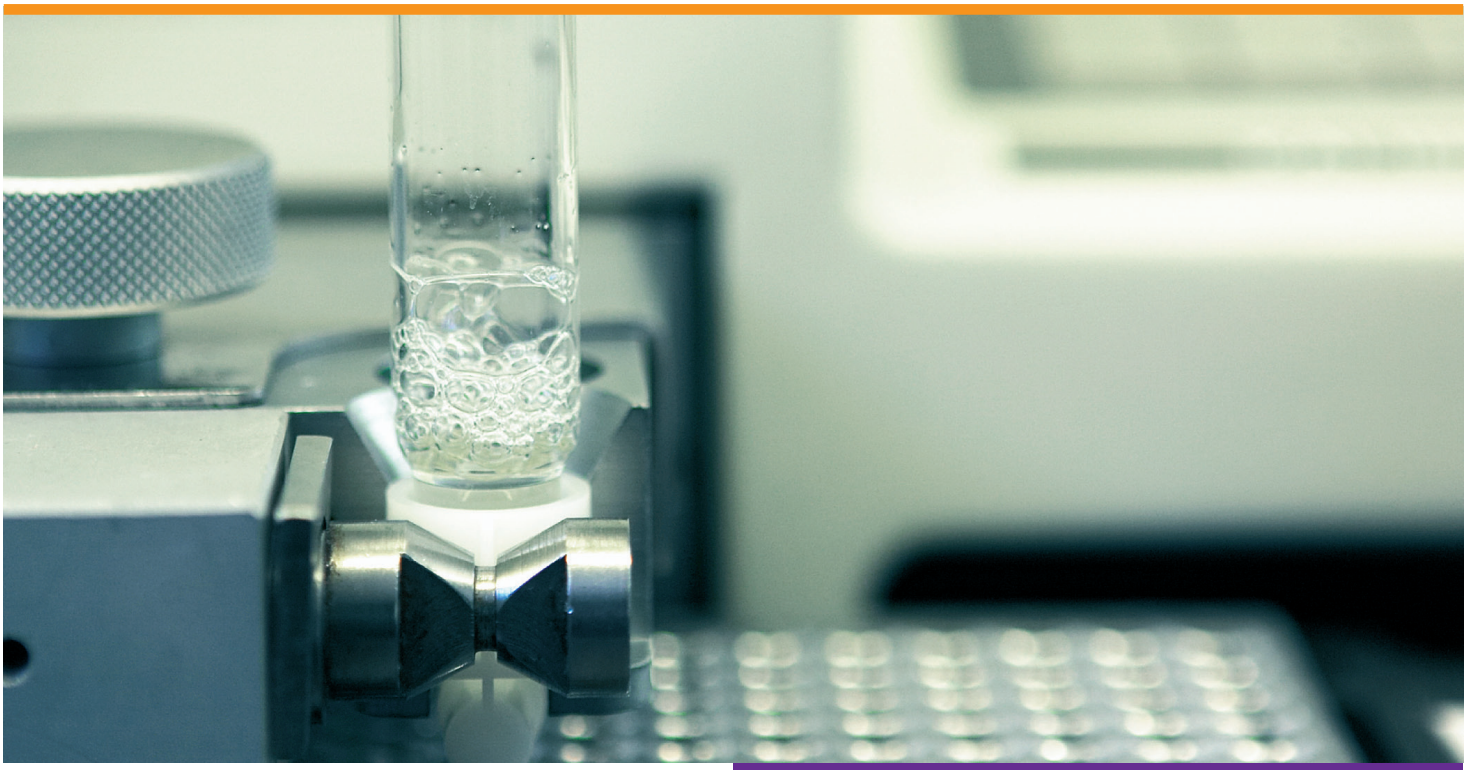
The 5th CRL Profiency Testing *Salmonella* and *Campylobacter* 2008



DTU Food
National Food Institute

Community Reference Laboratory Antimicrobial Resistance

Community Reference Laboratory – Antimicrobial Resistance

THE 5TH CRL PROFICIENCY TESTING
SALMONELLA AND *CAMPYLOBACTER* - 2008

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1. Introduction

In this report, results are summarised from the fifth 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 third 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 identify areas or laboratories, for which guidance or assistance would be required as means of producing reliable susceptibility data. The goal is having all laboratories performing antimicrobial susceptibility testing (AST) with less than 7% incorrect interpretations.

The technical advisory group for the CRL EQAS scheme consists of competent representatives from all National Reference Laboratories (NRLs), 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 8th of August 2008 by e-mail to the 36 NRLs in the CRL-network (including Norway and Switzerland). The pre-notification was sent to NRLs in all EU countries except Luxemburg, where no contact was established. All 36 laboratories responded. One laboratory declined to participate as there had been a delay in their process of initiating the use of a new method (microbroth). This laboratory had subsequently also taken part in hands-on as well as theoretical training at the CRL regarding the microbroth method. A second laboratory declined to participate as they had neither *Salmonella* nor *Campylobacter* as their field of responsibility.

Appendix 2 shows that 31 of the 34 participating NRLs were appointed by the individual member states. Three NRLs had not been appointed, but had – along with Norway and Switzerland – been enrolled on equal terms as the designated NRLs, 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 laboratories in Norway and Switzerland were charged a fee for their participation in the EQAS, whereas the NRLs from EU member states participated free of charge.

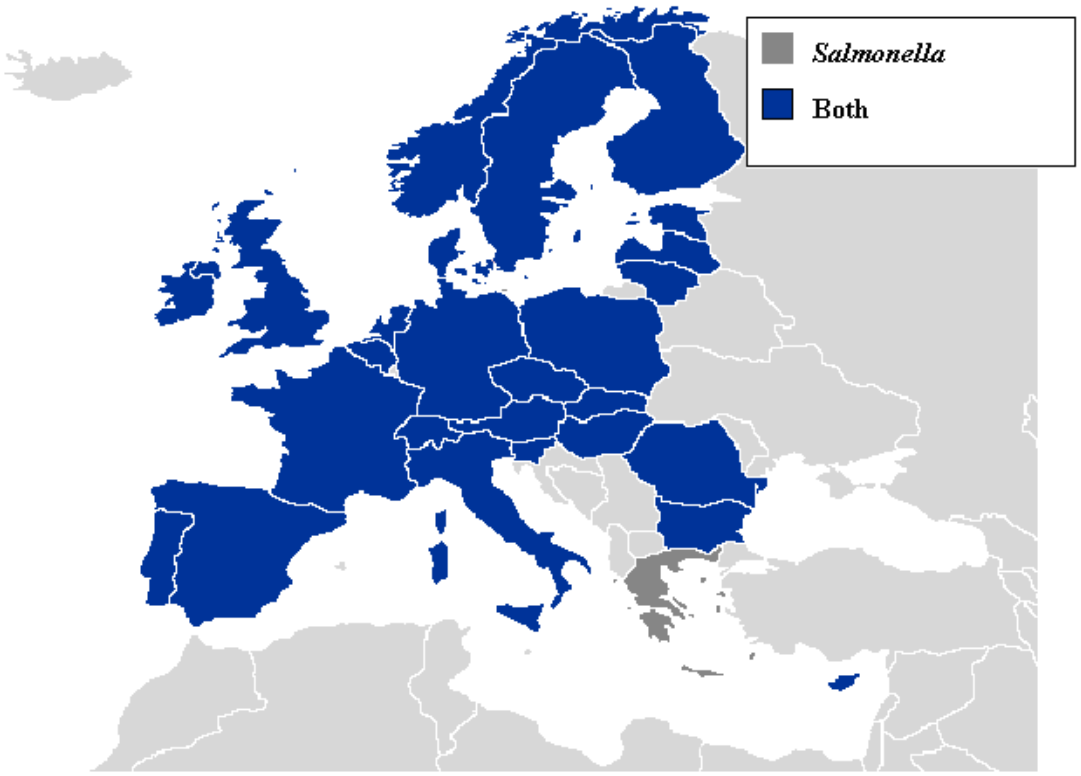

Figure 1: Participating countries that perform antimicrobial susceptibility testing of *Salmonella* or both *Salmonella* and *Campylobacter*

Figure 1 shows that out of 28 participating countries, one uploaded only the *Salmonella* results, whereas 27 tested both *Salmonella* and *Campylobacter*. The results from the designated NRLs are being presented and evaluated in this report; results from 25 countries consisting of 28 sets of *Salmonella* results and 26 sets of *Campylobacter* results.

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 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 Czech Collection of Micro-organisms (CCM); The Czech Republic. This was relevant only for the NRLs which had not been provided with these reference strains in previous 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 served 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*, furthermore, streptomycin and chloramphenicol for *Campylobacter*.

2.3 Antimicrobials

The antimicrobials used in the EQAS are listed in the protocol (App. 4b) and were 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: ampicillin, cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, ceftiofur, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfonamides (sulphamethoxazole), tetracycline and trimethoprim. Additionally, cefoxitin was used for detection of AmpC, and imipenem; imipenem/EDTA for detection of metallo-beta-lactamases.

Minimum Inhibitory Concentration (MIC) determination of the *Salmonella* test strains was performed using the Sensititre system from Trek diagnostics Ltd with the exception of cefotaxime + clavulanic acid, cefoxitin, ceftazidime + clavulanic acid, imipenem and imipenem

+ EDTA. 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-S18 (2008) "Performance Standards for Antimicrobial Susceptibility Testing" (Eighteenth Informational Supplement) and document M31-A3 (2008) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated From Animals" (Approved Standard – Third 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 on October 22nd 2008 to the selected laboratories as dangerous goods UN3373 according to the International Air Transport Association (IATA) regulations. Immediately prior to dispatch, each laboratory was informed about the shipment.

2.5 Procedure

On the website, http://www.crl-ar.eu/, the laboratories were provided with protocols and information regarding the handling of the test strains and reference strains (App. 4b, c, d, e). 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 NRLs should work towards harmonising to MIC methods for these AST analyses. Additionally, it was agreed upon all NRLs working 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 carried out when performing monitoring for EFSA.

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 *Salmonella* with the exception of 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. A categorisation as 'intermediate' was not accepted. 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 does 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. Alternatively, the record sheets from the protocol could be sent by fax to DTU Food. The website was open for data entry in the period from the 29th of October 2008 to the 28th of January 2009.

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 would consist of MIC values for the reference strains *E. coli* (ATCC 25922) and *C. jejuni* (ATCC 33560), or for *E. coli* (ATCC 25922), the zone diameter in millimetres. The results should be in

agreement with the quality control ranges according to the relevant guideline of the following: the CLSI documents M31-A3 (2008) / M100-S18 (2008) / 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 reports assessed the submitted results, reporting all deviations from the expected. Deviations were categorised as 'incorrect'.

In the database, questions for use when evaluating the EQAS were also included as well as questions regarding the routine work with AST in the participating laboratory. These were 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.

At the CRL-AR Workshop (2008), the network agreed that if only 75% of the results were correct, based on strain/antimicrobial combination, these results should be further analysed and possibly omitted from evaluation. For this EQAS this was the case for two of the *Salmonella* test strains and one *Campylobacter* test strain (App. 10a and 10b). The combinations in question are S3.1/ciprofloxacin (75% correct) and S3.3/ceftazidime (60% correct). These results are not omitted on these grounds which will be addressed in the discussion. The third combination with a performance level below 75% was C3.6/erythromycin (72% correct). This low performance level could not easily be explained, and the results have been omitted from evaluation in this report. In the appendices 10b and 11b the omitted results are presented.

3.1 Methods used by EQAS-participants

In the *Salmonella* trials, 21 laboratories used MIC determination, one used E-test, and six laboratories used disk diffusion, however, some laboratories supplement one method with the other. The majority of laboratories (n=22) used MIC determination (microbroth or agar dilution) for the *Campylobacter* trial. One NRL reported the use of E-test (#4), whereas three laboratories (#23, #38 and #40) used disk diffusion.

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

3.2 Deviations by strain and antimicrobial

The list of deviations is shown in Appendix 11a and 11b. Figure 2 shows the total percentage of deviations from the expected results of AST performed by participating laboratories. For the *Salmonella* strains, 98.0% of the AST's were interpreted correctly. For the *Campylobacter* strains, 98.7% of AST's were correct. Compared to the CRL EQAS 2006 and 2007 this is a considerable improvement for both the *Salmonella* AST and in particular the *Campylobacter* AST.

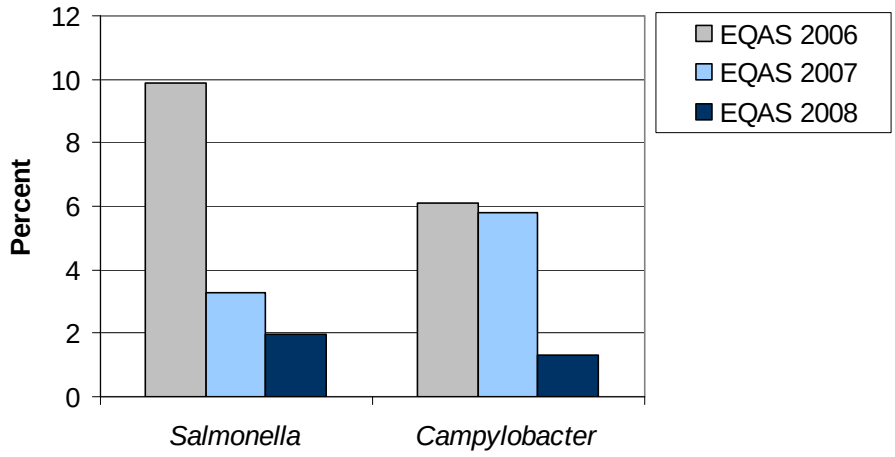

Figure 2: A comparison between EQAS 2006, EQAS 2007 and EQAS 2008 showing the percent of deviations in total for antimicrobial susceptibility testing performed by participating laboratories

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 considerably higher when performed by diffusion methods compared to MIC-methods.

