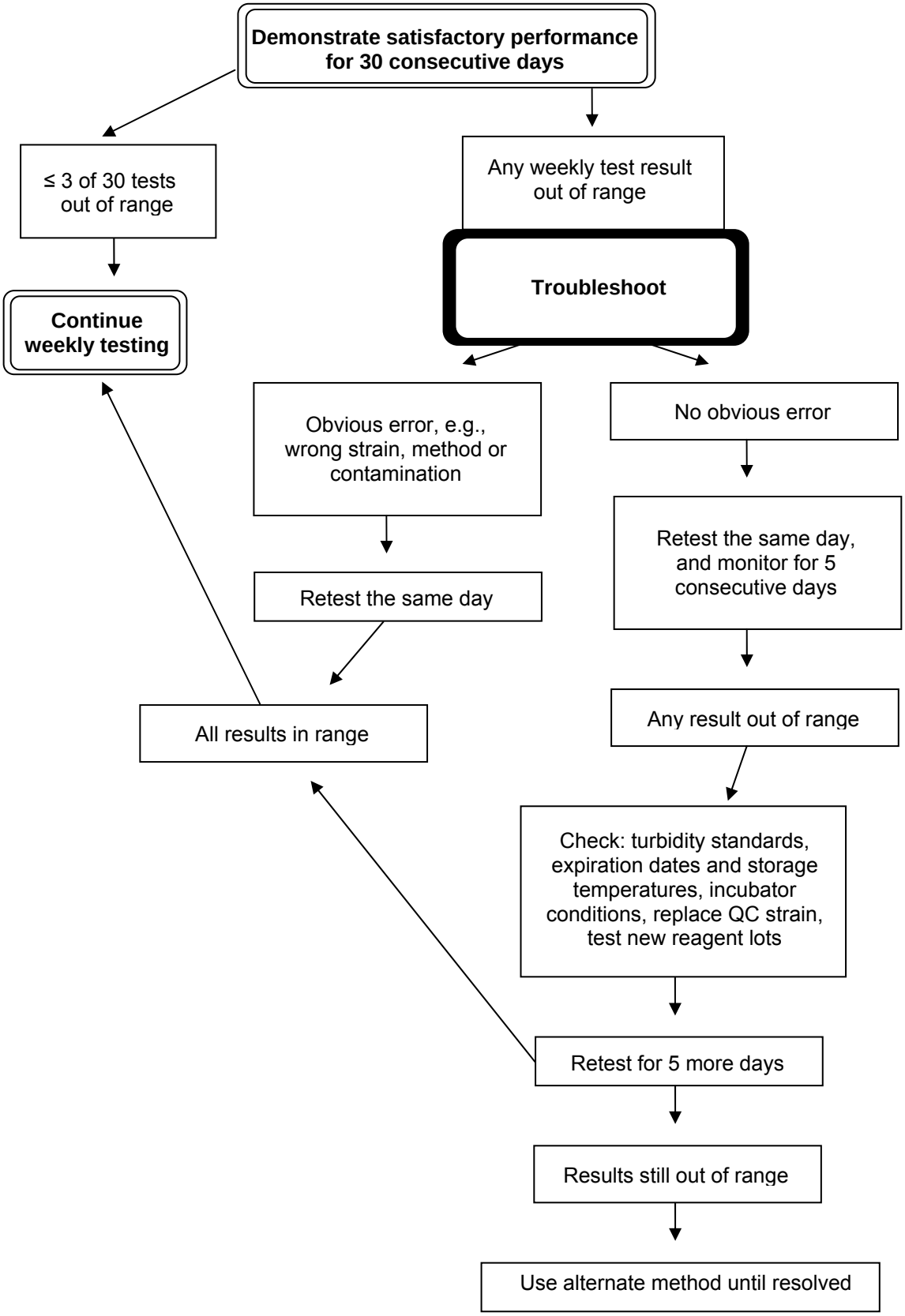
2 DAILY MIC QC CHART



Modified from CLSI M7-A6, page 35

National Food Institute

3 WEEKLY MIC QC CHART



Modified from CLSI M7-A6, page 36

National Food Institute

EVALUATION FORM

As means of improving the quality and usefulness of the CRL EQAS Salm/Camp 2007 we kindly ask you to take a moment to complete this evaluation form

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Name:

Country:

---

1. Information received during the EQAS and how the EQAS was performed:

	Very poor	Poor	Satisfactory	Good	Very good
Information about the EQAS in general	☐	☐	☐	☐	☐
The EQAS welcome letter (the letter in the parcel)	☐	☐	☐	☐	☐
The EQAS protocol and test forms	☐	☐	☐	☐	☐
The distribution of the samples	☐	☐	☐	☐	☐
What is your overall impression of the interactive web database	☐	☐	☐	☐	☐
How did participation in this EQAS meet your expectations	☐	☐	☐	☐	☐

Comments, suggestions:

2. Did you enter your results in the interactive web database?

☐ yes ☐ no

If not, please specify why:

3. Did you meet limitations or problems when entering data into the interactive web database?

☐ yes ☐ no

If yes, please specify:

4. General comments or suggestions for the EQAS (procedures, species, number of strains, antimicrobials etc.):

Questionnaire
CRL EQAS Salm/Camp 2007

As means of having updated information on your laboratory's work with *Salmonella* and *Campylobacter*, we ask you to please fill in the information listed below.

Considering the antimicrobials we ask for information on your *routine diagnostic methods* in you laboratory as well as the test ranges.

Please send the questionnaire by email to Michael Krause (mik@food.dtu.dk).

Participant

Name:

Country:

Annual isolates and susceptibility tests

How many *Salmonella* isolates does your laboratory annually isolate:

How many *Campylobacter* isolates does your laboratory annually isolate:

How many *Salmonella* isolates does your laboratory annually susceptibility test:

How many *Campylobacter* isolates does your laboratory annually susceptibility test:

Please list information on antimicrobials as described on the following pages.

Comments or additional information:

Routine diagnostic method used for AST of *Salmonella*

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

	Antimicrobial	**Disk content**	**Test-range for MIC**

Routine diagnostic method used for AST of *Campylobacter*

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

	Antimicrobial	**Disk content**	**Test-range for MIC**

Breakpoints used in daily routine (disk diffusion) - *Salmonella*

Antimicrobial	Lab No	Sensitive	Resistant
Amoxicillin+cl, AUG	29	<= 19	>= 12
	23	<= 18	>= 13
	9	>= 18	<= 13
	19	= 18	= 13
	5	>= 18	<= 13
	28	>= 18	<= 13
	30	= 18	= 13
	13	>= 21	< 14
	14	>= 21	< 14
	15	>= 21	< 14
Ampicillin, AMP	29	<= 18	>= 12
	23	<= 17	>= 13
	9	>= 17	<= 13
	19	= 17	= 13
	5	>= 17	<= 13
	28	>= 17	<= 13
	18	>= 17	<= 13
	30	= 17	= 13
	13	>= 19	< 14
	14	>= 21	< 14
	15	>= 21	< 14
Cefotaxime, CTX	23	<= 23	>= 14
	9	>= 23	<= 14
	19	= 23	= 14
	5	>= 23	<= 14
	28	>= 23	<= 14
	18	>= 23	<= 14
	30	= 23	= 14
	13	>= 21	< 15
	14	>= 21	< 15
	15	>= 21	< 15
	29	<= 22	>= 16
Ceftazidime, CAZ	23	<= 18	>= 14
	9	>= 18	<= 14
	19	= 18	= 14
	5	>= 18	<= 14
	28	>= 18	<= 14
	30	= 18	= 14
	13	>= 21	< 15
	14	>= 21	< 15
	15	>= 21	< 15
	29	<= 22	>= 16
Ceftiofur, XNL	29	<= 22	>= 16
	30	= 20	= 16
	9	>= 21	<= 17
	19	= 21	= 17
	14	>= 21	< 18
	15	>= 21	< 18

Antimicrobial	Lab No	Sensitive	Resistant
Chloramphenicol, CHL	29	<= 19	>= 11
	23	<= 18	>= 12
	9	>= 18	<= 12
	19	= 18	= 12
	5	>= 18	<= 12
	28	>= 18	<= 12
	18	>= 18	<= 12
	30	= 18	= 12
	13	>= 23	= 19
	14	>= 23	< 19
	15	>= 23	< 19
Ciprofloxacin, CIP	29	<= 24	>= 15
	9	>= 21	<= 15
	19	= 21	= 15
	5	>= 21	<= 15
	28	>= 21	<= 15
	18	>= 21	<= 15
	30	= 21	= 15
	15	>= 22	< 17
	13	>= 22	< 17
	14	>= 25	< 22
Gentamicin, GEN	29	<= 16	>= 11
	23	<= 15	>= 12
	9	>= 15	<= 12
	19	= 15	= 12
	5	>= 15	<= 12
	28	>= 15	<= 12
	18	>= 15	<= 12
	30	= 15	= 12
	13	>= 18	< 16
	14	>= 18	< 16
	15	>= 18	< 16
Nalidixic acid, NAL	23	<= 19	>= 13
	9	>= 19	<= 13
	19	= 19	= 13
	5	>= 19	<= 13
	28	>= 19	<= 13
	18	>= 19	<= 13
	30	= 19	= 13
	13	>= 20	< 15
	29	<= 24	>= 15
	14	>= 20	< 15
	15	>= 20	< 15
Streptomycin, STR	29	<= 16	>= 11
	23	<= 15	>= 11
	9	>= 15	<= 11
	19	= 15	= 11
	28	>= 15	<= 11
	18	>= 15	<= 11
	30	= 15	= 11
	13	>= 15	< 13
	15	>= 15	< 13

Antimicrobial	Lab No	Sensitive	Resistant
Sulphonamides, SMX	29	<= 18	>= 11
	13	>= 17	< 12
	23	<= 17	>= 12
	9	>= 17	<= 12
	14	>= 17	< 12
	19	= 17	= 12
	28	>= 17	<= 12
	15	>= 17	< 12
	18	>= 17	<= 12
	30	= 17	= 12
Tetracycline,TET	19	= 15	= 11
	28	>= 15	<= 11
	29	<= 20	>= 13
	23	<= 19	>= 14
	9	>= 19	<= 14
	5	>= 19	<= 14
	18	>= 19	<= 14
	30	= 19	= 14
	13	>= 19	< 17
	14	>= 19	< 17
	15	>= 19	< 17
TMP+SMX, SXT	29	<= 17	>= 9
	13	>= 16	< 10
	23	<= 16	>= 10
	9	>= 16	<= 10
	14	>= 16	< 10
	19	= 16	= 10
	5	>= 16	<= 10
	28	>= 16	<= 10
	18	>= 16	<= 10
	30	= 16	= 10
Trimethoprim, TMP	29	<= 17	>= 9
	23	<= 16	>= 10
	9	>= 16	<= 10
	19	= 16	= 10
	28	>= 16	<= 10
	30	= 16	= 10
	13	>= 16	< 12
	14	>= 16	< 12
	15	>= 16	< 12

Test results from the reference strain *E. coli* ATCC 25922

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
1	Amoxicillin cl., AUG	=	8	2	8	1	MIC
	Ampicillin, AMP	=	8	2	8	1	MIC
	Cefpodoxime, POD	=	0.5	0.25	1	1	MIC
	Ceftiofur, XNL	<=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	<=	0.03	0.004	0.016	1	MIC
	Florphenicol, FFN	=	4	2	8	1	MIC
	Gentamicin, GEN	<=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Streptomycin, STR	<=	4	4	16	1	MIC
	Tetracycline, TET	<=	2	0.5	2	1	MIC
	Trimethoprim, TMP	<=	4	0.5	2	1	MIC
2	Amoxicillin cl., AUG	=	4	2	8	1	MIC
	Ampicillin, AMP	=	2	2	8	1	MIC
	Cefotaxime, CTX	=	31	29	35	1	DD
	Ceftiofur, XNL	<=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	<=	0.03	0.004	0.016	1	MIC
	Florphenicol, FFN	=	4	2	8	1	MIC
	Gentamicin, GEN	<=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	8	1	4	1	MIC
	Streptomycin, STR	<=	4	4	16	1	MIC
	Sulphonamides, SMX	<=	64	8	32	1	MIC
	Tetracycline, TET	<=	2	0.5	2	1	MIC
	Trimethoprim, TMP	<=	4	0.5	2	1	MIC
4	Ampicillin, AMP	=	4	2	8	1	ET
	Cefotaxime, CTX	=	0.094	0.03	0.12	1	ET
	Chloramphenicol, CHL	=	3	0	256	1	ET
	Nalidixic acid, NAL	=	3	1	4	1	ET
	Streptomycin, STR	=	4	2	8	1	ET
	Sulphonamides, SMX	=	64	32	128	1	ET
	Tetracycline, TET	=	3	0.5	2	0	ET
	TMP+SMX, SXT	=	0.094	0.064	0.25	1	ET
	Trimethoprim, TMP	=	1	0.5	2	1	ET
5	Amoxicillin cl., AUG	=	23	18	24	1	DD
	Ampicillin, AMP	=	20	16	22	1	DD
	Cefotaxime, CTX	=	33	29	35	1	DD
	Ceftazidime, CAZ	=	26	25	32	1	DD
	Chloramphenicol, CHL	=	24	21	27	1	DD
	Ciprofloxacin, CIP	=	34	30	40	1	DD
	Gentamicin, GEN	=	24	19	26	1	DD
	Nalidixic acid, NAL	=	24	22	28	1	DD
	Tetracycline, TET	=	24	18	25	1	DD
	TMP+SMX, SXT	=	25	23	29	1	DD
6	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	<	0.06	0.03	0.12	1	MIC
	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	<	0.008	0.004	0.016	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	<	4	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulphonamides, SMX	=	16	8	32	1	MIC
	Tetracycline, TET	<	1	0.5	2	1	MIC
	Trimethoprim, TMP	<	0.5	0.5	2	1	MIC
9	Amoxicillin cl., AUG	=	21	18	24	1	DD
	Ampicillin, AMP	=	16	16	22	1	DD
	Cefotaxime, CTX	=	30	29	35	1	DD
	Cefoxitin, FOX	=	24	23	29	1	DD
	Ceftazidime, CAZ	=	27	25	32	1	DD
	Ceftiofur, XNL	=	26	26	31	1	DD
	Chloramphenicol, CHL	=	25	21	27	1	DD
	Ciprofloxacin, CIP	=	33	30	40	1	DD
	Florphenicol, FFN	=	23	22	28	1	DD
	Gentamicin, GEN	=	19	19	26	1	DD
	Nalidixic acid, NAL	=	25	22	28	1	DD
	Streptomycin, STR	=	16	0	50	1	DD
	Sulphonamides, SMX	=	22	15	23	1	DD
	Tetracycline, TET	=	23	18	25	1	DD
	TMP+SMX, SXT	=	25	23	29	1	DD
	Trimethoprim, TMP	=	26	21	28	1	DD