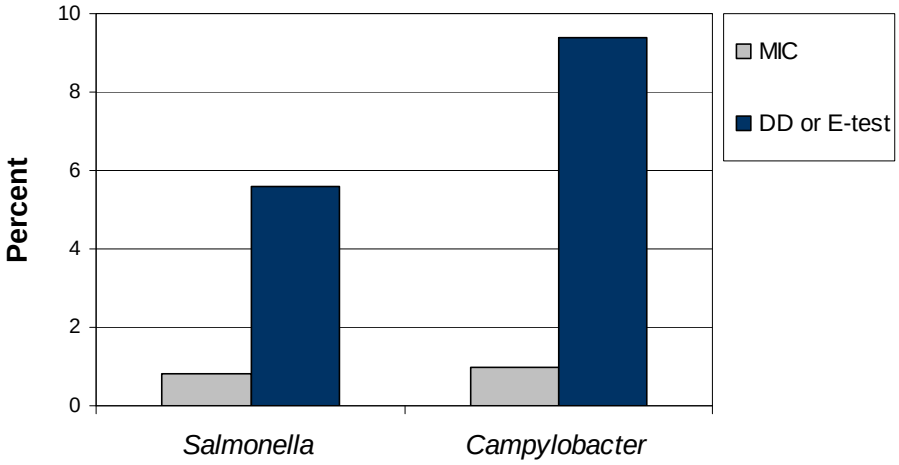

Figure 3: The percent of deviations in total for EQAS 2008 for AST's is shown comparing the results when using MIC-methods as opposed to disk diffusion or E-test.

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. Variations were observed between strains of the same species, from 94.7-100% for *Salmonella* and from 96.4-99.4% for *Campylobacter*.

EQAS 2008 - *Salmonella*			EQAS 2008 – *Campylobacter*		
Test strain	AST in total	% correct	Test strain	AST in total	% correct
S-3.1	305	97.7	C-3.1 (*C. coli*)	163	97.5
S-3.2	302	99.0	C-3.2 (*C. jejuni*)	164	99.4
S-3.3	301	94.7	C-3.3 (*C. coli*)	164	97.6
S-3.4	279	97.8	C-3.4 (*C. coli*)	171	98.2
S-3.5	303	100	C-3.5 (*C. jejuni*)	164	97.6
S-3.6	299	96.7	C-3.6 (*C. jejuni*)	139*	96.4*
S-3.7	302	98.7	C-3.7 (*C. coli*)	171	97.7
S-3.8	301	99.7	C-3.8 (*C. jejuni*)	164	98.2

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

*Results from AST's performed with erythromycin excluded.

For *Salmonella*, the strain with the highest deviation percent was S3.3 (94.7% correct). This strain was also included in EQAS 2006 and EQAS 2007 as internal reference strain, with 85.3% and 92.3% correct results, respectively. This strain is resistant to ampicillin, cefotaxime, ceftiofur, ciprofloxacin, nalidixic acid and tetracycline. Additionally, for ceftazidime the MIC value is <0.5, but as the strain is ESBL-producing, it should be regarded resistant towards this drug as well (interpretation of cephalosporins described in the protocol).

In Table 1, the *Campylobacter* test strain C3.6 is listed with a percentage of correct results of 96.4%, however, the original percentage was 92.7%. This strain is resistant to ciprofloxacin, erythromycin, nalidixic acid and tetracycline, and it was the expected result for erythromycin that caused problems (72% correct results for this strain/antimicrobial combination). Six laboratories out of 25 obtained a MIC-value of 1µg/mL or below, and therefore categorised the strain sensitive towards this antimicrobial. However, the expected result was an MIC-value >32µg/mL. It is not clear what the reason for this deviation is and therefore it was decided to omit the data from this strain/antimicrobial combination in this evaluation.

In Table 2, the percentage of correct AST per antimicrobial by species is shown. When testing *Salmonella* it appeared that one antimicrobial had a considerably lower percentage than the

EQAS 2008 Antimicrobial	% correct	
	Salmonella	*Campylobacter*
Ampicillin, AMP	99.5	-
Cefotaxime, CTX	99.5	-
Ceftazidime, CAZ	94.7	-
Ceftiofur, XNL	100.0	-
Chloramphenicol, CHL	99.5	100.0
Ciprofloxacin, CIP	**90.5**	97.5
Erythromycin, ERY	-	97.7*
Gentamicin, GEN	98.6	99.0
Nalidixic acid, NAL	99.5	96.4
Streptomycin, STR	96.8	98.4
Sulphonamides, SMX	99.5	-
Tetracycline, TET	99.1	96.4
Trimethoprim, TMP	100.0	-

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

*Results from AST's performed with erythromycin excluded.

others. For ciprofloxacin the levels of correct results based on the susceptibility categorisation were low (90.5%). In EQAS 2006 and EQAS 2007 this was also the case, with 79.8% and 90.0% correct results, respectively. Thus, in this case, an improvement in performance from last year could not be detected.

For *Campylobacter* it does not seem that any of the antimicrobials stand out with a difference in deviation percent compared to the other antimicrobials on the list. In last year's EQAS's, tetracycline seemed to pose a problem (87.2% correct). However, this year the performance regarding tetracycline was satisfactory (96.4% correct).

It was decided on the CRL Workshop 2008 that the testing of ESBL-production in *Salmonella* should be mandatory, and the laboratories were asked to detect the ESBL producing *Salmonella* strains (S3.1, S3.3 and S3.5) according to the description in the protocol. In this protocol it is described that ESBL producing strains that are resistant to one cephalosporin should be interpreted resistant to all cephalosporins regardless of the value detected from the results. Out of the 28 laboratories which tested *Salmonella*, four did not upload results on confirmatory ESBL-testing, and therefore results from 24 laboratories are evaluated below.

All ESBL-producing strains were so-called 'true ESBLs' with a CTX M-15- and SHV 12-gene (S3.1), CTX M-9-gene (S3.3) and CTX M-15-like-gene (S3.5) (Table 3). It appears that the laboratories quite confidently detected and confirmed two of the ESBL-producers (S3.1 and S3.5; 96%) but two laboratories did not detect the test strain S3.3 as ESBL-producing.

	Strain S3.1 (CTX M-15 / SHV 12)	**Strain S3.3 (CTX M-9)**	**Strain S3.5 (CTX M-15 like)**
CTX, CAZ, XNL	6/6 (100%)	4/5 (80%)	5/6 (83%)
CTX, CAZ	11/12 (92%)	12/13 (92%)	13/13 (100%)
CTX, XNL	2/2 (100%)	2/2 (100%)	2/2 (100%)
CTX	2/2 (100%)	2/2 (100%)	2/2 (100%)
CTX/CI:CTX	22/23 (96%)	20/22 (91%)	21/21 (100%)
CAZ/CI:CAZ	22/23 (96%)	10/18 (56%)	22/23 (96%)
Confirmed ESBL	23/24 (96%)	22/24 (92%)	23/24 (96%)
FOX^{S}	24/24 (100%)	24/24 (100%)	24/24 (100%)
AmpC not confirmed	23/24 (96%)	24/24 (100%)	23/24 (96%)

Table 3: Proportion of laboratories that obtained the expected result. Number and percentages of laboratories which correctly detected and confirmed the three ESBL producing *Salmonella* strains.

There is a difference in the number of cephalosporins used by the laboratories in their routine test for ESBL-production; five compounds 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 combination disk method).

For two laboratories, the use of cefotaxime in combination with ceftazidime did not result in detection of the ESBL's: In both cases confirmatory tests were performed by evaluating the increase in zone diameter, but this was found not to confirm ESBL-production (increase < 5mm). In additional two cases, the use of all three antimicrobials, cefotaxime, ceftazidime and ceftiofur, did not produce confirmation of ESBL production: In one of these cases all antimicrobials were found sensitive and no confirmatory test was performed, and in the other case, a negative result was obtained when comparing zone diameters (CAZ/CAZ:Cl).

The results for S3.3 for CAZ/CAZ:Cl appeared to be in disagreement, some found this test to be confirming ESBL-production (56%) and some did not (44%). Furthermore, this test strain did not show resistance towards ceftazidime (MIC <0.5), but it should be regarded resistant to this cephalosporin also. This is the reason for the low percentage of correct results (94.7%) presented in Table 2.

In Table 4, the results obtained when comparing the different methods for ESBL confirmatory testing are shown. Eleven laboratories uploaded increase of zone diameter as the result, and 13 uploaded an MIC-ratio (data shown refer to all three ESBL-producing strains). For the laboratories that obtained an MIC-result, all conclusions were correct, whereas the labs that performed disk diffusion failed to confirm ESBL-production in four cases.

		Increase in zone diameter	MIC-ratio
Expected result / no. of results in total	CAZ/Cl:CAZ	27/37 (73%)	23/25 (92%)
	CTX/Cl:CTX	29/32 (91%)	35/36 (97%)
Confirmed ESBL / no. of laboratories		29/33 (91%)	39/39 (100%)

Table 4: Comparison of obtained results when performing confirmatory tests by either of the two methods: measurement of zonediameters (disk diffusion) or by obtaining a MIC-ratio (E-test). Results compiled for all three ESBL-producing strains.

In addition to the confirmation of ESBL-production, one laboratory confirmed two test strains to be of the AmpC-type (S3.1 and S3.5), however, no resistance towards cefoxitin was

reported. According to the expected, no laboratories reported resistance towards cephalosporins for any of the non-ESBL's.

3.3 Deviations by laboratory

Figure 4 and 6 illustrate the percentage of deviations 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 including only tests with antimicrobials recommended by EFSA are additionally indicated; these results will be the focus of the evaluation in the following. In Figure 5 and 7 the total amount of deviations in percentages is illustrated by number of laboratories.

3.3.1 *Salmonella* trial

Seventeen of the laboratories obtained a result of 100% correctly tested *Salmonella* strains. The maximum percentage of deviations was 10.9%.

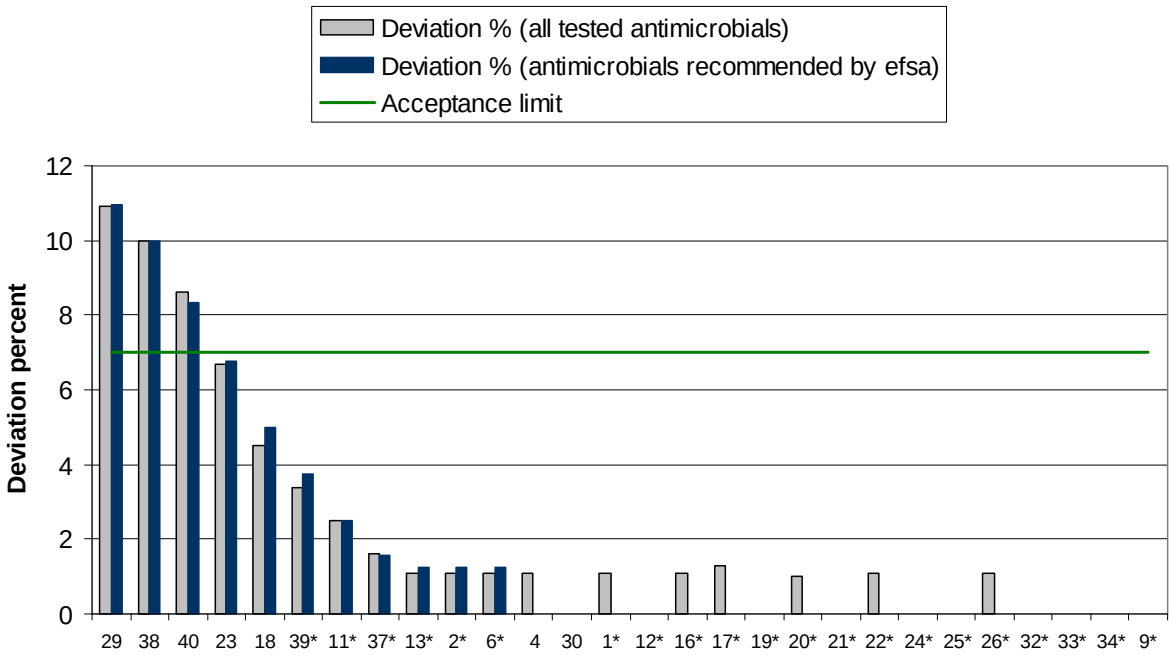

Figure 4: Individual participants' deviations in percent of their total number of *Salmonella* AST's. An asterisk indicates that the laboratory has performed AST using microbroth dilution or agar dilution

The vast majority of the laboratories have a deviation percentage below 7, and none of the laboratories can be categorized as outliers. All in all, 25 of the 28 participating laboratories lived up to the level of performance expected by the CRL. A significant difference ($p<0.01$) was observed when comparing results obtained by the use of disk diffusion and a MIC method.

Figure 5 also illustrates that the majority of laboratories had less than 7% deviation, whereas three laboratories (#29, #38, #40) obtained levels of deviations above the acceptance limit.