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
11	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	<=	0.06	0.03	0.12	1	MIC
	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.03	0.004	0.016	0	MIC
	Florphenicol, FFN	<=	4	2	8	1	MIC
	Gentamicin, GEN	=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	=	4	1	4	1	MIC
	Streptomycin, STR	=	8	4	16	1	MIC
	Sulphonamides, SMX	=	24	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
12	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	<=	0.06	0.03	0.12	1	MIC
	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.03	0.004	0.016	0	MIC
	Gentamicin, GEN	=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	=	2	1	4	1	MIC
	Streptomycin, STR	=	8	4	16	1	MIC
	Sulphonamides, SMX	<=	16	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
13	Amoxicillin cl., AUG	=	22	18	24	1	DD
	Ampicillin, AMP	=	14	16	22	0	DD
	Cefotaxime, CTX	=	35	29	35	1	DD
	Cefoxitin, FOX	=	27	23	29	1	DD
	Ceftazidime, CAZ	=	32	25	32	1	DD
	Chloramphenicol, CHL	=	26	21	27	1	DD
	Ciprofloxacin, CIP	=	35	30	40	1	DD
	Gentamicin, GEN	=	26	19	26	1	DD
	Imipenem, IMI	=	35	26	32	0	DD
	Nalidixic acid, NAL	=	26	22	28	1	DD
	Streptomycin, STR	=	18	0	50	1	DD
	Sulphonamides, SMX	=	22	15	23	1	DD
	Tetracycline, TET	=	24	18	25	1	DD
	TMP+SMX, SXT	=	29	23	29	1	DD
	Trimethoprim, TMP	=	25	21	28	1	DD
15	Amoxicillin cl., AUG	=	26	18	24	0	DD
	Amoxicillin, AMX	=	26	0	50	1	DD
	Cefotaxime, CTX	=	37	29	35	0	DD
	Cefoxitin, FOX	=	32	23	29	0	DD
	Ceftazidime, CAZ	=	34	25	32	0	DD
	Ceftiofur, XNL	=	32	26	31	0	DD
	Chloramphenicol, CHL	=	29	21	27	0	DD
	Florphenicol, FFN	=	27	22	28	1	DD
	Gentamicin, GEN	=	26	19	26	1	DD
	Imipenem, IMI	=	38	26	32	0	DD
	Nalidixic acid, NAL	=	28	22	28	1	DD
	Streptomycin, STR	=	17	0	50	1	DD
	Sulphonamides, SMX	=	30	15	23	0	DD
	Tetracycline, TET	=	26	18	25	0	DD
	Trimethoprim, TMP	=	27	21	28	1	DD
16	Amoxicillin cl., AUG	=	8	2	8	1	MIC
	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Cefoxitin, FOX	=	8	2	8	1	MIC
	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
	Ceftiofur, XNL	=	1	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Florphenicol, FFN	=	4	2	8	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	=	2	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulphonamides, SMX	=	32	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	TMP+SMX, SXT	<=	1	0	0.5	1	MIC
	Trimethoprim, TMP	=	2	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
17	Amoxicillin cl., AUG	=	8	2	8	1	MIC
	Ampicillin, AMP	=	4	2	8	1	MIC
	Ceftiofur, XNL	<=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	<=	0.03	0.004	0.016	1	MIC
	Florphenicol, FFN	=	4	2	8	1	MIC
	Gentamicin, GEN	<=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Streptomycin, STR	<=	4	4	16	1	MIC
	Sulphonamides, SMX	<=	32	8	32	1	MIC
	Tetracycline, TET	<=	2	0.5	2	1	MIC
	TMP+SMX, SXT	<=	1	0	0.5	1	MIC
	Trimethoprim, TMP	<=	4	0.5	2	1	MIC
18	Ampicillin, AMP	=	19.5	16	22	1	DD
	Cefotaxime, CTX	=	31.6	29	35	1	DD
	Chloramphenicol, CHL	=	24	21	27	1	DD
	Ciprofloxacin, CIP	=	33	30	40	1	DD
	Gentamicin, GEN	=	22	19	26	1	DD
	Nalidixic acid, NAL	=	24.7	22	28	1	DD
	Streptomycin, STR	=	14.5	0	50	1	DD
	Sulphonamides, SMX	=	20.6	15	23	1	DD
	Tetracycline, TET	=	24	18	25	1	DD
	TMP+SMX, SXT	=	24	23	29	1	DD
19	Amoxicillin cl., AUG	=	22	18	24	1	DD
	Ampicillin, AMP	=	17	16	22	1	DD
	Cefotaxime, CTX	=	32	29	35	1	DD
	Cefpodoxime, POD	=	26	23	28	1	DD
	Ceftazidime, CAZ	=	28	25	32	1	DD
	Ceftiofur, XNL	=	26	26	31	1	DD
	Chloramphenicol, CHL	=	23	21	27	1	DD
	Ciprofloxacin, CIP	=	31	30	40	1	DD
	Florphenicol, FFN	=	26	22	28	1	DD
	Gentamicin, GEN	=	21	19	26	1	DD
	Nalidixic acid, NAL	=	27	22	28	1	DD
	Streptomycin, STR	=	15	0	50	1	DD
	Sulphonamides, SMX	=	21	15	23	1	DD
	Tetracycline, TET	=	25	18	25	1	DD
	TMP+SMX, SXT	=	29	23	29	1	DD
	Trimethoprim, TMP	=	23	21	28	1	DD
20	Ampicillin, AMP	=	8	2	8	1	MIC
	Cefotaxime, CTX	<	0.06	0.03	0.12	1	MIC
	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Florphenicol, FFN	=	4	2	8	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	<	4	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulphonamides, SMX	=	16	8	32	1	MIC
	Tetracycline, TET	<	1	0.5	2	1	MIC
	Trimethoprim, TMP	<	0.5	0.5	2	1	MIC
21	Ampicillin, AMP	=	2	2	8	1	MIC
	Cefotaxime, CTX	=	0.06	0.03	0.12	1	MIC
	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	2	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	=	4	1	4	1	MIC
	Sulphonamides, SMX	=	8	8	32	1	MIC
	Tetracycline, TET	=	2	0.5	2	1	MIC
	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
22	Ampicillin, AMP	=	2	2	8	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Gentamicin, GEN	=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	=	2	1	4	1	MIC
	Streptomycin, STR	=	8	4	16	1	MIC
	Sulphonamides, SMX	<=	16	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
23	Amoxicillin cl., AUG	=	22	18	24	1	DD
	Ampicillin, AMP	=	23	16	22	0	DD
	Cefotaxime, CTX	=	30	29	35	1	DD
	Ceftazidime, CAZ	=	27	25	32	1	DD
	Chloramphenicol, CHL	=	25	21	27	1	DD
	Ciprofloxacin, CIP	=	32	30	40	1	DD
	Florphenicol, FFN	=	25	22	28	1	DD
	Gentamicin, GEN	=	20	19	26	1	DD
	Nalidixic acid, NAL	=	24	22	28	1	DD
	Streptomycin, STR	=	17	0	50	1	DD
	Sulphonamides, SMX	=	23	15	23	1	DD
	Tetracycline, TET	=	23	18	25	1	DD
	TMP+SMX, SXT	=	27	23	29	1	DD
	Trimethoprim, TMP	=	25	21	28	1	DD
24	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	<=	0.06	0.03	0.12	1	MIC
	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Florphenicol, FFN	=	4	2	8	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulphonamides, SMX	<=	8	8	32	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
25	Ampicillin, AMP	=	8	2	8	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	8	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Florphenicol, FFN	=	4	2	8	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
26	Ampicillin, AMP	=	4	2	8	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Gentamicin, GEN	=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	=	4	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulphonamides, SMX	=	32	8	32	1	MIC
	Tetracycline, TET	=	2	0.5	2	1	MIC
	Trimethoprim, TMP	=	2	0.5	2	1	MIC
27	Amoxicillin, AMX	<=	4	0	256	1	MIC
	Ampicillin, AMP	<=	4	2	8	1	MIC
	Cefotaxime, CTX	=	0.06	0.03	0.12	1	MIC
	Cefoxitin, FOX	=	4	2	8	1	MIC
	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
	Ciprofloxacin, CIP	=	0.016	0.004	0.016	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
28	Amoxicillin cl., AUG	=	20	18	24	1	DD
	Ampicillin, AMP	=	22	16	22	1	DD
	Cefotaxime, CTX	=	31	29	35	1	DD
	Ceftazidime, CAZ	=	29	25	32	1	DD
	Chloramphenicol, CHL	=	25	21	27	1	DD
	Ciprofloxacin, CIP	=	32	30	40	1	DD
	Gentamicin, GEN	=	20	19	26	1	DD
	Nalidixic acid, NAL	=	28	22	28	1	DD
	Streptomycin, STR	=	16	0	50	1	DD
	Sulphonamides, SMX	=	23	15	23	1	DD
	Tetracycline, TET	=	24	18	25	1	DD
	TMP+SMX, SXT	=	29	23	29	1	DD
	Trimethoprim, TMP	=	28	21	28	1	DD
29	Amoxicillin cl., AUG	=	23	18	24	1	DD
	Cefotaxime, CTX	=	24	29	35	0	DD
	Ceftiofur, XNL	=	27	26	31	1	DD
	Chloramphenicol, CHL	=	30	21	27	0	DD
	Ciprofloxacin, CIP	=	33	30	40	1	DD
	Florphenicol, FFN	=	28	22	28	1	DD
	Gentamicin, GEN	=	21	19	26	1	DD
	Nalidixic acid, NAL	=	26	22	28	1	DD
	Streptomycin, STR	=	24	0	50	1	DD
	Sulphonamides, SMX	=	22	15	23	1	DD
	Tetracycline, TET	=	23	18	25	1	DD
	TMP+SMX, SXT	=	25	23	29	1	DD