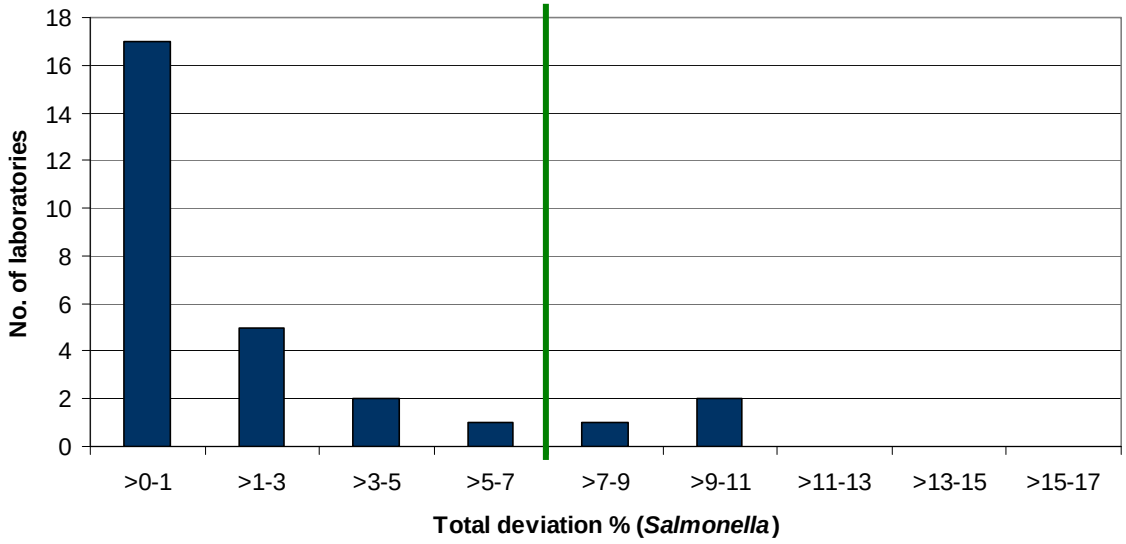

Figure 5: The number of laboratories listed in intervals of percent of total deviations. The green line marks the acceptance limit set by the CRL

3.3.2 *Campylobacter* trial

In the *Campylobacter* trial most laboratories performed very well. Applying the earlier mentioned acceptance threshold, 25 of 26 participating laboratories performed acceptably, with twelve laboratories having no deviations at all. One laboratory (#40) had a very high level of deviation (28.2%) and is considered as an outlier (Figure 6 and 7).

Laboratory #40 used disk diffusion which is not recommended for AST of *Campylobacter*.

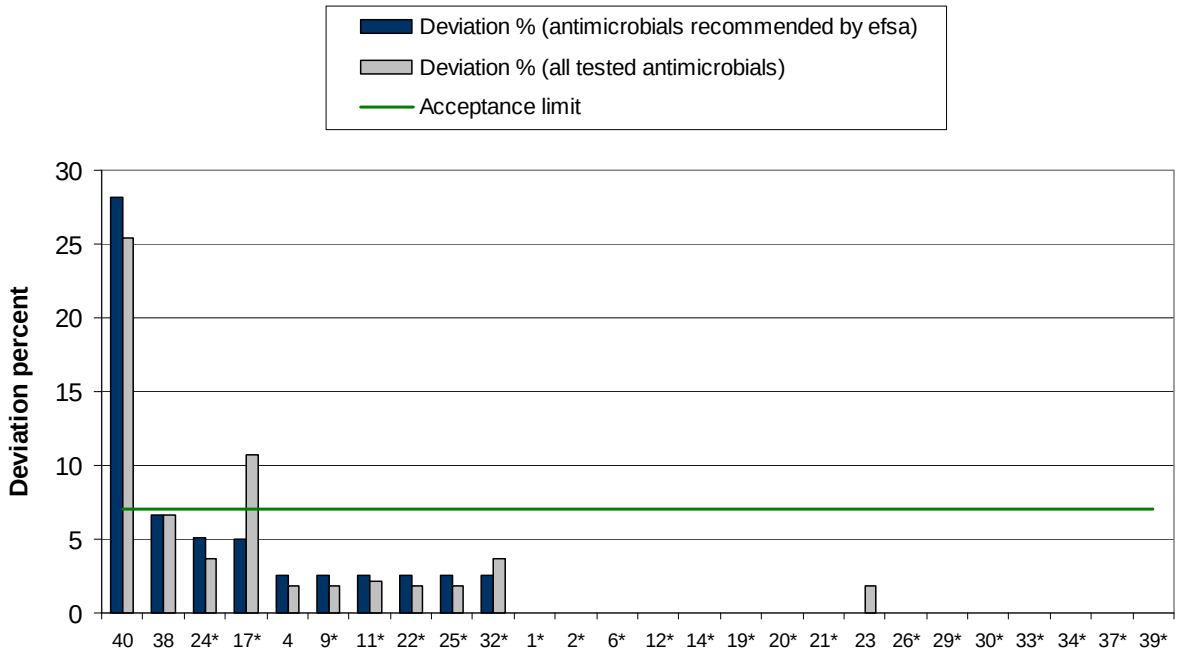

Figure 6: Individual participants' deviations in percent of their total number of *Campylobacter* AST's. An asterisk indicates that the laboratory has performed AST using microbroth dilution or agar dilution. Results from AST's from the strain/antimicrobial combination C3.6/erythromycin excluded.

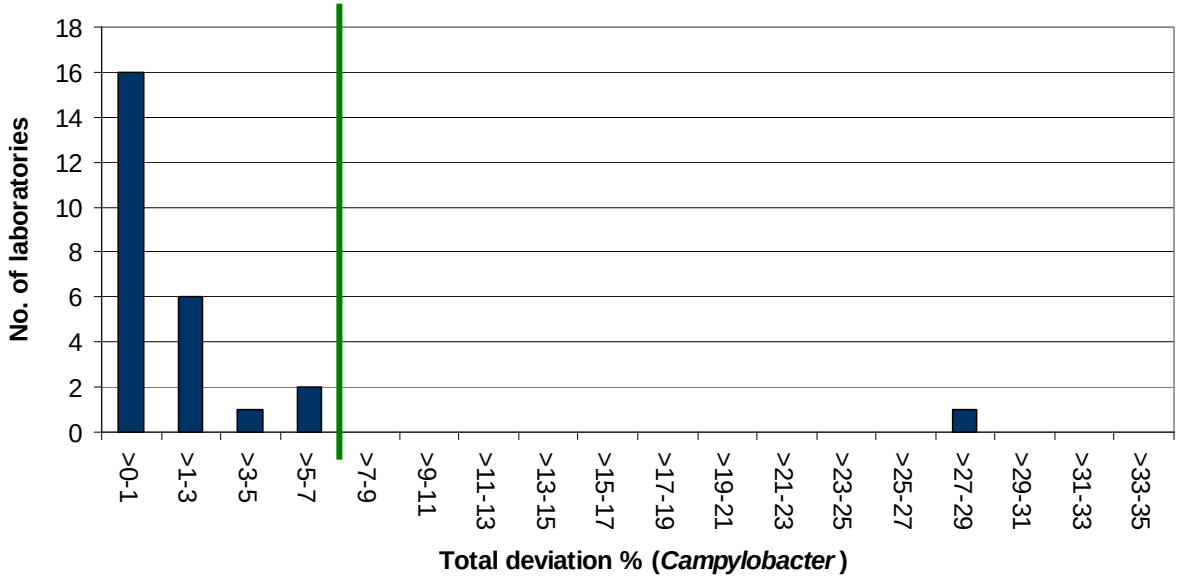

Figure 7: The number of laboratories listed in intervals of percent of total deviations. Results from AST's from the strain/antimicrobial combination C3.6/erythromycin excluded.

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. For the *Salmonella* trial, all laboratories except one performed QC testing of the reference strain. For the *Campylobacter* trial, all laboratories performing AST by MIC-method, also performed QC-testing on the reference strain.

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. Six laboratories used the disk diffusion method, and out of 70 disk diffusion QC tests, one was out of range (sulfisoxasole, 1mm below the lower limit).

EQAS 2008	**Disk diffusion *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in mm zones (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/6 (0%)	-	-
Cefotaxime, CTX	0/4 (0%)	-	-
Cefoxitin, FOX	0/5 (0%)	-	-
Ceftazidime, CAZ	0/4 (0%)	-	-
Ceftiofur, XNL	0/3 (0%)	-	-
Chloramphenicol, CHL	0/6 (0%)	-	-
Ciprofloxacin, CIP	0/6 (0%)	-	-
Gentamicin, GEN	0/6 (0%)	-	-
Imipenem, IMI	0/4 (0%)	-	-
Nalidixic acid, NAL	0/6 (0%)	-	-
Streptomycin, STR	0/6 (0%)	-	-
Sulphonamides, SMX	1/3 (33%)	1	-
Tetracycline, TET	0/6 (0%)	-	-
Trimethoprim, TMP	0/5 (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 the outcome presented in Table 6. Twenty-one laboratories submitted MIC data (including one laboratory which performed E-test). No mistakes were seen for 12 antimicrobials, but for ciprofloxacin deviation level of 14% was detected. This was caused by three laboratories with an MIC-value one step higher than the QC interval.

Quality control was also performed using MIC determination against the *C. jejuni* reference strain ATCC 33560, with participation of 23 laboratories (including one laboratory which used E-test). One laboratory which used a different incubation than recommended by CLSI (#14) was excluded in this summary (App. 6b).

EQAS 2008	**MIC determination *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/20 (0%)	-	-
Cefotaxime, CTX	0/21 (0%)	-	-
Cefoxitin, FOX	0/1 (0%)	-	-
Ceftazidime, CAZ	0/16 (0%)	-	-
Ceftiofur, XNL	0/3 (0%)	-	-
Chloramphenicol, CHL	0/20 (0%)	-	-
Ciprofloxacin, CIP	3/21 (14%)	-	1 step
Gentamicin, GEN	0/21 (0%)	-	-
Nalidixic acid, NAL	0/21 (0%)	-	-
Streptomycin, STR	0/21 (0%)	-	-
Sulphonamides, SMX	0/14 (0%)	-	-
Tetracycline, TET	0/21 (0%)	-	-
Trimethoprim, TMP	0/21 (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. For all antimicrobials, deviations were seen, however the highest values of deviation were detected for erythromycin, nalidixic acid and tetracycline (18%, 15% and 15%). Erythromycin deviations were also observed at about the same level in last year's EQAS,

EQAS 2008	**MIC determination *C. jejuni* ATCC 33560**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Chloramphenicol, CHL	1/16 (6%)	1 step	-
Ciprofloxacin, CIP	1/22 (4%)	-	1 step
Erythromycin, ERY	4/22 (18%)	2 steps	1 step
Gentamicin, GEN	1/20 (5%)	1 step	-
Nalidixic acid, NAL	3/20 (15%)	3 steps	-
Tetracycline, TET	3/20 (15%)	1 step	2 steps

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

whereas the performance regarding nalidixic acid and tetracycline appeared to have decreased. In comparison to EQAS 2006 and 2007, ciprofloxacin had a low deviation percentage (4%) which was 29% and 24% in 2006 and 2007, respectively. The 13 MIC-values outside the QC-ranges are caused by five different laboratories, of which two laboratories have four deviations each.

4. Discussion

4.1 *Salmonella* trial

Overall, the percentage of correct susceptibility test results of *Salmonella* was 98.0%. The majority of participants (25) 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 a MIC method.

Compared to the performance in EQAS 2006 and EQAS 2007 with 90.1% and 96.7% correct results, respectively, it would therefore appear that the quality of the results has improved.

Three laboratories had a deviation level higher than 7% (#29, #38 and #40), with values of 10.9%, 10.0% and 8.6%, respectively. All laboratories performed AST by disk diffusion, and for laboratories #29 and #38 all QC-results were within range. For laboratory #40, one antimicrobial was just below the QC-limit (sulfisoxazole). When performing disk diffusion for AST, it should be noted that a higher cut off value for ciprofloxacin is used in comparison to MIC methods. This is the antimicrobial that caused more than 50% of the mistakes for these three laboratories. However, in the protocol this problem is addressed; *Salmonella* strains resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin. When disregarding the deviations caused by ciprofloxacin, the deviation level for all three laboratories is below the 7% acceptance limit.

In general, ciprofloxacin caused unsatisfactory results when testing *Salmonella*; the over-all level of correct tests for all test strains was 90.5%. This was largely caused by the already mentioned issue regarding the low MIC cut off value. When extracting the 14 deviations caused by this from the strains S3.3, S3.4 and S3.6, the level of correct results was 96.8%. These specific test strains have a low MIC value for ciprofloxacin, which should however, be categorized as resistant. One of the six laboratories performing disk diffusion on the *Salmonella*

test strains obtained correct ciprofloxacin results for these strains just by following the guidelines described in the protocol.

The isolate S3.1 was a *Salmonella* strain which contained a *qnrB*-gene. The *qnr*-gene confers low-level resistance to ciprofloxacin, but not to nalidixic acid, which would in general be expected. The participants generally found this isolate sensitive to nalidixic acid (97%), whereas only 75% found the isolate resistant to ciprofloxacin. The low-level ciprofloxacin resistance caused by a *qnr*-gene is difficult to detect when performing disk diffusion (MIC-value: 0.25µg/mL) as the usual connection between ciprofloxacin and nalidixic acid is not seen. All five laboratories performing disk diffusion obtained a diffusion zone indicating that the test strain is sensitive.

The *Salmonella* test strain with the highest deviation percentage (S3.3; 94.7% correct) was the ESBL-producing isolate which had an MIC value for ceftazidime below the cut off value. The strain was resistant towards cefotaxime, which according to the guidelines would render an interpretation as resistant to all cephalosporins. Nine laboratories reported ceftazidime sensitive, and eight of these reported cefotaxime resistant. When speculating that these eight had also categorised ceftazidime as resistant and re-calculating the deviation level, it turned out to be 97.3% correct for test strain S3.3. Moreover, the performance level for ceftazidime would then increase from 94.7% to 99.4%. Disregarding these misinterpretations, the strain/antimicrobial-combination with a level of correct result on 60% would have been 95.5%.

For the *E. coli* reference strain, the results to a very high extent lived up to the CLSI recommendations. The number of laboratories performing AST on *Salmonella* by the use of disk diffusion has decreased to six. All of these laboratories uploaded data for the testing of the reference strain, and a total of 98.6% were within range. For the laboratories performing AST on *Salmonella* by an MIC-method, all but one uploaded QC-results to the database. The proportion of values within the expected range was 99.5%.

A follow-up on the highest level of deviations in EQAS 2007 showed considerable improvement, as laboratory #32 had 13.6% deviations in 2007, whereas at this year's EQAS they had no deviations at all. The other laboratory which had a deviation level above the acceptance limit in EQAS 2007, laboratory #5, is no longer part of the CRL network.