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
30	Amoxicillin cl., AUG	=	21	18	24	1	DD
	Ampicillin, AMP	=	21	16	22	1	DD
	Cefotaxime, CTX	=	35	29	35	1	DD
	Cefpodoxime, POD	=	28	23	28	1	DD
	Ceftazidime, CAZ	=	27	25	32	1	DD
	Chloramphenicol, CHL	=	25	21	27	1	DD
	Ciprofloxacin, CIP	=	35	30	40	1	DD
	Gentamicin, GEN	=	23	19	26	1	DD
	Nalidixic acid, NAL	=	27	22	28	1	DD
	Streptomycin, STR	=	16	0	50	1	DD
	Sulphonamides, SMX	=	22	15	23	1	DD
	Tetracycline, TET	=	24	18	25	1	DD
	TMP+SMX, SXT	=	28	23	29	1	DD
	Trimethoprim, TMP	=	25	21	28	1	DD
32	Ampicillin, AMP	=	8	2	8	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	16	2	8	0	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Florphenicol, FFN	=	8	2	8	1	MIC
	Gentamicin, GEN	=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Streptomycin, STR	=	16	4	16	1	MIC
	Sulphonamides, SMX	>	1024	8	32	0	MIC
	Tetracycline, TET	=	2	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
33	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	<=	0.06	0.03	0.12	1	MIC
	Cefoxitin, FOX	=	4	2	8	1	MIC
	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.016	0.004	0.016	1	MIC
	Gentamicin, GEN	=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	=	4	1	4	1	MIC
	Streptomycin, STR	=	8	4	16	1	MIC
	Sulphonamides, SMX	=	32	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
34	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	<=	0.06	0.03	0.12	1	MIC
	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulphonamides, SMX	=	16	8	32	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
35	Amoxicillin, AMX	=	8	0	256	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	>=	0.06	0.004	0.016	0	MIC
	Florphenicol, FFN	<=	4	2	8	1	MIC
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	=	2	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
36	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.03	0.004	0.016	0	MIC
	Gentamicin, GEN	=	1	0.25	1	1	MIC
	Nalidixic acid, NAL	=	2	1	4	1	MIC
	Streptomycin, STR	=	8	4	16	1	MIC
	Sulphonamides, SMX	=	32	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC

Test results from the reference strain *C. jejuni* ATCC 33560

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36ºC/48h	42ºC/24h
1	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
2	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	0.5	0.25	2	1	MIC	X	
4	Chloramphenicol, CHL	=	2	-	-	-	ET	X	
	Ciprofloxacin, CIP	=	0.094	0.125	1	1	ET	X	
	Erythromycin, ERY	=	1	1	8	1	ET	X	
	Gentamicin, GEN	=	0.38	0.5	2	0	ET	X	
	Nalidixic acid, NAL	=	3	-	-	-	ET	X	
	Tetracycline, TET	=	0.125	-	-	-	ET	X	
6	Chloramphenicol, CHL	<	2	1	4	1	MIC		X
	Ciprofloxacin, CIP	<	0.06	0.03	0.12	1	MIC		X
	Erythromycin, ERY	<	0.5	0.25	2	1	MIC		X
	Gentamicin, GEN	=	0.25	0.25	2	1	MIC		X
	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
	Tetracycline, TET	=	0.5	0.25	1	1	MIC		X
11	Ciprofloxacin, CIP	=	0.5	0.06	0.25	0	MIC	X	
	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
	Gentamicin, GEN	=	8	0.5	2	0	MIC	X	
	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
12	Ciprofloxacin, CIP	=	0.5	0.06	0.25	0	MIC	X	
	Erythromycin, ERY	=	4	0.5	2	0	MIC	X	
	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
14	Ciprofloxacin, CIP	=	0.25				MIC	37°C/24h	
	Erythromycin, ERY	=	2				MIC	37°C/24h	
	Tetracycline, TET	=	1				MIC	37°C/24h	
15	Ciprofloxacin, CIP	=	0.094	0.064	0.5	1	ET		X
	Erythromycin, ERY	=	1	1	4	1	ET		X
	Gentamicin, GEN	=	0.75	0.5	4	1	ET		X
	Nalidixic acid, NAL	=	4	-	-	-	ET		X
	Tetracycline, TET	=	0.5	-	-	-	ET		X
17	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	1	0.06	0.25	0	MIC	X	
	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
19	Ciprofloxacin, CIP	=	0.12	0.03	0.12	1	MIC		X
	Erythromycin, ERY	=	0.5	0.25	2	1	MIC		X
	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
	Tetracycline, TET	=	2	0.25	1	0	MIC		X
20	Chloramphenicol, CHL	=	8	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
	Tetracycline, TET	=	2	0.25	2	1	MIC	X	

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36ºC/48h	42ºC/24h
21	Chloramphenicol, CHL	=	1	1	4	1	MIC		X
	Ciprofloxacin, CIP	=	0.12	0.03	0.12	1	MIC		X
	Erythromycin, ERY	=	1	0.25	2	1	MIC		X
	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
	Tetracycline, TET	=	1	0.25	1	1	MIC		X
22	Ciprofloxacin, CIP	=	0.5	0.03	0.12	0	MIC		X
	Erythromycin, ERY	=	4	0.25	2	0	MIC		X
	Gentamicin, GEN	=	2	0.25	2	1	MIC		X
	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
	Tetracycline, TET	=	1	0.25	1	1	MIC		X
23	Chloramphenicol, CHL	=	40				DD		
	Ciprofloxacin, CIP	=	31				DD		
	Erythromycin, ERY	=	35				DD		
	Nalidixic acid, NAL	=	24				DD		
	Tetracycline, TET	=	35				DD		
24	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
25	Chloramphenicol, CHL	=	8	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	4	0.5	2	0	MIC	X	
	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
26	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
28	Chloramphenicol, CHL	<=	8	0	256	1	AGA		
	Ciprofloxacin, CIP	<=	0.5	0.12	1	1	AGA		
	Erythromycin, ERY	<=	2	1	8	1	AGA		
	Nalidixic acid, NAL	<=	8	8	32	1	AGA		
	Tetracycline, TET	<=	1	1	4	1	AGA		
30	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	0.5	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
32	Chloramphenicol, CHL	=	4	1	4	1	MIC		X
	Ciprofloxacin, CIP	=	0.25	0.03	0.12	0	MIC		X
	Erythromycin, ERY	=	1	0.25	2	1	MIC		X
	Gentamicin, GEN	=	1	0.25	2	1	MIC		X
	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
	Tetracycline, TET	=	1	0.25	1	1	MIC		X
33	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
34	Chloramphenicol, CHL	=	8	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
36	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	

E. coli ATCC 25922			
Antimicrobial	**MIC**	**E-test**	**DD (disc content)**
Amoxicillin cl., AUG	2/1-8/4	2/1-8/4	18-24 (20/10µg)
Amoxicillin, AMX	None	None	None
Ampicillin, AMP	2-8	2-8	16-22 (10µg)
Cefotaxime, CTX	0,03-0,12	0,03-0,12	29-35 (30µg)
Cefoxitin, FOX	2-8	None	23-29 (30µg)
Cefpodoxime, POD	0,25-1	0,25-1	23-28 (10µg)
Ceftazidime, CAZ	0,06-0,5	0,06-0,5	25-32 (30µg)
Ceftiofur, XNL	0,25-1	None	26-31 (30µg)
Chloramphenicol, CHL	2-8	None	21-27 (30µg)
Ciprofloxacin, CIP	0,004-0,016	None	30-40 (5µg)
Florphenicol, FFN	2-8	None	22-28 (30µg)
Gentamicin, GEN	0,25-1	None	19-26 (10µg)
Imipenem, IMI	0,06-0,25	0,06-0,25	26-32 (10µg)
Nalidixic acid, NAL	1-4	1-4	22-28 (30µg)
Streptomycin, STR	4-16	2-8	None
Sulphonamides, SMX	8-32	32-128	15-23 (250/300µg)
TMP+SMX, SXT	0-0,5	0,064-0,25	23-29 (1,25/23,75µg)
Tetracycline, TET	0,5-2	0,5-2	18-25 (30µg)
Trimethoprim, TMP	0,5-2	0,5-2	21-28 (5µg)

MIC ranges and disc diffusion ranges are according to CLSI M100 S15 with one exception: The MIC range for streptomycin is according to Sensititre. Additionally, the range for ciprofloxacin is extended to include 0,016 as well.