ESBL-producing *Salmonella* test strains

ESBL-producing microorganisms are an emerging problem worldwide, and it should be of a high priority for the NRLs to be able to detect these strains. It was therefore decided at the CRL Workshop in June 2008, that the detection of ESBL producing test strains should be included as a mandatory test in this EQAS.

Three of the *Salmonella* test strains were ESBL producing (S3.1, S3.3 and S3.5), and the participants were asked to interpret their results according to the description in the protocol that an ESBL-producing strain resistant to one cephalosporin should be interpreted as resistant to all cephalosporins. Of the 28 laboratories which tested *Salmonella*, 24 uploaded results from ESBL-testing, and the proportion of laboratories that could confirm that S3.1, S3.3 and S3.5 as an ESBL-producer was 96%, 92% and 96%, respectively.

For the detection of an ESBL-producing *Salmonella* when initially screening the isolate, it is recommended that more than one cephalosporin is used. This is however not very well supported by the results obtained in this EQAS (Table 3), where laboratories using two (CTX, CAZ) and three (CTX, CAZ, XNL) antimicrobial agents appeared to have difficulties obtaining the expected result.

Another issue to take into account is the actual gene causing the ESBL-production. The CTX-M9-gene is not detected by ceftazidime, consequently, the cephalosporin combinations CTX/CAZ, and CTX/CAZ/XNL would be expected to detect resistance only for CTX and XNL. For other ESBL-genes, however, ceftazidime would also be effective.

4.2 *Campylobacter* trial

The percentage of correct susceptibility test results of *Campylobacter* was 98.7%. Between the laboratories, the performance varied from no deviations at all to 28.2% deviations, with 25 laboratories performing satisfactorily according to the acceptance ranges established by the CRL. Compared to the performance in EQAS 2006 and EQAS 2007 (93.9% and 94.2% correct results) it would therefore seem that the quality of the results has improved.

One laboratory (#40) was found to be an outlier. Laboratory #40 used the methodology based on disk diffusion. The CLSI guidelines (M45-A) state that appearance of any zone of inhibition

would require MIC determination for accurate categorization of susceptibility (ciprofloxacin and erythromycin, only). 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. Moreover, as the CRL EQAS is an assessment of the method carried out when performing monitoring for EFSA, results obtained by disk diffusion on *Campylobacter* will not be included in future EQASs.

The proportion of obtained MIC-values for the *C. jejuni* reference strain within the QC intervals was 89.2% which was an increase in comparison to EQAS 2007, where the proportion was 83.8%. In this year's trial, erythromycin, nalidixic acid and tetracycline all had high deviation percentages (18%, 15% and 15%, respectively). All laboratories uploading MIC-values to the database for the *Campylobacter* trial also uploaded data from tests on the reference strain. Two laboratories each had four of the 13 deviations (#21 and #29).

A follow-up on the laboratories which were outliers in the *Campylobacter* trial in EQAS 2007 (#5, #17, #22), showed that laboratories #17 and #22 this year obtained a deviation level of 6.7% and 2.6%, respectively. Laboratory #5 is no longer part of the CRL network.

Follow-up on the outlier in the *Campylobacter* EQAS's included discussions regarding methodical issues and a training course focussing on these issues in February 2009.

5. Conclusions

The goal of the CRL EQAS is having all participating NRLs performing susceptibility testing of *Salmonella* and *Campylobacter* with a deviation level less than 7%. This seems within reach for *Salmonella*, and also for *Campylobacter*. However, for *Campylobacter* one laboratory would need to apply methodological changes to be able to improve the quality of the results.

The NRLs' performance appear to have improved for *Salmonella* AST's this EQAS (98.0%) when compared to the results from the EQAS 2007 (96.7%), as also with regard to *Campylobacter* AST (94.2% in 2007 and 98.7% in 2008).

The laboratory which was detected as an outlier in the *Campylobacter* trial took part in a training course focussing on these issues in February 2009. Results obtained by disk diffusion on *Campylobacter* will not be included in future EQASs.

Harmonising breakpoints, antimicrobials and ranges of these, are issues of importance 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 the EQAS's will be addressed at the annual workshop this year.

CRL-AR EQAS pre-notification

EQAS 2008 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 content 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 2008.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

Shipment of isolates and protocol: The isolates will be shipped in October 2008. The protocol will be provided electronically.

Returning of results: Results must be returned to the National Food Institute, by December 12th 2008. 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 2009.

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

Sincerely,

Rene S. Hendriksen
EQAS-Coordinator

Participant list

Campy	Salm	Institute	Country
X	X	Austrian Agency for Health and Food Safety	Austria
X	X	Institute of Public Health	Belgium
X	X	Nacional Diagnostic and Research Veterinary Institute	Bulgaria
X	X	Veterinary Services	Cyprus
X	X	State Veterinary Institute Praha	Czech Republic
X	X	The National Food Institute	Denmark
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 Veterinary Service	Latvia
X	X	National Veterinary Laboratory	Lithuania
X	X	Centre for Infections Health Protection Agency (UK)	Malta (UK)
X	X	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	Veterinærinstituttet	Norway
X	X	National Veterinary Research Institute	Poland
X	X	Laboratorio National de Investigacáo Veterinaria)	Portugal
X	X	National Institute of Research-Development for Microbiology and Immunology “Cantacuzino”	Romania
-	-	Institute for Hygiene and Veterinary Public Health	Romania
X	X	State Veterinary and Food Institute (SVFI)	Slovakia
X	X	National Veterinary Institute	Slovenia
-	-	Laboratorio Central de Sanidad, Animal de Santa Fe (only Staph)	Spain
X	X	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	X	Complutense University of Madrid	Spain
X	X	Centro nacional de Alimentacion. Agencia Espanola de Seguridad Alimentria y Nutricio	Spain
X	X	National Veterinary Institute, SVA	Sweden
X	X	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
X	X	The Veterinary Laboratory Agency	United Kingdom

	Designated NRL-AR by the compentent authority of the member state
	Non-NRL-AR enroled by the CRL
	Not a Member State of the EU
X	Participated in the specific trial
-	Did not participate in the specific trial

Salmonella test strains and reference values (MIC)

Kode	AMP	CTX	CTX/CL	CAZ	CAZ/CL	XNL	ESBL gene	CHL	CIP	GEN	NAL	STR	SMX	TET	TMP	IP/IPE	FOX
CRL S-3.1	>32	>4	0.016	>32	0.125	>8	CTX M-15/SHV12	>64	0.25	>16	8	>128	>1024	>32	>32	<1.0 / <0.4	4
CRL S-3.2	4	≤0.12	0.03	<0.5	<0.125	1	-	8	>4	1	>64	≤8	≤64	<=2	≤1	<1.0 / <0.4	2
CRL S-3.3	>32	>4	<0.016	<0.5	<0.125	8	CTX M-9	4	0.25	≤0.5	>64	≤8	≤64	32	≤1	<1.0 / <0.4	2
CRL S-3.4	≤1	≤0.12	0.016	<0.5	0.125	≤0.5	-	4	0.25	≤0.5	>64	32	≤64	>32	≤1	<1.0 / <0.4	2
CRL S-3.5	>32	>4	0.03	>32	0.125	>8	CTX M-15 like	>64	>4	>16	>64	>128	>1024	>32	>32	<1.0 / <0.4	2
CRL S-3.6	2	0.25	0.016	<0.5	0.125	2	-	64	0.5	4	>64	16	>1024	>32	>32	<1.0 / <0.4	4
CRL S-3.7	2	0.25	0.03	<0.5	0.125	1	-	8	0.03	≤0.5	≤4	≤8	≤64	≤2	≤1	<1.0 / <0.4	2
CRL S-3.8	>32	≤0.12	0.016	<0.5	1	≤0.5	-	8	0.03	≤0.5	≤4	>128	>1024	≤2	≤1	<1.0 / <0.4	2

Kode	AMP	CTX	CTX/CL	CAZ	CAZ/CL	XNL	ESBL gene	CHL	CIP	GEN	NAL	STR	SMX	TET	TMP	IP/IPE	FOX
CRL S-3.1	R	R	MIC ratio ≥8	R	MIC ratio ≥8	R	CTX M-15/SHV12	R	R	R	S	R	R	R	R	none Metallo beta lactamase	none-AmpC
CRL S-3.2	S	S	MIC ratio <8	S	MIC ratio <8	S	none-ESBL	S	R	S	R	S	S	S	S	none Metallo beta lactamase	none-AmpC
CRL S-3.3	R	R	MIC ratio ≥8	R*	Synergy	R	CTX M-9	S	R	S	R	S	S	R	S	none Metallo beta lactamase	none-AmpC
CRL S-3.4	S	S	MIC ratio <8	S	MIC ratio <8	S	none-ESBL	S	R	S	R	S	S	R	S	none Metallo beta lactamase	none-AmpC
CRL S-3.5	R	R	Synergy	R	Synergy	R	CTX M-15 like	R	R	R	R	R	R	R	R	none Metallo beta lactamase	none-AmpC
CRL S-3.6	S	S	MIC ratio <8	S	MIC ratio <8	S	none-ESBL	R	R	R	R	S	R	R	R	none Metallo beta lactamase	none-AmpC
CRL S-3.7	S	S	MIC ratio <8	S	MIC ratio <8	S	none-ESBL	S	S	S	S	S	S	S	S	none Metallo beta lactamase	none-AmpC
CRL S-3.8	R	S	MIC ratio <8	S	MIC ratio <8	S	none-ESBL	S	S	S	S	R	R	S	S	none Metallo beta lactamase	none-AmpC

Resistant

ESBL/AmpC

*MIC value is not resistant, but due to the rule about cephalosporins the interpretation should be resistant

Campylobacter test strains and reference values (MIC)

Strain no.	Species	CHL	CIP	ERY	GEN	NAL	STR	TET
C-3.1	*C. coli*	4	0.25	>32	0.5	8	≤1	2
C-3.2	*C. jejuni*	≤2	0.12	2	0.25	8	≤1	>16
C-3.3	*C. coli*	≤2	>4	≤0.5	0.5	64	>16	>16
C-3.4	*C. coli*	8	>4	16	0.5	>64	>16	>16
C-3.5	*C. jejuni*	4	≤0.06	1	0.5	4	≤1	0.5
C-3.6	*C. jejuni*	4	>4	>32	0.25	>64	≤1	>16
C-3.7	*C. coli*	8	0.12	2	0.5	8	>16	0.5
C-3.8	*C. jejuni*	4	>4	2	0.5	>64	≤1	>16

Strain no.	Species	CHL	CIP	ERY	GEN	NAL	STR	TET
C-3.1	*C. coli*	S	S	R	S	S	S	S
C-3.2	*C. jejuni*	S	S	S	S	S	S	R
C-3.3	*C. coli*	S	R	S	S	R	R	R
C-3.4	*C. coli*	S	R	S	S	R	R	R
C-3.5	*C. jejuni*	S	S	S	S	S	S	S
C-3.6	*C. jejuni*	S	R	R	S	R	S	R
C-3.7	*C. coli*	S	S	S	S	S	R	S
C-3.8	*C. jejuni*	S	R	S	S	R	S	R

Resistant

DTU Food
National Food Institute

Community Reference Laboratory
Antimicrobial Resistance

CRL-AR Inter-laboratory Proficiency Test 2008
- *Salmonella* and *Campylobacter*

Id:

Copenhagen, October 2008

Dear >>name<<,

Please find enclosed the bacterial strains for the CRL AR EQAS 2008.

On the CRL-website (www.crl-ar.eu) the following documents relevant for the CRL EQAS are available:

- **Protocol for *Salmonella* and *Campylobacter* including test forms**
- **Instructions for Opening and Reviving Lyophilised Cultures**
- **Subculture and Maintenance of Quality Strains**

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: >>username<<

Your password: >>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 31st 2008.

Please acknowledge receipt of parcel immediately on arrival (by email to rshe@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
Ph: +45 7234 6288, Fax: +45 7234 6001, e-mail: rshe@food.dtu.dk

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 2008 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 manual 'Subculture and Maintenance of QC Strains'. Please use them for future internal quality control for susceptibility testing in your laboratory.

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

2 OBJECTIVES

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 to EFSA by different laboratories on *Salmonella* and *Campylobacter* and to harmonise the breakpoints used within the EU.

3 OUTLINE OF THE EQAS 2008

3.1 Shipping, receipt and storage of strains

In October 2008 the 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 are shipped lyophilised, the *Campylobacter* test strains are shipped as a charcoal swabs and the *Salmonella* test strains are stab cultures. On arrival, the stab cultures and the charcoal swabs must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

Please see the document 'Instructions for opening and reviving lyophilised cultures' on the CRL-website (see www.crl-ar.eu).

3.3 Susceptibility testing

The strains should be susceptibility tested towards as many as possible of the following antimicrobials by the method used in the laboratory when performing monitoring for EFSA. For MIC the cut off values listed in tables 3.3.1 and 3.3.2 should be used. The epidemiological cut-off values allow two categories of characterisation – resistant or sensitive.

Participants using disk diffusion are recommended to interpret the results according to their individual breakpoints, categorising them into the terms resistant and sensitive. A categorization as intermediary is not accepted. 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).

With regard to MIC range and/or disc conctent we ask you to fill in these pieces of information in the database. Also, if you ***do not use*** the cut-off values listed in the protocol for interpretation of the susceptibility results, please fill in or update the breakpoints used, in the database.

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.

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 >**
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

* ARBAO ** CLSI

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

ESBL production

The following tests regarding ESBL production are mandatory: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) should 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.

Also, when testing cephalosporins, please note that when an isolate is found resistant to one cephalosporin, the isolate is regarded resistant to all cephalosporins.

3.3.2 *Campylobacter*

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

*Not part of the EFSA monitoring programme

Please find information on the test forms showing which test strains are *C. jejuni* and *C. coli* respectively.

The sub-cultured *Campylobacter* should be used for the MIC-testing after incubation at 36ºC for 48 hours or 42ºC for 24 hours.

4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the test forms, and enter your results into the interactive web database. Please read the detailed description below before entering your results. 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 31st, 2008.

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 be summarized in a report which will be made available to all participants. The data in the report will be 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.

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

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E-mail: rshe@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 2008 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 is exactly 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 relevant information, either disk content or MIC range. If you use disk diffusion, please upload the breakpoints used.

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.

For *Salmonella*, you also type in results for the ESBL tests.

If you have not used an antimicrobial, 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.

Please fill in the evaluation form.

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.

TEST FORMS

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

Comments:

TEST FORM

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

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

Brand**:**

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

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

Comments or additional information:

Antimicrobial	General info The relevant information in the two columns below should be filled in		Zonediameter (mm) Please, *only* fill in breakpoint information if you did not use the cut-off values listed in the protocol		
	Disk content (µg)	Test-range for MIC (µg/mL)	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Ampicillin, AMP			≤		≥
Cefotaxime, CTX			≤		≥
Ceftazidime, CAZ			≤		≥
Ceftiofur, XNL			≤		≥
Chloramphenicol, CHL			≤		≥
Ciprofloxacin, CIP			≤		≥
Gentamicin, GEN			≤		≥
Nalidixic acid, NAL			≤		≥
Streptomycin, STR			≤		≥
Sulphamethoxazole, SMX			≤		≥
Tetracycline, TET			≤		≥
Trimethoprim, TMP			≤		≥

TEST FORM

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

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

Brand**:**

Incubation conditions: °C/ h

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

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

Comments or additional information:

Antimicrobial	General info The relevant information should be filled in below
	Test-range for MIC (µg/mL)
Chloramphenicol	
Ciprofloxacin	
Erythromycin	
Gentamicin	
Nalidixic Acid	
Streptomycin	
Tetracycline	

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 3._	Ampicillin, AMP			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) should be included 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 ☐ Phantom zone (synergy) ☐ Deformation (synergy) ☐ Not determinable	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8 ☐ Phantom zone (synergy) ☐ Deformation (synergy) ☐ Not determinable	Incr. in zone diam	☐ Incr. ≥ 5 mm (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 ☐ Phantom zone (synergy) ☐ Deformation (synergy) ☐ Not determinable		

Comments:

TEST FORM

Susceptibility testing of *E. coli* referencestrain ATCC 25922

Strain	Antimicrobial	Zonediameter (mm) or MIC-value (µg/ml)
E. coli ATCC 25922	Ampicillin, AMP	
	Cefotaxime, CTX	
	Cefoxitin, FOX	
	Ceftazidime, CAZ	
	Ceftiofur, XNL	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Gentamicin, GEN	
	Imipenem, IMI	
	Nalidixic acid, NAL	
	Streptomycin, STR	
	Sulfisoxazole, FIS	
	Tetracycline, TET	
	Trimethoprim, TMP	

TEST FORM

Strain	Antimicrobial	Interpretation	
		Zonediam (mm) or MIC-value (µg/ml)	S / R
Campylobacter CRL C. 3.1 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 3.2 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 3.3 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 3.4 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

TEST FORM

Strain	Antimicrobial	Interpretation	
		Zonediam (mm) or MIC-value (µg/ml)	S / R
Campylobacter CRL C. 3.5 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 3.6 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 3.7 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 3.8 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

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		

For Agar dilution:

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (µg/ml)
C. jejuni ATCC 33560	Ciprofloxacin	
	Doxycycline	
	Erythromycin	
	Gentamicin	
	Meropenem	
	Nalidixic Acid	
	Tetracycline	

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!

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-S18, January 2008 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A7, January 2006 (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% fecal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen. (Alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

Working cultures

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

1.6 Frequency of Testing

Weekly vs. daily testing

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

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

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

Corrective Actions

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

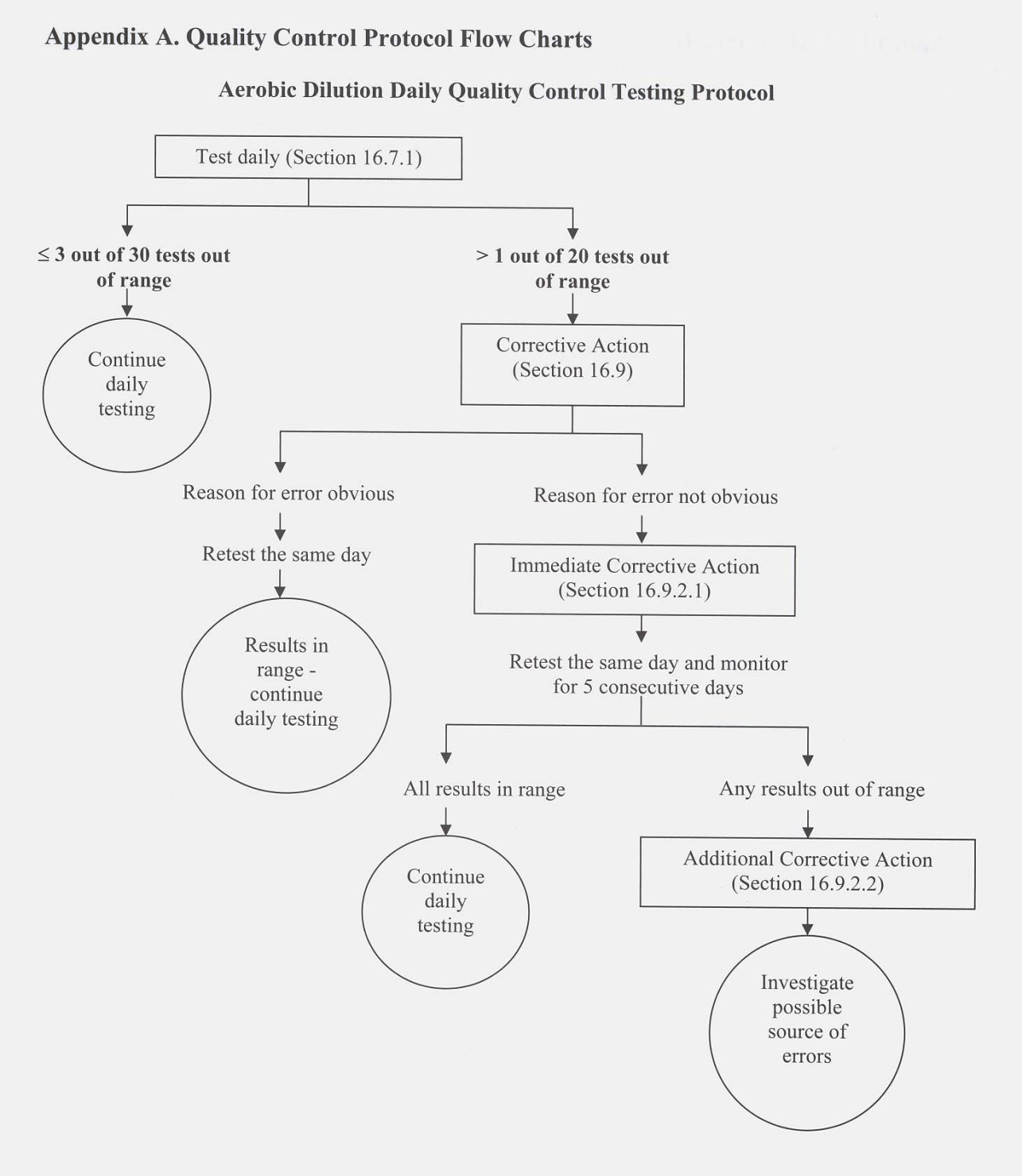
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

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

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

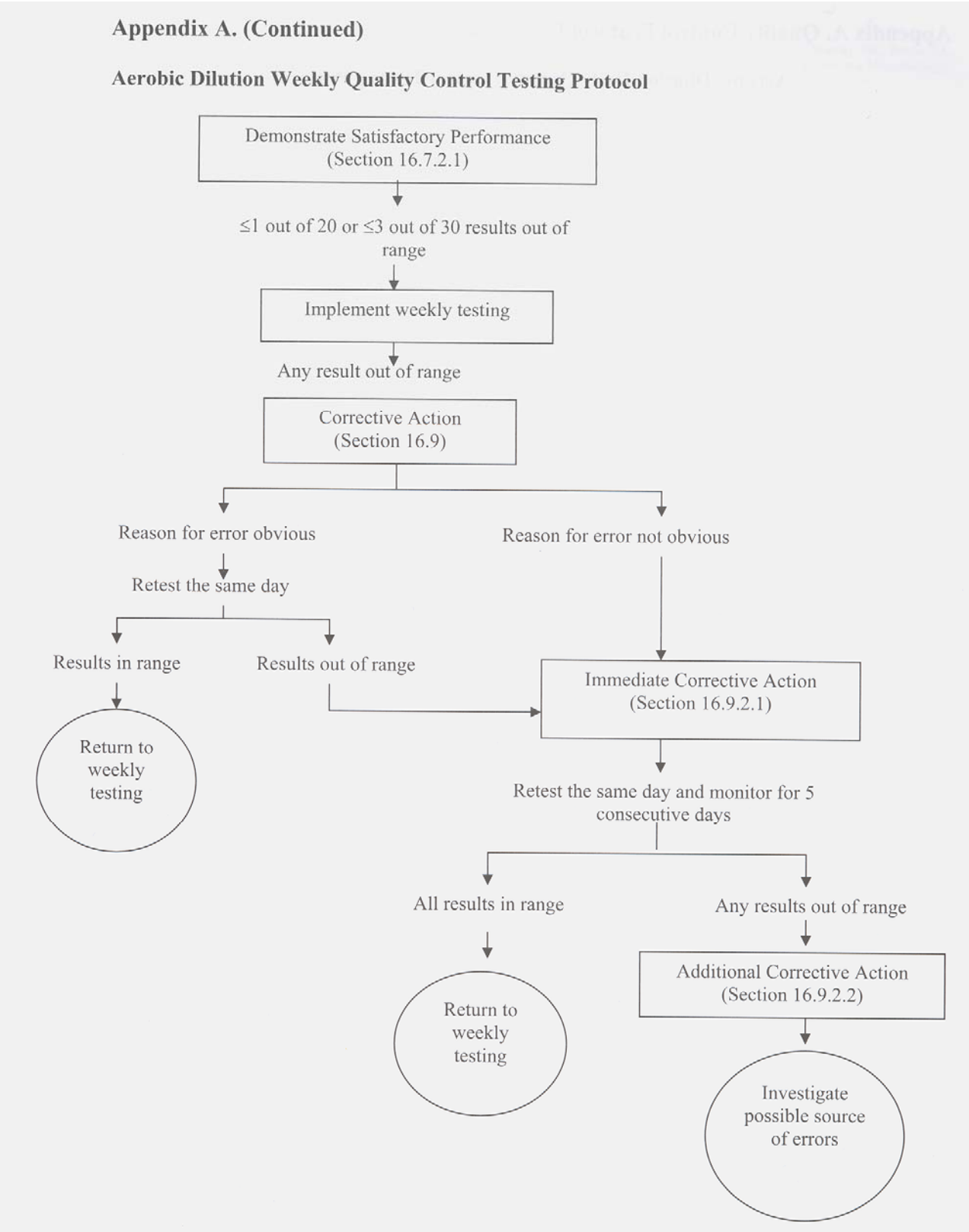
Repeat the 30 days validation before resuming weekly testing.

DAILY MIC QC CHART



Reference: CLSI M7-A7, page 39

WEEKLY MIC QC CHART



Reference: CLSI M7-A7, page 40

Disk content and breakpoints used in daily routine (disk diffusion) - *Salmonella*

Antimicrobial	Lab No	Disk content (ug)	R val <= (mm)	I val = (mm)	S val >= (mm)
Ampicillin, AMP	18	10	13	14-16	17
	23		13	14-16	17
	29	10	13	14-16	17
	30	10	13	14-16	17
	38	10	13	14-16	17
Cefotaxime, CTX	30	30	14	15-22	23
	38	30	14	15-22	23
	18	30	26		27
Ceftazidime, CAZ	23		14	15-17	18
	30	30	14	15-17	18
	18	30	21		22
Ceftiofur, XNL	30	30	16	17-19	20
	20	30			
Chloramphenicol, CHL	18	30	12	13-17	18
	23		12	13-17	18
	29	30	12	11-17	18
	30	30	12	13-17	18
	38	30	12	13-17	18
Ciprofloxacin, CIP	18	5	15	16-20	21
	23		15	16-20	21
	30	5	15	16-20	21
	38	5	15	16-20	21
	29	5			
Gentamicin, GEN	18	10	12	13-14	15
	23		12	13-14	15
	29	10	12	13-14	15
	30	10	12	13-14	15
	38	10	12	13-14	15
Nalidixic acid, NAL	18	30	13	14-18	19
	23		13	14-18	19
	29	30	13	14-18	19
	30	30	13	14-18	19
	38	30	13	14-18	19
Streptomycin, STR	18	10	11	12-14	15
	23		11	12-14	15
	29	10	11	12-14	15
	30	10	11	12-14	15
	38	10	11	12-14	15
Sulfamethoxazole, SMX	18	300	12	13-16	17
	23		12	13-16	17
	30	250-300	12	13-16	17
Tetracycline,TET	18	30	11	12-14	15
	30	30	11	12-14	15
	38	30	11	12-14	15
	23		14	15-18	19
	29	30	14	15-18	19
Trimethoprim, TMP	18	5	10	11-15	16
	23		10	11-15	16
	30	5	10	11-15	16
	38	5	10	11-15	16