E-test ranges are according to AB-Biodisk

Campylobacter jejuni ATCC 33560			
Antimicrobial	**Microbroth (36°C/48h)**	**Microbroth (42°C/24h)**	**Agar dilution**
Chloramphenicol, CHL	1-8	1-4	None
Ciprofloxacin, CIP	0,06-0,25	0,03-0,12	0,12-1
Doxycycline, DOX	None	None	0,5-2
Erythromycin, ERY	0,5-2	0,25-2	1-8
Gentamicin, GEN	0,5-2	0,25-2	0,5-4
Meropenem, MERO	None	None	0,004-0,015
Nalidixic acid, NAL	4-16	4-16	8-32
Tetracycline, TET	0,25-2	0,25-1	1-4

Ranges are according to CLSI:

- For microbroth MIC: CLSI guideline M45-A
- For agardilution MIC: CLSI guideline M31-A2

Campylobacter jejuni ATCC 33560		
Antimicrobial	**E-test (36°C/48h)**	**E-test (42°C/24h)**
Ciprofloxacin, CIP	0,125-1	0,064-0,5
Doxycycline, DOX	0,5-2	0,25-2
Erythromycin, ERY	1-8	1-4
Gentamicin, GEN	0,5-2	0,5-4
Meropenem, MERO	0,004-0,016	0,008-0,032

Ranges are according to AB Biodisk

Evaluation forms, summarised

Participants' evaluation of the CRL EQAS Salm/Camp 2007

The number of participating laboratories in the CRL EQAS Salm/Camp 2007 was 31. The participants were asked to fill in an evaluation form as a means of improving the quality and usefulness of the EQAS. In the following, the information obtained through the 7 completed evaluation forms is collected and commented. Please find comments from the CRL in *italic* in the following.

1. Information received during the CRL AR EQAS 2007 and how the EQAS was performed:

Opinion Percentage (number of laboratories)	Very poor	Poor	Satisfactory	Good	Very good
Information about the EQAS in general	-	-	14% (1)	43% (3)	43% (3)
The EQAS welcome letter (the letter in the parcel)	-	-	-	71% (5)	28% (2)
The EQAS protocol and test forms	-	-	14% (1)	57% (4)	28% (2)
The distribution of the samples	-	-	28% (2)	28% (2)	43% (3)
What is your overall impression of the interactive web database	-	-	17% (1)	67% (4)	17% (1)
How did participation in this EQAS meet your expectations	-	-	-	57% (4)	43% (3)

Comments and proposals from participants:

One participant mentions that two of the *Campylobacter* did not grow, other participants state that they have problems with opening the ampoules and prefer strains in swabs to ampoules. *In this EQAS we had some problems with the viability of some* Campylobacter *test strains. In the future we aim towards not sending lyophilised strains, but will instead ship* Campylobacter *strains as charcoal swabs.*

2. Did you enter your results in the interactive database?

Opinion Percentage (number of laboratories)	Yes	86% (6)
	No	14% (1)

Comments and expectations and suggestions from participants:

Writing on paper is simpler. *Sending the results by fax or email is an option.In general, if you have problems with the login or with the functionality of the the database you are always welcome to contact the CRL.*

3. Did you meet limitations or problems when entering data into the interactive web database?

Opinion Percentage (number of laboratories)	Yes	50% (3)
	No	50% (3)

When using a scroll mouse, you have to beware that the last selected field (e.g. interpretation) can change when using the wheel. *It is correct that this is a downside to when uploading data.*

First we had no code, then slow speed of our net impede our interaction with the web database. *The username and password is on the letter following the test strains.*

It was not possible to enter the *C. jejuni* and *C. coli* breakpoints as there was only room for one set of breakpoints. *The systems developer has been asked to add the possibility to upload both sets of breakpoints.*

4. General comments or suggestions for the EQAS (procedures, species, number of strains, antimicrobials etc.):

One participant states that there are too many isolates and EQAS is very often, another states that they would suggest to include more staphylococci and *Listeria*, to have the procedure for *Campylobacter* extended and then no more than five strains per trimester. *In general, the EFSA recommendations regarding microorganisms will be followed. At the conference and the workshop in June 2008 the selection of microorganisms and antimicrobials can be discussed.Eight strains of each species gives a number of tests that can be evaluated with some certainty with regard to*

determining the level of performance of the laboratory. This will be the number of strains of each species that we will be using in the future.

We are testing *Salmonella* and *Campylobacter* using two different methods, but can upload only one set of results. *It is possible to upload two sets of results. If it is the case that you supplement one method with another, and thus would like to mix the methods used when you upload the data – this is no problem. The database evaluates on the interpretations and therefore it does not make a difference whether the obtained result is given as a zone diameter or a MIC-value. If you choose to mix the methods like this, please note that the method you have used for the QC-strain is the method you should mark on the first page! In case of using two different methods paralel on all antimicrobials it is possible for us to provide you with an extra username and password and give you the opportunity to upload two sets of results. If you choose to get an extra username and password please note that this extra set of results will not be evaluated in the report – only one set of results from each NRL will be evaluated in the report.*

Why do you want to have results for ciprofloxacin, if these are the same as the results for nalidixic acid? Salmonella *isolates do not always react the same way to ciprofloxacin and nalidixic acid, e.g. qnr positive strains which are cip-resistant and nal-sensitive.*

Additional comments from the CRL

It is of great value to have comments from the participants, it helps us to optimise the EQAS. Thank you very much for taking your time to write them down. In general, we welcome any comments or enquiries that you may have. You are welcome to write us an email and we will make an effort to get back to you a.s.a.p. with an answer or some relevant advice.

Questionnaire, summarised - Test range for MIC (µg/mL) - *Salmonella*

Antimicrobial	Lab #								
	1	**6**	**11**	**17**	**12**	**11**	**20**	**24**	**29**
Ampicillin/Clavulanic acid	2/1-32/16			2/1-32/16					
Ampicillin	1-32	0.5-32	0.25-32	1-32	0.25-32	0.25-32	0.5-32	0.5-32	0,5 - 64
Ampicillin/Sulbactam (1:1)									0,5 - 64
Apramycin	4-32								
Cefotaxime		0.06-4	0.06-2		0.06-2	0.06-2	0.06-4	0.06-4	0,25 - 32
Cefpodoxime	0,125-4								
Ceftazidime		0.25-16					0.25-16	0.25-16	0,25 - 32
Ceftiofur	0,5-8		0.12-16	0.5-8	0.12-16	0.12-16			
Cephalothin	4-32								0,12 - 16
Chloramphenicol	2-64	2-64	1-128	2-64	1-128	1-128	2-64	2-64	0,25 - 32
Ciprofloxacin	0,03-4	0.008-4	0.008-1	0.03-4	0.008-1	0.008-1	0.008-8	0.008-8	0,06 - 4
Colistin	4-16			4-64			8-16	8-16	
Florfenicol	1-32		4-32	2-64	4-32	4-32	2-64	2-64	
Gentamicin	1-32	0.25-32	0.5-64	1-32	0.5-64	0.5-64	0.25-32	0.25-32	0,25 - 32
Kanamycin			2-16	4-64	2-16	2-16	4-128	4-128	
Nalidixic acid	4-64	4-64	1-128	4-128	1-128	1-128	4-64	4-64	
Neomycin	2-32			2-32					
Oxolinic acid									0,5 - 64
Spectinomycin	16-256			2-128					
Streptomycin	4-64	2-128	2-256	4-64	2-256	2-256	2-128	2-128	0,25 - 32
Sulphonamides (sulphamethoxazole)	64-1024	8-1024	16-2048	32-512	16-2048	16-2048	8-1024	8-1024	
Tetracyclin	2-32	1-64	0.5-64	2-32	0.5-64	0.5-64	1-64	1-64	0,25 - 32
Trimethoprim	4-32	0.5-32	0.25-32	4-32	0.25-32	0.25-32	0.5-32	0.5-32	0,5 - 64
Sulf./Trimethoprim				19/1-152/8					