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

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
1	Ampicillin, AMP	=	8	2	8	1	MIC
	Cefotaxime, CTX	<=	0.125	0.03	0.12	1	MIC
	Ceftiofur, XNL	<=	0.5	0.25	1	1	MIC
	Chloramphenicol, CHL	=	4	2	8	1	MIC
	Ciprofloxacin, CIP	<=	0.015	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	<=	8	4	16	1	MIC
	Tetracycline, TET	<=	2	0.5	2	1	MIC
	Trimethoprim, TMP	<=	1	0.5	2	1	MIC
2	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
	Sulfisoxazole, FIS	=	16	8	32	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
4	Ampicillin, AMP	=	2	2	8	1	ET
	Cefotaxime, CTX	=	0.06	0.03	0.12	1	ET
	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	ET
	Chloramphenicol, CHL	=	4	0	256	1	ET
	Ciprofloxacin, CIP	=	0.015	0	256	1	ET
	Gentamicin, GEN	=	0.5	0	256	1	ET
	Nalidixic acid, NAL	=	4	1	4	1	ET
	Streptomycin, STR	=	8	2	8	1	ET
	Tetracycline, TET	=	1	0.5	2	1	ET
	Trimethoprim, TMP	=	1	0.5	2	1	ET
6	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	=	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
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	<	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
9	Ampicillin, AMP	=	8	2	8	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Cefoxitin, FOX	=	4	2	8	1	MIC
	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
	Chloramphenicol, CHL	=	8	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	=	8	4	16	1	MIC
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	=	2	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
11	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	=	0.12	0.03	0.12	1	MIC
	Chloramphenicol, CHL	=	8	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	=	4	1	4	1	MIC
	Streptomycin, STR	=	8	4	16	1	MIC
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	=	2	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
12	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	=	8	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	=	4	1	4	1	MIC
	Streptomycin, STR	=	8	4	16	1	MIC
	Sulfisoxazole, FIS	<=	16	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
13	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
	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
16	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
	Imipenem, IMI	<=	4	0.06	0.25	1	MIC
	Nalidixic acid, NAL	=	2	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
17	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	=	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
	Sulfisoxazole, FIS	=	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
18	Ampicillin, AMP	=	16	16	22	1	DD
	Cefotaxime, CTX	=	33	29	35	1	DD
	Cefoxitin, FOX	=	25	23	29	1	DD
	Ceftazidime, CAZ	=	30	25	32	1	DD
	Chloramphenicol, CHL	=	24	21	27	1	DD
	Ciprofloxacin, CIP	=	35	30	40	1	DD
	Gentamicin, GEN	=	22	19	26	1	DD
	Imipenem, IMI	=	27	26	32	1	DD
	Nalidixic acid, NAL	=	24	22	28	1	DD
	Streptomycin, STR	=	15	12	20	1	DD
	Sulfisoxazole, FIS	=	22	15	23	1	DD
	Tetracycline, TET	=	23	18	25	1	DD
	Trimethoprim, TMP	=	24	21	28	1	DD
19	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
	Ceftiofur, XNL	=	26	26	31	1	DD
	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
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
20	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
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	=	2	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	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	=	0.06	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	=	0.25	0.25	1	1	MIC
	Nalidixic acid, NAL	=	1	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	=	0.5	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
22	Ampicillin, AMP	=	4	2	8	1	MIC
	Cefotaxime, CTX	<	4	0.03	0.12	1	MIC
	Ceftazidime, CAZ	<	16	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
	Sulfisoxazole, FIS	=	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	Ampicillin, AMP	=	20	16	22	1	DD
	Cefoxitin, FOX	=	25	23	29	1	DD
	Ceftazidime, CAZ	=	28	25	32	1	DD
	Chloramphenicol, CHL	=	25	21	27	1	DD
	Ciprofloxacin, CIP	=	33	30	40	1	DD
	Gentamicin, GEN	=	22	19	26	1	DD
	Nalidixic acid, NAL	=	26	22	28	1	DD
	Streptomycin, STR	=	16	12	20	1	DD
	Tetracycline, TET	=	20	18	25	1	DD
	Trimethoprim, TMP	=	24	21	28	1	DD
24	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	=	8	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
	Tetracycline, TET	=	2	0.5	2	1	MIC
	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
25	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
	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
26	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	=	8	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
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
29	Ampicillin, AMP	=	18	16	22	1	DD
	Chloramphenicol, CHL	=	25	21	27	1	DD
	Ciprofloxacin, CIP	=	34	30	40	1	DD
	Gentamicin, GEN	=	19	19	26	1	DD
	Nalidixic acid, NAL	=	23	22	28	1	DD
	Streptomycin, STR	=	15	12	20	1	DD
	Tetracycline, TET	=	21	18	25	1	DD
30	Ampicillin, AMP	=	16	16	22	1	DD
	Cefotaxime, CTX	=	35	29	35	1	DD
	Cefoxitin, FOX	=	24	23	29	1	DD
	Ceftazidime, CAZ	=	31	25	32	1	DD
	Ceftiofur, XNL	=	28	26	31	1	DD
	Chloramphenicol, CHL	=	24	21	27	1	DD
	Ciprofloxacin, CIP	=	36	30	40	1	DD
	Gentamicin, GEN	=	24	19	26	1	DD
	Imipenem, IMI	=	30	26	32	1	DD
	Nalidixic acid, NAL	=	27	22	28	1	DD
	Streptomycin, STR	=	16	12	20	1	DD
	Sulfisoxazole, FIS	=	22	15	23	1	DD
	Tetracycline, TET	=	25	18	25	1	DD
	Trimethoprim, TMP	=	23	21	28	1	DD

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
32	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	=	8	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	=	8	4	16	1	MIC
	Tetracycline, TET	<	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
33	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	=	0.016	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
	Sulfisoxazole, FIS	=	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	=	8	2	8	1	MIC
	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
	Gentamicin, GEN	<=	0.25	0.25	1	1	MIC
	Nalidixic acid, NAL	<=	4	1	4	1	MIC
	Streptomycin, STR	=	4	4	16	1	MIC
	Sulfisoxazole, FIS	=	32	8	32	1	MIC
	Tetracycline, TET	<=	1	0.5	2	1	MIC
	Trimethoprim, TMP	=	1	0.5	2	1	MIC
37	Cefotaxime, CTX	=	0.06	0.03	0.12	1	AGA
	Ciprofloxacin, CIP	=	0.03	0.004	0.016	**0**	AGA
	Gentamicin, GEN	=	0.25	0.25	1	1	AGA
	Nalidixic acid, NAL	=	2	1	4	1	AGA
	Streptomycin, STR	=	4	4	16	1	AGA
	Tetracycline, TET	=	1	0.5	2	1	AGA
	Trimethoprim, TMP	=	0.5	0.5	2	1	AGA
38	Ampicillin, AMP	=	18.9	16	22	1	DD
	Cefotaxime, CTX	=	32.3	29	35	1	DD
	Cefoxitin, FOX	=	24.7	23	29	1	DD
	Chloramphenicol, CHL	=	26.2	21	27	1	DD
	Ciprofloxacin, CIP	=	34.3	30	40	1	DD
	Gentamicin, GEN	=	23.9	19	26	1	DD
	Imipenem, IMI	=	27.3	26	32	1	DD
	Nalidixic acid, NAL	=	22.1	22	28	1	DD
	Streptomycin, STR	=	14.0	12	20	1	DD
	Tetracycline, TET	=	22.0	18	25	1	DD
	Trimethoprim, TMP	=	23.5	21	28	1	DD
40	Ampicillin, AMP	=	19	16	22	1	DD
	Cefotaxime, CTX	=	29	29	35	1	DD
	Cefoxitin, FOX	=	23	23	29	1	DD
	Ceftazidime, CAZ	=	29	25	32	1	DD
	Ceftiofur, XNL	=	27	26	31	1	DD
	Chloramphenicol, CHL	=	26	21	27	1	DD
	Ciprofloxacin, CIP	=	30	30	40	1	DD
	Gentamicin, GEN	=	21	19	26	1	DD
	Imipenem, IMI	=	26	26	32	1	DD
	Nalidixic acid, NAL	=	26	22	28	1	DD
	Streptomycin, STR	=	20	12	20	1	DD
	Sulfisoxazole, FIS	=	14	15	23	**0**	DD
	Tetracycline, TET	=	20	18	25	1	DD
	Trimethoprim, TMP	=	28	21	28	1	DD

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

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37ºC/48h	42ºC/24h
1	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	=	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	=	4	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.12	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	=	1	0.25	2	1	MIC	X	
4	Ciprofloxacin, CIP	=	0.094	0.03	0.125	1	ET	X	
	Erythromycin, ERY	=	1	0.25	2	1	ET	X	
	Gentamicin, GEN	=	0.5	0.25	2	1	ET	X	
	Nalidixic acid, NAL	=	4	4	16	1	ET	X	
	Tetracycline, TET	=	0.25	0.25	1	1	ET	X	
6	Chloramphenicol, CHL	<	2	1	4	1	MIC		X
	Ciprofloxacin, CIP	=	0.12	0.03	0.125	1	MIC		X
	Erythromycin, ERY	<	0.5	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
9	Chloramphenicol, CHL	=	4	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	=	8	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
11	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
12	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	
14	Ciprofloxacin, CIP	=	0.25	0.06	0.5	1	AGA	37ºC/24h	
	Erythromycin, ERY	=	2	1	4	1	AGA	37ºC/24h	
	Gentamicin, GEN	=	0.5	0.5	4	1	AGA	37ºC/24h	
	Nalidixic acid, NAL	=	4	0	256	1	AGA	37ºC/24h	
	Tetracycline, TET	=	1	0	256	1	AGA	37ºC/24h	
17	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.5	0.06	0.25	**0**	MIC	X	
	Erythromycin, ERY	=	4	0.5	2	**0**	MIC	X	
	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
	Tetracycline, TET	<=	0.25	0.25	2	1	MIC	X	
19	Chloramphenicol, CHL	=	2	1	4	1	MIC		X
	Ciprofloxacin, CIP	=	0.06	0.03	0.125	1	MIC		X
	Erythromycin, ERY	=	0.5	0.25	2	1	MIC		X
	Gentamicin, GEN	=	0.5	0.25	2	1	MIC		X
	Nalidixic acid, NAL	=	2	4	16	**0**	MIC		X
	Tetracycline, TET	=	2	0.25	1	**0**	MIC		X
20	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.12	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	=	1	0.25	2	1	MIC	X	
21	Chloramphenicol, CHL	=	1	1	4	1	MIC		X
	Ciprofloxacin, CIP	=	0.06	0.03	0.125	1	MIC		X
	Erythromycin, ERY	=	0.12	0.25	2	**0**	MIC		X
	Gentamicin, GEN	=	0.12	0.25	2	**0**	MIC		X
	Nalidixic acid, NAL	=	0.12	4	16	**0**	MIC		X
	Tetracycline, TET	=	0.12	0.25	1	**0**	MIC		X

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37ºC/48h	42ºC/24h
22	Chloramphenicol, CHL	=	4	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	=	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	
24	Chloramphenicol, CHL	=	4	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	=	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	<=	0.12	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	<=	0.5	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	
26	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.12	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	
29	Chloramphenicol, CHL	=	0.5	1	4	**0**	MIC		X
	Ciprofloxacin, CIP	=	0.125	0.03	0.125	1	MIC		X
	Erythromycin, ERY	<	0.125	0.25	2	**0**	MIC		X
	Nalidixic acid, NAL	=	2	4	16	**0**	MIC		X
	Tetracycline, TET	=	4	0.25	1	**0**	MIC		X
30	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
	Erythromycin, ERY	<=	0.5	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	=	1	0.25	2	1	MIC	X	
32	Chloramphenicol, CHL	=	2	1	4	1	MIC		X
	Ciprofloxacin, CIP	=	0.125	0.03	0.125	1	MIC		X
	Erythromycin, ERY	<	0.25	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	=	0.25	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	=	16	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	=	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	=	1	0.25	2	1	MIC	X	
37	Ciprofloxacin, CIP	=	0.06	0.06	0.5	1	AGA		X
	Erythromycin, ERY	=	0.25	1	4	**0**	AGA		X
	Gentamicin, GEN	=	1	0.5	4	1	AGA		X
	Nalidixic acid, NAL	=	4	0	256	1	AGA		X
	Tetracycline, TET	=	1	0	256	1	AGA		X
39	Chloramphenicol, CHL	<	8	0	256	1	AGA	X	
	Ciprofloxacin, CIP	<	1	0.12	1	1	AGA	X	
	Erythromycin, ERY	<	4	1	8	1	AGA	X	
	Gentamicin, GEN	<	4	0.5	2	1	AGA	X	
	Nalidixic acid, NAL	<	16	0	256	1	AGA	X	
	Tetracycline, TET	<	8	0	256	1	AGA	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	12-20 (10µg)
Sulfisoxazole, FIS	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 S18 with the following exceptions: The MIC range for streptomycin is according to Sensititre and the ranges for ceftiofur and florphenicol is according to M31-A3. Additionally, the range for ciprofloxacin is extended to include 0.016 µg/ml.

E-test ranges are according to AB-Biodisk

Campylobacter jejuni ATCC 33560				
Antimicrobial	**Microbroth (36-37°C/48h)**	**Microbroth (42°C/24h)**	**Agar dilution (36-37°C/48h)**	**Agar dilution (42°C/24h)**
Chloramphenicol, CHL	1-8	1-4	None	None
Ciprofloxacin, CIP	0.06-0.25	0.03-0.12	0.12-1	0.06-0.5
Doxycycline, DOX	0.12-0.5	0.12-0.5	0.5-2	0.25-2
Erythromycin, ERY	0.5-2	0.25-2	1-8	1-4
Gentamicin, GEN	0.5-2	0.25-2	0.5-2	0.5-4
Meropenem, MERO	0.008-0.03	0.008-0.03	0.004-0.015	0.008-0.03
Nalidixic acid, NAL	4-16	4-16	-	-
Tetracycline, TET	0.25-2	0.25-1	-	-

Ranges are according to CLSI (M31-A3)

Campylobacter jejuni ATCC 33560		
Antimicrobial	**E-test (36-37°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 comments, summarised

Participants' evaluation of the CRL EQAS Salm/Camp 2008

As means of improving the quality and usefulness of the EQAS, the participants of the CRL EQAS Salm/Camp were asked to fill in an evaluation form in the database.