Antimicrobials recommended by EFSA are marked in grey
Participants using ranges recommended by EFSA are marked in grey

Questionnaire, summarised - Disk content (µg) - *Salmonella*

Antimicrobial	Lab #								
	4	**2**	**6**	**11**	**19**	**21**	**22**	**28**	**29**
Amoxycillin + clavulanic acid	30/15		20/10		30		30	20/10	30
Amoxycillin									10
Ampicillin	33	10	10	10	10	10	10	10	
Apramycin					15				
Cefotaxime		30	30	30	30	30	30	30	
Cefoxitin								30	
Cefquinome									30
Ceftazidime			30		30	30		30	
Cephalexin									30
Cephalotin					30				
Ceftiofur	30		30	30			30		
Chloramphenicol	60	30	30	30	30	30	30	30	
Ciprofloxacin		5	5	5		5	5	5	
Colistin					10				
Doxycyclin									30
Enrofloxacin					5				5
Florfenicol				30	30				30
Gentamicin	40	10	10	10	10	10	10	10	10
Imipenem								10	
Kanamycin		30		30		30		30	30
Nalidixic acid	130	30	30	30	30	30	30	30	30
Neomycin				30	30				
Nitrofurantoin		300							
Oxytetracycline									30
Spectinomycin					100				100
Streptomycin		10	10	10	10	10	10	10	10
Sulphonamides		300	300	300	300	250	300	250/300	300
Tetracycline	80	30	30	30	30	30	30	30	
Trimethoprim	5.2	5	30	5		5	5/25	5	5
Trimethoprim + sulfameth.	5.2/240		1.25/2.75		23.75/1.25			1,25/23,75	25

Questionnaire, summarised - test range for MIC (µg/mL) - *Campylobacter*

Antimicrobial	Lab #									
	1	**2**	**6**	**12**	**17**	**11**	**19**	**20**	**21**	**24**
Amoxicillin/Clavulanic acid		1/05-128/64								
Amoxicillin					0.25-32				0.12-128	
Ampicillin		1-128								0.25-32
Chloramphenicol	2-32	2-32	2-32		2-128				1-32	2-128
Clindamycin							0.03-16			
Ciprofloxacin	0.03-4	0.06-32	0.06-4	0.06-8	0.12-16	0.06-8		0.06-8	0.06-128	0.12-16
Clarithromycin										0.5-64
Colistin		4-64								
Erythromycin	0.5-32	0.25-128	0.5-32	0.5-64	0.5-64	0.5-64	0.03-64	0.5-64	0.12-128	0.5-64
Enrofloxacin							0.015-64			
Gentamicin	0.125-16	0.25-64	0.12-16	0.12-16	0.25-32	0.12-16		0.12-16	0.12-128	0.25-32
Metronidazole					0.5-64				0.5-128	
Nalidixic acid	2-64	2-128	2-64	1-64	1-128	1-64	4-64	1-64	0.12-128	1-128
Neomycin		1-64			0.5-64					0.5-64
Streptomycin	2-16	1-64	1-16	0.5-64	1-128	0.5-64		0.5-64		1-128
Sulfonamides (sulfamethoxazole)					8-512					8-1024`
Tetracycline	0.25-16	0.25-128	0.25-16	0.12-16	0.5-64	0.12-16	0.06-64	0.12-16	0.12-128	0.5-64
Trimethoprim		0.5-64								
Sulfonamides+trimethoprim					0.25/4.75-32/608					
Tulathromycin										0.5-64

Antimicrobials recommended by EFSA are marked in grey

Participants using ranges recommended by EFSA are marked in grey

Salmonella - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S
Amoxicillin cl., AUG	CRL S-2.1	R	91	9
	CRL S-2.2	R	33	67
	CRL S-2.3	R	36	64
	CRL S-2.4	S	0	100
	CRL S-2.5	S	14	86
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	R	100	0
Ampicillin, AMP	CRL S-2.1	R	100	0
	CRL S-2.2	R	100	0
	CRL S-2.3	R	100	0
	CRL S-2.4	S	7	93
	CRL S-2.5	R	100	0
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	R	100	0
Cefotaxime, CTX	CRL S-2.1	S	0	100
	CRL S-2.2	R	88	12
	CRL S-2.3	S	0	100
	CRL S-2.4	S	7	93
	CRL S-2.5	S	0	100
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	R	92	8
Ceftazidime, CAZ	CRL S-2.1	S	0	100
	CRL S-2.2	R	56	44
	CRL S-2.3	S	0	100
	CRL S-2.4	S	6	94
	CRL S-2.5	S	0	100
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	R	100	0
Ceftiofur, XNL	CRL S-2.1	S	7	93
	CRL S-2.2	R	100	0
	CRL S-2.3	S	7	93
	CRL S-2.4	S	0	100
	CRL S-2.5	S	0	100
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	R	100	0
Chloramphenicol, CHL	CRL S-2.1	S	11	89
	CRL S-2.2	S	0	100
	CRL S-2.3	R	100	0
	CRL S-2.4	S	3	97
	CRL S-2.5	S	0	100
	CRL S-2.6	S	0	100
	CRL S-2.7	R	97	3
	CRL S-2.8	R	100	0
Ciprofloxacin, CIP	CRL S-2.1	R	85	15
	CRL S-2.2	R	74	26
	CRL S-2.3	S	4	96
	CRL S-2.4	S	7	93
	CRL S-2.5	R	78	22
	CRL S-2.6	S	0	100
	CRL S-2.7	S	3	97
	CRL S-2.8	S	4	96

Antimicrobial	Strain	Expected	% R	% S
Gentamicin, GEN	CRL S-2.1	R	96	4
	CRL S-2.2	S	0	100
	CRL S-2.3	R	93	7
	CRL S-2.4	S	3	97
	CRL S-2.5	R	100	0
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	S	0	100
Nalidixic acid, NAL	CRL S-2.1	R	100	0
	CRL S-2.2	R	100	0
	CRL S-2.3	S	3	97
	CRL S-2.4	S	0	100
	CRL S-2.5	R	100	0
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	S	0	100
Streptomycin, STR	CRL S-2.1	R	93	7
	CRL S-2.2	S	0	100
	CRL S-2.3	R	100	0
	CRL S-2.4	R	100	0
	CRL S-2.5	S	23	77
	CRL S-2.6	S	0	100
	CRL S-2.7	R	93	7
	CRL S-2.8	R	100	0
Sulphonamides, SMX	CRL S-2.1	R	100	0
	CRL S-2.2	S	0	100
	CRL S-2.3	R	100	0
	CRL S-2.4	R	100	0
	CRL S-2.5	R	96	4
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	R	100	0
Tetracycline, TET	CRL S-2.1	S	7	93
	CRL S-2.2	R	100	0
	CRL S-2.3	R	100	0
	CRL S-2.4	R	100	0
	CRL S-2.5	R	100	0
	CRL S-2.6	S	4	96
	CRL S-2.7	R	100	0
	CRL S-2.8	R	100	0
TMP+SMX, SXT	CRL S-2.1	S	6	94
	CRL S-2.2	S	0	100
	CRL S-2.3	R	100	0
	CRL S-2.4	S	0	100
	CRL S-2.5	R	93	7
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	S	0	100
Trimethoprim, TMP	CRL S-2.1	S	4	96
	CRL S-2.2	S	0	100
	CRL S-2.3	R	100	0
	CRL S-2.4	S	4	96
	CRL S-2.5	R	100	0
	CRL S-2.6	S	0	100
	CRL S-2.7	S	0	100
	CRL S-2.8	S	0	100