The relevant information obtained through the eight completed evaluation forms is collected and commented below. Comments from the CRL are in *italic* in the following.

Information received during the CRL AR EQAS 2007 and how the EQAS was performed: Percentage (number of laboratories)	Very poor	Poor	Satisfactory	Good	Very good
Information about the EQAS in general	-	-	13% (1)	13% (1)	75% (6)
The EQAS welcome letter (the letter in the parcel)	-	-	-	25% (2)	75% (6)
The EQAS protocol and test forms	-	-	-	25% (2)	75% (6)
The distribution of the samples	-	-	13% (1)	13% (1)	75% (6)
What is your overall impression of the interactive web database	-	-	13% (1)	38% (3)	50% (4)
The evaluation report	-	-	-	71% (5)	29% (2)
How did participation in this EQAS meet your expectations	-	-	13% (1)	38% (3)	50% (4)

Comments and proposals from participants:

A single print out (pdf file) would be nice. *This has been passed on to the systems developer.*

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 to us. 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.

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

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Ampicillin, AMP	12	MIC	0.25-32
	2	MIC	0.5-32
	6	MIC	0.5-32
	9	MIC	0.5-32
	13	MIC	0.5-32
	17	MIC	0.5-32
	20	MIC	0.5-32
	25	MIC	0.5-32
	26	MIC	0.5-32
	32	MIC	0.5-32
	11	MIC	0.5-64
	33	MIC	0.5-64
	1	MIC	1-32
	39	AGA	8 and 128*
Cefotaxime, CTX	32	MIC	0.006-4
	37	AGA	0.015-512
	12	MIC	0.06-2
	2	MIC	0.06-4
	6	MIC	0.06-4
	9	MIC	0.06-4
	13	MIC	0.06-4
	17	MIC	0.06-4
	20	MIC	0.06-4
	25	MIC	0.06-4
	26	MIC	0.06-4
	11	MIC	0.06-8
	33	MIC	0.06-8
	1	MIC	0.125-4
Ceftazidime, CAZ	1	MIC	0.25-128
	2	MIC	0.25-16
	6	MIC	0.25-16
	9	MIC	0.25-16
	13	MIC	0.25-16
	17	MIC	0.25-16
	20	MIC	0.25-16
	25	MIC	0.25-16
	26	MIC	0.25-16
	32	MIC	0.25-16
Ceftiofur, XNL	12	MIC	0.12-16
	33	MIC	0.12-16
	1	MIC	0.5-8
Chloramphenicol, CHL	12	MIC	1-128
	11	MIC	2-256
	33	MIC	2-256
	1	MIC	2-64
	2	MIC	2-64
	6	MIC	2-64
	9	MIC	2-64
	13	MIC	2-64
	17	MIC	2-64
	20	MIC	2-64
	25	MIC	2-64
	26	MIC	2-64
	32	MIC	2-64
Ciprofloxacin, CIP	11	MIC	0.008-1
	12	MIC	0.008-1
	2	MIC	0.008-8
	6	MIC	0.008-8
	9	MIC	0.008-8
	13	MIC	0.008-8
	17	MIC	0.008-8
	20	MIC	0.008-8
	25	MIC	0.008-8
	26	MIC	0.008-8
	32	MIC	0.008-8
	33	MIC	0.008-8
	1	MIC	0.015-4
	37	AGA	0.015-512
	39	AGA	0.125 and 1*
Gentamicin, GEN	37	AGA	0.015-512
	2	MIC	0.25-32
	6	MIC	0.25-32
	9	MIC	0.25-32
	11	MIC	0.25-32
	13	MIC	0.25-32
	17	MIC	0.25-32
	20	MIC	0.25-32
	25	MIC	0.25-32
	26	MIC	0.25-32
	32	MIC	0.25-32
	33	MIC	0.25-32
	1	MIC	0.5-16
	12	MIC	0.5-64

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Nalidixic acid, NAL	37	AGA	0.015-512
	12	MIC	1-128
	11	MIC	2-256
	33	MIC	2-256
	1	MIC	4-64
	2	MIC	4-64
	6	MIC	4-64
	9	MIC	4-64
	17	MIC	4-64
	20	MIC	4-64
	25	MIC	4-64
	26	MIC	4-64
	32	MIC	4-64
	13	MIC	8-64
Streptomycin, STR	37	AGA	0.015-512
	2	MIC	2-128
	6	MIC	2-128
	9	MIC	2-128
	13	MIC	2-128
	17	MIC	2-128
	20	MIC	2-128
	25	MIC	2-128
	26	MIC	2-128
	32	MIC	2-128
	11	MIC	2-256
	12	MIC	2-256
	33	MIC	2-256
	39	AGA	8 and 128*
	1	MIC	8-128
Sulfamethoxazole, SMX	37	AGA	0.015-512
	12	MIC	16-2048
	1	MIC	64-1024
	2	MIC	8-1024
	6	MIC	8-1024
	9	MIC	8-1024
	11	MIC	8-1024
	13	MIC	8-1024
	17	MIC	8-1024
	20	MIC	8-1024
	25	MIC	8-1024
	26	MIC	8-1024
	32	MIC	8-1024
	33	MIC	8-1024
Tetracycline,TET	37	AGA	0.015-512
	11	MIC	0.5-32
	12	MIC	0.5-64
	33	MIC	0.5-64
	2	MIC	1-64
	6	MIC	1-64
	9	MIC	1-64
	13	MIC	1-64
	17	MIC	1-64
	20	MIC	1-64
	25	MIC	1-64
	26	MIC	1-64
	32	MIC	1-64
	1	MIC	2-32
	39	AGA	8 and 128*
Trimethoprim, TMP	37	AGA	0.015-512
	11	MIC	0.25-32
	12	MIC	0.25-32
	33	MIC	0.25-32
	2	MIC	0.5-32
	6	MIC	0.5-32
	9	MIC	0.5-32
	13	MIC	0.5-32
	17	MIC	0.5-32
	20	MIC	0.5-32
	25	MIC	0.5-32
	26	MIC	0.5-32
	32	MIC	0.5-32
	1	MIC	1-32

Antimicrobials recommended by EFSA are marked in grey
Participants' ranges covering the EFSA range are marked in grey

MIC: Microbroth dilution
AGA: Agar dilution

* This laboratory uses a breakpoint system with just one or two antimicrobial concentrations for each antibiotic

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

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Chloramphenicol, CHL	33	MIC	0.12-16
	29	MIC	0.125-256
	32	MIC	0.5-32
	21	MIC	1-32
	34	MIC	1-32
	25	MIC	2-128
	1	MIC	2-32
	6	MIC	2-32
	9	MIC	2-32
	17	MIC	2-32
	20	MIC	2-32
	26	MIC	2-32
	2	MIC	2-64
Ciprofloxacin, CIP	29	MIC	0.01-32
	37	AGA	0.015-512
	34	MIC	0.032-32
	21	MIC	0.06-128
	2	MIC	0.06-32
	1	MIC	0.06-4
	6	MIC	0.06-4
	9	MIC	0.06-4
	17	MIC	0.06-4
	20	MIC	0.06-4
	26	MIC	0.06-4
	11	MIC	0.06-8
	12	MIC	0.06-8
	14	AGA	0.06-8
	32	MIC	0.06-8
	33	MIC	0.06-8
	25	MIC	0.12-16
Erythromycin, ERY	37	AGA	0.015-512
	21	MIC	0.12-128
	34	MIC	0.125-128
	29	MIC	0.125-256
	2	MIC	0.25-128
	32	MIC	0.25-64
	1	MIC	0.5-32
	6	MIC	0.5-32
	9	MIC	0.5-32
	17	MIC	0.5-32
	20	MIC	0.5-32
	26	MIC	0.5-32
	11	MIC	0.5-64
	12	MIC	0.5-64
	14	AGA	0.5-64
	25	MIC	0.5-64
	33	MIC	0.5-64
Gentamicin, GEN	37	AGA	0.015-512
	29	MIC	0.03-64
	21	MIC	0.12-128
	6	MIC	0.12-16
	9	MIC	0.12-16
	11	MIC	0.12-16
	12	MIC	0.12-16
	17	MIC	0.12-16
	20	MIC	0.12-16
	26	MIC	0.12-16
	33	MIC	0.12-16
	1	MIC	0.125-16
	2	MIC	0.125-16
	14	AGA	0.125-16
	32	MIC	0.125-16
	34	MIC	0.125-32
	25	MIC	0.25-32

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Nalidixic acid, NAL	37	AGA	0.015-512
	21	MIC	0.12-128
	34	MIC	0.5-64
	25	MIC	1-128
	14	AGA	1-256
	11	MIC	1-64
	12	MIC	1-64
	33	MIC	1-64
	2	MIC	2-256
	1	MIC	2-64
	6	MIC	2-64
	9	MIC	2-64
	17	MIC	2-64
	20	MIC	2-64
	26	MIC	2-64
	32	MIC	2-64
Streptomycin, STR	37	AGA	0.015-512
	29	MIC	0.06-128
	21	MIC	0.12-128
	34	MIC	0.25-64
	2	MIC	0.5-32
	14	AGA	0.5-32
	32	MIC	0.5-32
	11	MIC	0.5-64
	12	MIC	0.5-64
	33	MIC	0.5-64
	25	MIC	1-128
	1	MIC	1-16
	6	MIC	1-16
	9	MIC	1-16
	17	MIC	1-16
	20	MIC	1-16
	26	MIC	1-16
Tetracycline,TET	37	AGA	0.015-512
	21	MIC	0.12-128
	11	MIC	0.12-16
	12	MIC	0.12-16
	33	MIC	0.12-16
	34	MIC	0.125-128
	14	AGA	0.125-16
	32	MIC	0.125-16
	29	MIC	0.125-256
	2	MIC	0.125-64
	1	MIC	0.25-16
	6	MIC	0.25-16
	9	MIC	0.25-16
	17	MIC	0.25-16
	20	MIC	0.25-16
	26	MIC	0.25-16
	25	MIC	0.5-64

Antimicrobials recommended by EFSA are marked in grey
Participants' ranges covering the EFSA range are marked in grey

MIC: Microbroth dilution
AGA: Agar dilution

Salmonella - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Ampicillin, AMP	CRL S-3.1	R	100%	0%	27	0
	CRL S-3.2	S	0%	100%	27	0
	CRL S-3.3	R	100%	0%	27	0
	CRL S-3.4	S	4%	96%	24	1
	CRL S-3.5	R	100%	0%	27	0
	CRL S-3.6	S	0%	100%	27	0
	CRL S-3.7	S	0%	100%	27	0
	CRL S-3.8	R	100%	0%	27	0
Cefotaxime, CTX	CRL S-3.1	R	100%	0%	27	0
	CRL S-3.2	S	0%	100%	27	0
	CRL S-3.3	R	96%	4%	25	1
	CRL S-3.4	S	0%	100%	24	0
	CRL S-3.5	R	100%	0%	26	0
	CRL S-3.6	S	0%	100%	26	0
	CRL S-3.7	S	0%	100%	26	0
	CRL S-3.8	S	0%	100%	26	0
Ceftazidime, CAZ	CRL S-3.1	R	100%	0%	22	0
	CRL S-3.2	S	0%	100%	21	0
	CRL S-3.3	R	59%	41%	13	9
	CRL S-3.4	S	0%	100%	19	0
	CRL S-3.5	R	100%	0%	22	0
	CRL S-3.6	S	0%	100%	21	0
	CRL S-3.7	S	0%	100%	21	0
	CRL S-3.8	S	0%	100%	21	0
Ceftiofur, XNL	CRL S-3.1	R	100%	0%	9	0
	CRL S-3.2	S	0%	100%	7	0
	CRL S-3.3	R	100%	0%	6	0
	CRL S-3.4	S	0%	100%	7	0
	CRL S-3.5	R	100%	0%	8	0
	CRL S-3.6	S	0%	100%	7	0
	CRL S-3.7	S	0%	100%	9	0
	CRL S-3.8	S	0%	100%	7	0
Chloramphenicol, CHL	CRL S-3.1	R	100%	0%	27	0
	CRL S-3.2	S	4%	96%	26	1
	CRL S-3.3	S	0%	100%	27	0
	CRL S-3.4	S	0%	100%	25	0
	CRL S-3.5	R	100%	0%	27	0
	CRL S-3.6	R	100%	0%	27	0
	CRL S-3.7	S	0%	100%	27	0
	CRL S-3.8	S	0%	100%	27	0
Ciprofloxacin, CIP	CRL S-3.1	R	75%	25%	21	7
	CRL S-3.2	R	100%	0%	28	0
	CRL S-3.3	R	82%	18%	23	5
	CRL S-3.4	R	81%	19%	21	5
	CRL S-3.5	R	100%	0%	28	0
	CRL S-3.6	R	86%	14%	24	4
	CRL S-3.7	S	0%	100%	28	0
	CRL S-3.8	S	0%	100%	28	0

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Gentamicin, GEN	CRL S-3.1	R	100%	0%	28	0
	CRL S-3.2	S	0%	100%	28	0
	CRL S-3.3	S	0%	100%	28	0
	CRL S-3.4	S	0%	100%	26	0
	CRL S-3.5	R	100%	0%	28	0
	CRL S-3.6	R	89%	11%	24	3
	CRL S-3.7	S	0%	100%	28	0
	CRL S-3.8	S	0%	100%	28	0
Nalidixic acid, NAL	CRL S-3.1	S	0%	100%	28	0
	CRL S-3.2	R	100%	0%	28	0
	CRL S-3.3	R	100%	0%	28	0
	CRL S-3.4	R	100%	0%	26	0
	CRL S-3.5	R	100%	0%	28	0
	CRL S-3.6	R	100%	0%	28	0
	CRL S-3.7	S	4%	96%	27	1
	CRL S-3.8	S	0%	100%	28	0
Streptomycin, STR	CRL S-3.1	R	100%	0%	28	0
	CRL S-3.2	S	7%	93%	26	2
	CRL S-3.3	S	4%	96%	27	1
	CRL S-3.4	R	100%	0%	26	0
	CRL S-3.5	R	100%	0%	28	0
	CRL S-3.6	S	11%	89%	24	3
	CRL S-3.7	S	4%	96%	26	1
	CRL S-3.8	R	100%	0%	28	0
Sulphonamides, SMX	CRL S-3.1	R	100%	0%	26	0
	CRL S-3.2	S	0%	100%	26	0
	CRL S-3.3	S	0%	100%	26	0
	CRL S-3.4	S	0%	100%	24	0
	CRL S-3.5	R	100%	0%	26	0
	CRL S-3.6	R	100%	0%	26	0
	CRL S-3.7	S	4%	96%	25	1
	CRL S-3.8	R	100%	0%	26	0
Tetracycline, TET	CRL S-3.1	R	100%	0%	28	0
	CRL S-3.2	S	0%	100%	28	0
	CRL S-3.3	R	100%	0%	28	0
	CRL S-3.4	R	100%	0%	26	0
	CRL S-3.5	R	100%	0%	28	0
	CRL S-3.6	R	100%	0%	28	0
	CRL S-3.7	S	4%	96%	27	1
	CRL S-3.8	S	4%	96%	27	1
Trimethoprim, TMP	CRL S-3.1	R	100%	0%	27	0
	CRL S-3.2	S	0%	100%	27	0
	CRL S-3.3	S	0%	100%	27	0
	CRL S-3.4	S	0%	100%	25	0
	CRL S-3.5	R	100%	0%	27	0
	CRL S-3.6	R	100%	0%	27	0
	CRL S-3.7	S	0%	100%	27	0
	CRL S-3.8	S	0%	100%	27	0

Campylobacter - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Chloramphenicol, CHL	CRL C-3.1	S	0%	100%	19	0
	CRL C-3.2	S	0%	100%	19	0
	CRL C-3.3	S	0%	100%	19	0
	CRL C-3.4	S	0%	100%	20	0
	CRL C-3.5	S	0%	100%	19	0
	CRL C-3.6	S	0%	100%	19	0
	CRL C-3.7	S	0%	100%	20	0
	CRL C-3.8	S	0%	100%	19	0
Ciprofloxacin, CIP	CRL C-3.1	S	4%	96%	24	1
	CRL C-3.2	S	0%	100%	25	0
	CRL C-3.3	R	100%	0%	25	0
	CRL C-3.4	R	100%	0%	26	0
	CRL C-3.5	S	0%	100%	25	0
	CRL C-3.6	R	92%	8%	23	2
	CRL C-3.7	S	0%	100%	26	0
	CRL C-3.8	R	92%	8%	23	2
Erythromycin, ERY	CRL C-3.1	R	100%	0%	25	0
	CRL C-3.2	S	0%	100%	25	0
	CRL C-3.3	S	0%	100%	25	0
	CRL C-3.4	S	8%	92%	24	2
	CRL C-3.5	S	4%	96%	24	1
	CRL C-3.6	R	72%	28%	18	7
	CRL C-3.7	S	0%	100%	26	0
	CRL C-3.8	S	4%	96%	24	1
Gentamicin, GEN	CRL C-3.1	S	0%	100%	24	0
	CRL C-3.2	S	0%	100%	24	0
	CRL C-3.3	S	0%	100%	24	0
	CRL C-3.4	S	0%	100%	25	0
	CRL C-3.5	S	8%	92%	22	2
	CRL C-3.6	S	0%	100%	24	0
	CRL C-3.7	S	0%	100%	25	0
	CRL C-3.8	S	0%	100%	24	0
Nalidixic acid, NAL	CRL C-3.1	S	0%	100%	24	0
	CRL C-3.2	S	4%	96%	23	1
	CRL C-3.3	R	88%	13%	21	3
	CRL C-3.4	R	96%	4%	24	1
	CRL C-3.5	S	0%	100%	24	0
	CRL C-3.6	R	96%	4%	23	1
	CRL C-3.7	S	4%	96%	24	1
	CRL C-3.8	R	100%	0%	24	0
Streptomycin, STR	CRL C-3.1	S	0%	100%	23	0
	CRL C-3.2	S	0%	100%	23	0
	CRL C-3.3	R	96%	4%	22	1
	CRL C-3.4	R	100%	0%	24	0
	CRL C-3.5	S	0%	100%	23	0
	CRL C-3.6	S	4%	96%	22	1
	CRL C-3.7	R	96%	4%	23	1
	CRL C-3.8	S	0%	100%	23	0
Tetracycline, TET	CRL C-3.1	S	13%	87%	20	3
	CRL C-3.2	R	100%	0%	24	0
	CRL C-3.3	R	100%	0%	24	0
	CRL C-3.4	R	100%	0%	25	0
	CRL C-3.5	S	4%	96%	23	1
	CRL C-3.6	R	96%	4%	23	1
	CRL C-3.7	S	8%	92%	23	2
	CRL C-3.8	R	100%	0%	24	0

Deviations - Salmonella

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
1	CRL S-3.3	Ceftazidime, CAZ	S	1	R	<=0.5	MIC
2	CRL S-3.6	Gentamicin, GEN	S	2	R	4	MIC
4	CRL S-3.3	Ceftazidime, CAZ	S	1	R	<=0.5	ET
6	CRL S-3.1	Confirmed ESBL	No		Yes		MIC
	CRL S-3.4	Ampicillin,Â AMP	R	32	S	<=1	MIC
	CRL S-3.5	Confirmed ESBL	No		Yes		MIC
11	CRL S-3.2	Chloramphenicol, CHL	R	32	S	8	MIC
	CRL S-3.7	Streptomycin, STR	R	64	S	<=8	MIC
13	CRL S-3.7	Sulfamethoxazole, SMX	R	>1024	S	<=64	MIC
16	CRL S-3.3	Ceftazidime, CAZ	S	1	R	<=0.5	MIC
17	CRL S-3.1	Confirmed ESBL	No		Yes		MIC
	CRL S-3.3	Ceftazidime, CAZ	S	0.5	R	<=0.5	MIC
	CRL S-3.3	Confirmed ESBL	No		Yes		MIC
	CRL S-3.5	Confirmed ESBL	No		Yes		MIC
18	CRL S-3.1	Ciprofloxacin, CIP	S	30	R	0.25	DD
	CRL S-3.3	Ciprofloxacin, CIP	S	27	R	0.25	DD
	CRL S-3.4	Ciprofloxacin, CIP	S	29	R	0.25	DD
	CRL S-3.6	Ciprofloxacin, CIP	S	26	R	0.5	DD
20	CRL S-3.3	Ceftazidime, CAZ	S	0.5	R	<=0.5	MIC
22	CRL S-3.3	Ceftazidime, CAZ	S	1	R	<=0.5	MIC
	CRL S-3.3	Confirmed ESBL	No		Yes		MIC
23	CRL S-3.1	Ciprofloxacin, CIP	S	31	R	0.25	DD
	CRL S-3.1	Confirmed ESBL	No		Yes		DD
	CRL S-3.3	Ceftazidime, CAZ	S	25	R	<=0.5	DD
	CRL S-3.3	Ciprofloxacin, CIP	S	27	R	0.25	DD
	CRL S-3.3	Confirmed ESBL	No		Yes		DD
	CRL S-3.4	Ciprofloxacin, CIP	S	28	R	0.25	DD
	CRL S-3.5	Confirmed ESBL	No		Yes		DD
	CRL S-3.6	Ciprofloxacin, CIP	S	24	R	0.5	DD
	CRL S-3.6	Gentamicin, GEN	S	15	R	4	DD
26	CRL S-3.3	Ceftazidime, CAZ	S	1	R	<=0.5	MIC
29	CRL S-3.1	Ciprofloxacin, CIP	S	22	R	0.25	DD
	CRL S-3.2	Streptomycin, STR	R	13	S	<=8	DD
	CRL S-3.3	Ciprofloxacin, CIP	S	22	R	0.25	DD
	CRL S-3.4	Ciprofloxacin, CIP	S	22	R	0.25	DD
	CRL S-3.6	Streptomycin, STR	R	12	S	16	DD
	CRL S-3.7	Tetracycline, TET	R	15	S	<=2	DD
	CRL S-3.8	Tetracycline, TET	R	18	S	<=2	DD
37	CRL S-3.1	Ciprofloxacin, CIP	S	0.06	R	0.25	AGA
38	CRL S-3.1	Ciprofloxacin, CIP	S	>=21	R	0.25	DD
	CRL S-3.1	Confirmed ESBL	No		Yes		DD
	CRL S-3.2	Streptomycin, STR	R	<=11	S	<=8	DD
	CRL S-3.3	Ciprofloxacin, CIP	S	>=21	R	0.25	DD
	CRL S-3.3	Confirmed ESBL	No		Yes		DD
	CRL S-3.3	Streptomycin, STR	R	<=11	S	<=8	DD
	CRL S-3.4	Ciprofloxacin, CIP	S	>=21	R	0.25	DD
	CRL S-3.5	Confirmed ESBL	No		Yes		DD
	CRL S-3.6	Ciprofloxacin, CIP	S	>=21	R	0.5	DD
	CRL S-3.6	Streptomycin, STR	R	<=11	S	16	DD
39	CRL S-3.1	Ciprofloxacin, CIP	S	<0.125	R	0.25	AGA
	CRL S-3.1	Confirmed ESBL	No		Yes		AGA
	CRL S-3.3	Confirmed ESBL	No		Yes		AGA
	CRL S-3.5	Confirmed ESBL	No		Yes		AGA
	CRL S-3.6	Gentamicin, GEN	S	<4	R	4	AGA
	CRL S-3.6	Streptomycin, STR	R	>8,<128	S	16	AGA

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
40	CRL S-3.1	Ciprofloxacin, CIP	S	25	R	0.25	DD
	CRL S-3.1	Confirmed AmpC	Yes		No		DD
	CRL S-3.3	Cefotaxime, CTX	S	16	R	>4	DD
	CRL S-3.3	Ceftazidime, CAZ	S	21	R	<=0.5	DD
	CRL S-3.3	Ciprofloxacin, CIP	S	21	R	0.25	DD
	CRL S-3.3	Confirmed ESBL	No		Yes		DD
	CRL S-3.4	Ciprofloxacin, CIP	S	23	R	0.25	DD
	CRL S-3.5	Confirmed AmpC	Yes		No		DD
	CRL S-3.6	Ciprofloxacin, CIP	S	22	R	0.5	DD
	CRL S-3.7	Nalidixic acid, NAL	R	12	S	<=4	DD

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

Deviations - Campylobacter

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
4	CRL C-3.5	Gentamicin, GEN	R	1.5	S	0.5	ET
9	CRL C-3.1	Tetracycline, TET	R	4	S	2	MIC
11	CRL C-3.7	Tetracycline, TET	R	>16	S	0.5	MIC
12	CRL C-3.6	Erythromycin, ERY	S	<=0.5	R	>32	MIC
17	CRL C-3.3	Nalidixic acid, NAL	S	<=2	R	64	MIC
	CRL C-3.4	Erythromycin, ERY	R	8	S	16	MIC
	CRL C-3.4	Nalidixic acid, NAL	S	16	R	>64	MIC
20	CRL C-3.6	Erythromycin, ERY	S	<=0.5	R	>32	MIC
22	CRL C-3.6	Streptomycin, STR	R	4	S	<=1	MIC
23	CRL C-3.3	Nalidixic acid, NAL	S	18	R	64	DD
24	CRL C-3.1	Tetracycline, TET	R	4	S	2	MIC
	CRL C-3.4	Erythromycin, ERY	R	8	S	16	MIC
	CRL C-3.6	Erythromycin, ERY	S	<=0.5	R	>32	MIC
25	CRL C-3.1	Tetracycline, TET	R	4	S	2	MIC
32	CRL C-3.6	Ciprofloxacin, CIP	S	<=0.125	R	>4	MIC
	CRL C-3.6	Erythromycin, ERY	S	<=0.25	R	>32	MIC
	CRL C-3.6	Nalidixic acid, NAL	S	<=4	R	>64	MIC
33	CRL C-3.6	Erythromycin, ERY	S	<=0.5	R	>32	MIC
34	CRL C-3.6	Erythromycin, ERY	S	1	R	>32	MIC
38	CRL C-3.8	Ciprofloxacin, CIP	S	11.8 mm	R	>4	DD
40	CRL C-3.1	Ciprofloxacin, CIP	R	15	S	0.25	DD
	CRL C-3.2	Nalidixic acid, NAL	R	12	S	8	DD
	CRL C-3.3	Nalidixic acid, NAL	S	17	R	64	DD
	CRL C-3.3	Streptomycin, STR	S	23	R	>16	DD
	CRL C-3.5	Erythromycin, ERY	R	15	S	1	DD
	CRL C-3.5	Gentamicin, GEN	R	13	S	0.5	DD
	CRL C-3.5	Tetracycline, TET	R	12	S	0.5	DD
	CRL C-3.6	Ciprofloxacin, CIP	S	27	R	>4	DD
	CRL C-3.6	Erythromycin, ERY	S	29	R	>32	DD
	CRL C-3.6	Tetracycline, TET	S	22	R	>16	DD
	CRL C-3.7	Nalidixic acid, NAL	R	17	S	8	DD
	CRL C-3.7	Streptomycin, STR	S	21	R	>16	DD
	CRL C-3.7	Tetracycline, TET	R	11	S	0.5	DD
	CRL C-3.8	Ciprofloxacin, CIP	S	17	R	>4	DD
	CRL C-3.8	Erythromycin, ERY	R	13	S	2	DD

DD Disk diffusion
ET E-test
MIC Microbroth dilution

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