Campylobacter - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S
Chloramphenicol, CHL	CRL C-2.1	S	0	100
	CRL C-2.2	S	0	100
	CRL C-2.3	S	0	100
	CRL C-2.4	S	0	100
	CRL C-2.5	S	0	100
	CRL C-2.6	S	11	89
	CRL C-2.7	S	8	92
	CRL C-2.8	S	0	100
Ciprofloxacin, CIP	CRL C-2.1	S	4	96
	CRL C-2.2	S	0	100
	CRL C-2.3	R	100	0
	CRL C-2.4	S	8	92
	CRL C-2.5	S	9	91
	CRL C-2.6	R	80	20
	CRL C-2.7	R	91	9
	CRL C-2.8	R	91	9
Erythromycin, ERY	CRL C-2.1	S	4	96
	CRL C-2.2	S	4	96
	CRL C-2.3	S	4	96
	CRL C-2.4	S	4	96
	CRL C-2.5	R	100	0
	CRL C-2.6	R	100	0
	CRL C-2.7	S	9	91
	CRL C-2.8	R	96	4
Gentamicin, GEN	CRL C-2.1	S	5	95
	CRL C-2.2	S	0	100
	CRL C-2.3	S	4	96
	CRL C-2.4	S	4	96
	CRL C-2.5	S	0	100
	CRL C-2.6	R	87	13
	CRL C-2.7	S	9	91
	CRL C-2.8	S	5	95
Nalidixic acid, NAL	CRL C-2.1	S	0	100
	CRL C-2.2	S	0	100
	CRL C-2.3	R	100	0
	CRL C-2.4	S	4	96
	CRL C-2.5	S	5	95
	CRL C-2.6	R	93	7
	CRL C-2.7	R	91	9
	CRL C-2.8	R	100	0
Streptomycin, STR	CRL C-2.1*	S*	86*	14*
	CRL C-2.2	R	100	0
	CRL C-2.3	S	10	90
	CRL C-2.4	R	100	0
	CRL C-2.5	S	14	86
	CRL C-2.6	R	93	7
	CRL C-2.7	S	5	95
	CRL C-2.8	S	5	95
Tetracycline, TET	CRL C-2.1	S	0	100
	CRL C-2.2	S	0	100
	CRL C-2.3	S	0	100
	CRL C-2.4	R	100	0
	CRL C-2.5	R	23	77
	CRL C-2.6	R	87	13
	CRL C-2.7	R	91	9
	CRL C-2.8	R	91	9

*This combination of test strain and antimicrobial was left out of the EQAS evaluation

Deviations - Salmonella

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
1	CRL S-2.1	Streptomycin, STR	S	32	R	64	MIC
2	CRL S-2.5	Amoxicillin cl., AUG	R	8	S	4/2	MIC
4	CRL S-2.1	Ciprofloxacin, CIP	S	0.38	R	1	ET
4	CRL S-2.2	Ciprofloxacin, CIP	S	0.19	R	0.25	ET
4	CRL S-2.3	Gentamicin, GEN	S	0.5	R	32	ET
4	CRL S-2.5	Streptomycin, STR	R	8	S	32	ET
4	CRL S-2.5	Ciprofloxacin, CIP	S	0.25	R	0.5	ET
4	CRL S-2.6	Tetracycline, TET	R	4	S	<2	ET
5	CRL S-2.1	Amoxicillin cl., AUG	S	17	R	16/8	DD
5	CRL S-2.1	Gentamicin, GEN	S	15	R	16	DD
5	CRL S-2.1	Ciprofloxacin, CIP	S	26	R	1	DD
5	CRL S-2.2	Cefotaxime, CTX	S	22	R	128	DD
5	CRL S-2.2	Amoxicillin cl., AUG	S	23	R	8/4	DD
5	CRL S-2.2	Ceftazidime, CAZ	S	27	R	1.0	DD
5	CRL S-2.2	Ciprofloxacin, CIP	S	27	R	0.25	DD
5	CRL S-2.3	Amoxicillin cl., AUG	S	24	R	8/4	DD
5	CRL S-2.5	Ciprofloxacin, CIP	S	28	R	0.5	DD
5	CRL S-2.8	Cefotaxime, CTX	S	17	R	16	DD
6	CRL S-2.7	Chloramphenicol, CHL	S	>64	R	>64	MIC
6	CRL S-2.7	Streptomycin, STR	S	128	R	64	MIC
9	CRL S-2.2	Amoxicillin cl., AUG	S	19	R	8/4	DD
9	CRL S-2.3	Amoxicillin cl., AUG	S	19	R	8/4	DD
11	CRL S-2.8	Ciprofloxacin, CIP	R	0.12	S	<0.03	MIC
13	CRL S-2.1	Tetracycline, TET	R	14	S	4	DD
13	CRL S-2.2	Ceftazidime, CAZ	S	26	R	1.0	DD
13	CRL S-2.2	Ciprofloxacin, CIP	S	26	R	0.25	DD
13	CRL S-2.3	Amoxicillin cl., AUG	S	21	R	8/4	DD
13	CRL S-2.5	Streptomycin, STR	R	12	S	32	DD
13	CRL S-2.5	Ciprofloxacin, CIP	S	26	R	0.5	DD
15	CRL S-2.1	TMP+SMX, SXT	R		S	0.125	DD
15	CRL S-2.1	Tetracycline, TET	R	16	S	4	DD
15	CRL S-2.2	Amoxicillin cl., AUG	S	25	R	8/4	DD
15	CRL S-2.3	Amoxicillin cl., AUG	S	25	R	8/4	DD
15	CRL S-2.7	Ciprofloxacin, CIP	R		S	<0.03	DD
16	CRL S-2.2	Ceftazidime, CAZ	S	1	R	1.0	MIC
16	CRL S-2.5	Amoxicillin cl., AUG	R	16	S	4/2	MIC
16	CRL S-2.8	Confirmed AmpC	No		Yes		MIC
18	CRL S-2.2	Cefotaxime, CTX	S	20	R	128	DD
18	CRL S-2.5	Ciprofloxacin, CIP	S	19	R	0.5	DD
18	CRL S-2.8	Cefotaxime, CTX	S	18	R	16	DD
19	CRL S-2.1	Ciprofloxacin, CIP	S	21*	R	1	DD
19	CRL S-2.2	Amoxicillin cl., AUG	S	22	R	8/4	DD
19	CRL S-2.2	Ciprofloxacin, CIP	S	26*	R	0.25	DD
19	CRL S-2.3	Amoxicillin cl., AUG	S	20	R	8/4	DD
19	CRL S-2.5	Streptomycin, STR	R	11	S	32	DD
19	CRL S-2.5	Ciprofloxacin, CIP	S	30*	R	0.5	DD
20	CRL S-2.1	Chloramphenicol, CHL	R	=32	S	8	MIC
21	CRL S-2.2	Ceftazidime, CAZ	S	0.5	R	1.0	MIC
21	CRL S-2.4	Ampicillin, AMP	R	>32	S	1	MIC
21	CRL S-2.4	Ceftazidime, CAZ	R	0.5	S	0.5	MIC
21	CRL S-2.4	Cefotaxime, CTX	R	4	S	0.064	MIC
21	CRL S-2.4	Ciprofloxacin, CIP	R	0.25	S	0.03	MIC
22	CRL S-2.3	Ceftiofur, XNL	R	>16	S	1	MIC

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
23	CRL S-2.2	Amoxicillin cl., AUG	S	20	R	8/4	DD
23	CRL S-2.2	Ceftazidime, CAZ	S	26	R	1.0	DD
23	CRL S-2.3	Gentamicin, GEN	S	15	R	32	DD
23	CRL S-2.3	Amoxicillin cl., AUG	S	22	R	8/4	DD
24	CRL S-2.1	Streptomycin, STR	S	32	R	64	MIC
24	CRL S-2.2	Ceftazidime, CAZ	S	1	R	1.0	MIC
25	CRL S-2.1	Chloramphenicol, CHL	R	32	S	8	MIC
25	CRL S-2.5	Streptomycin, STR	R	64	S	32	MIC
26	CRL S-2.4	Ciprofloxacin, CIP	S	0.03	R	0.5	MIC
27	CRL S-2.2	Ciprofloxacin, CIP	S	<=0.12	R	0.25	MIC
27	CRL S-2.8	Confirmed AmpC	No		Yes		MIC
28	CRL S-2.1	Ciprofloxacin, CIP	S	20	R	1	DD
28	CRL S-2.2	Amoxicillin cl., AUG	S	20	R	8/4	DD
28	CRL S-2.2	Ciprofloxacin, CIP	S	23	R	0.25	DD
28	CRL S-2.3	Amoxicillin cl., AUG	S	20	R	8/4	DD
28	CRL S-2.5	Streptomycin, STR	R	13	S	32	DD
28	CRL S-2.5	Ciprofloxacin, CIP	S	22	R	0.5	DD
29	CRL S-2.2	Cefotaxime, CTX	S	23	R	128	DD
29	CRL S-2.2	Ciprofloxacin, CIP	S	24	R	0.25	DD
29	CRL S-2.5	TMP+SMX, SXT	S	27	R	>32	DD
29	CRL S-2.5	Sulphonamides, SMX	S	32	R	>1024	DD
30	CRL S-2.2	Amoxicillin cl., AUG	S	21	R	8/4	DD
30	CRL S-2.3	Amoxicillin cl., AUG	S	19	R	8/4	DD
32	CRL S-2.1	Chloramphenicol, CHL	R	32	S	8	MIC
32	CRL S-2.1	Trimethoprim, TMP	R	4	S	<4	MIC
32	CRL S-2.2	Ceftazidime, CAZ	S	2	R	1.0	MIC
32	CRL S-2.3	Ciprofloxacin, CIP	R	0.25	S	0.03	MIC
32	CRL S-2.3	Nalidixic acid, NAL	R	32	S	4	MIC
32	CRL S-2.4	Trimethoprim, TMP	R	>32	S	<4	MIC
32	CRL S-2.4	Cefotaxime, CTX	R	2	S	0.064	MIC
32	CRL S-2.4	Chloramphenicol, CHL	R	256	S	4	MIC
32	CRL S-2.4	Ampicillin, AMP	R	32	S	1	MIC
32	CRL S-2.4	Ciprofloxacin, CIP	R	0.5	S	0.03	MIC
32	CRL S-2.4	Gentamicin, GEN	R	8	S	1	MIC
32	CRL S-2.5	Streptomycin, STR	R	>128	S	32	MIC
33	CRL S-2.1	Ceftiofur, XNL	R	4	S	2	MIC
35	CRL S-2.2	Confirmed ESBL	No		Yes		MIC
35	CRL S-2.2	Amoxicillin cl., AUG	S	20	R	8/4	MIC
35	CRL S-2.2	Ceftazidime, CAZ	S	27	R	1.0	MIC
35	CRL S-2.3	Amoxicillin cl., AUG	S	19	R	8/4	MIC
35	CRL S-2.7	Streptomycin, STR	S	32	R	64	MIC
35	CRL S-2.8	Confirmed AmpC	No		Yes		MIC

DD Disk diffusion
ET E-test
MIC Microbroth dilution
*Remark made in the database: Disc diffusion results interpreted by CLSI clinical breakpoints

Deviations - Campylobacter

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
2	CRL C-2.5	Tetracycline, TET	S	1	R	4	MIC
4	CRL C-2.5	Tetracycline, TET	S	1	R	4	ET
5	CRL C-2.1	Erythromycin, ERY	R	25	S	<=0.5	DD
5	CRL C-2.2	Erythromycin, ERY	R	26	S	<=0.5	DD
5	CRL C-2.3	Erythromycin, ERY	R	21	S	<=0.5	DD
5	CRL C-2.4	Erythromycin, ERY	R	22	S	2	DD
5	CRL C-2.5	Streptomycin, STR	R	6	S	2	DD
5	CRL C-2.5	Ciprofloxacin, CIP	R	8	S	0.125	DD
5	CRL C-2.5	Nalidixic acid, NAL	R	8	S	8	DD
5	CRL C-2.5	Tetracycline, TET	S	26	R	4	DD
5	CRL C-2.6	Chloramphenicol, CHL	R	6	S	4	DD
5	CRL C-2.7	Gentamicin, GEN	R	10	S	<=0.125	DD
5	CRL C-2.7	Chloramphenicol, CHL	R	14	S	2	DD
5	CRL C-2.7	Erythromycin, ERY	R	6	S	<=0.5	DD
5	CRL C-2.7	Streptomycin, STR	R	8	S	2	DD
5	CRL C-2.8	Ciprofloxacin, CIP	S	26	R	>4	DD
5	CRL C-2.8	Tetracycline, TET	S	26	R	>16	DD
6	CRL C-2.3	Streptomycin, STR	R	16	S	<=2	MIC
11	CRL C-2.5	Tetracycline, TET	S	1	R	4	MIC
11	CRL C-2.6	Ciprofloxacin, CIP	S	0.25	R	>4	MIC
11	CRL C-2.6	Gentamicin, GEN	S	0.5	R	>16	MIC
11	CRL C-2.6	Tetracycline, TET	S	1	R	>16	MIC
11	CRL C-2.6	Nalidixic acid, NAL	S	4	R	>64	MIC
12	CRL C-2.5	Tetracycline, TET	S	1	R	4	MIC
14	CRL C-2.5	Streptomycin, STR	R	8	S	2	MIC
14	CRL C-2.5	Tetracycline, TET	S	2	R	4	MIC
15	CRL C-2.5	Tetracycline, TET	S	0.75	R	4	ET
15	CRL C-2.7	Ciprofloxacin, CIP	S	0.023	R	>4	ET
15	CRL C-2.7	Tetracycline, TET	S	0.5	R	>16	ET
15	CRL C-2.7	Nalidixic acid, NAL	S	3	R	>64	ET
17	CRL C-2.1	Ciprofloxacin, CIP	R	2	S	0.125	MIC
17	CRL C-2.1	Gentamicin, GEN	R	2	S	0.25	MIC
17	CRL C-2.3	Gentamicin, GEN	R	2	S	0.25	MIC
17	CRL C-2.3	Streptomycin, STR	R	8	S	<=2	MIC
17	CRL C-2.4	Ciprofloxacin, CIP	R	2	S	0.25	MIC
17	CRL C-2.4	Gentamicin, GEN	R	4	S	0.5	MIC
17	CRL C-2.5	Ciprofloxacin, CIP	R	2	S	0.125	MIC
17	CRL C-2.5	Streptomycin, STR	R	8	S	2	MIC
17	CRL C-2.8	Gentamicin, GEN	R	1	S	0.25	MIC
17	CRL C-2.8	Streptomycin, STR	R	4	S	<=2	MIC
20	CRL C-2.5	Tetracycline, TET	S	=2	R	4	MIC
21	CRL C-2.5	Tetracycline, TET	S	2	R	4	MIC
22	CRL C-2.5	Tetracycline, TET	S	<=0.12	R	4	MIC
22	CRL C-2.6	Gentamicin, GEN	S	<=0.12	R	>16	MIC
22	CRL C-2.6	Ciprofloxacin, CIP	S	0.12	R	>4	MIC
22	CRL C-2.6	Tetracycline, TET	S	0.5	R	>16	MIC
22	CRL C-2.6	Streptomycin, STR	S	2	R	>16	MIC
22	CRL C-2.7	Ciprofloxacin, CIP	S	<=0.06	R	>4	MIC
22	CRL C-2.7	Tetracycline, TET	S	<=0.12	R	>16	MIC
22	CRL C-2.7	Nalidixic acid, NAL	S	2	R	>64	MIC
23	CRL C-2.4	Nalidixic acid, NAL	R	10	S	16	DD
23	CRL C-2.4	Ciprofloxacin, CIP	R	15	S	0.25	DD
24	CRL C-2.5	Tetracycline, TET	S	2	R	4	MIC
26	CRL C-2.5	Tetracycline, TET	S	0.5	R	4	MIC
28	CRL C-2.5	Tetracycline, TET	S	<=1	R	4	AGA
30	CRL C-2.5	Tetracycline, TET	S	2	R	4	MIC
30	CRL C-2.7	Erythromycin, ERY	R	0.5	S	<=0.5	MIC
30	CRL C-2.7	Gentamicin, GEN	R	0.5	S	<=0.125	MIC
32	CRL C-2.5	Tetracycline, TET	S	2	R	4	MIC
32	CRL C-2.6	Ciprofloxacin, CIP	S	1	R	>4	MIC
32	CRL C-2.8	Tetracycline, TET	S	<=0.5	R	>16	MIC
32	CRL C-2.8	Ciprofloxacin, CIP	S	0.5	R	>4	MIC
32	CRL C-2.8	Erythromycin, ERY	S	0.5	R	>32	MIC
33	CRL C-2.5	Tetracycline, TET	S	1	R	4	MIC
36	CRL C-2.5	Tetracycline, TET	S	0.5	R	4	MIC

AGA	Agar dilution
DD	Disk diffusion
ET	E-test
MIC	Microbroth dilution

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