

## Results from the WHO GSS EQAS 2003

### - The External Quality Assurance System of the WHO Global *Salmonella* Surveillance and Laboratory Support Project

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#### Abstract

An international external quality assurance program on serotyping and antimicrobial susceptibility testing of eight *Salmonella enterica* strains is performed yearly to enhance the capacity of national and regional reference laboratories in WHO Global Salm-Surv. In 2003, a total of 152 laboratories from 78 countries participated. For testing of *Salmonella* strains, 81 % of the serotypings and 95 % of the susceptibility tests performed were correct. Quality control (QC) of susceptibility testing was performed with *E. coli* ATCC 25922 by 93 % of the laboratories. For 45 % of the laboratories, one or more of the QC results were outside the QC ranges given by the CLSI, indicating inadequate standardization of the methods used and a further need for internal QC and for taking part in quality assurance programs.

In 2003, identification of two thermophilic *Campylobacter* strains to species level and typing of one blinded bacterial strain, *E. coli* serotype O157, was included. For *Campylobacter*, a total of 97 laboratories participated and 85 % of the identifications were correct. For the blinded strain, a total of 115 laboratories participated and 99 % made correct species identification. Only four laboratories performed further typing on the strain, in all cases correct serotype.

In order to identify the barriers for serotyping, the level of difficulty in *Salmonella* serotyping was considerably higher in 2003 than in previous years. Number one barrier for serotyping was identified as lack of antisera. Misinterpretation of the Kaufmann-White serotyping scheme and of *Salmonella* taxonomy also played a role. The proportion of incorrect results in serotyping indicates a further need for the training provided by WHO GSS, and a further need for facilitating the availability of high-quality *Salmonella* antisera at low costs.

#### Introduction

In January 2000, WHO launched an international *Salmonella* surveillance and laboratory support project, the "WHO Global Salm-Surv" in order to enhance the member countries capacity to detect and respond to *Salmonella* problems, as well as to improve global surveillance of *Salmonella*. Today the WHO Global Salm-Surv embrace other important foodborne pathogens than *Salmonella*, especially *Campylobacter*, which also has become a problem of great concern in different parts of the world.

*Salmonella* and *Campylobacter* are among the most important foodborne pathogens worldwide, leading to millions of cases of diarrhoeal illness each year in developing as well as industrialized countries. Furthermore, there is a growing concern for the increasing resistance to antimicrobial

therapies in *Salmonella*. Infections with resistant *Salmonella* and *Campylobacter* are associated with increased morbidity and mortality.

To support laboratories participating in WHO Global Salm-Surv, an External Quality Assurance System (EQAS) was established in 2000. The EQAS supports the assessment of the quality of serotyping and antimicrobial susceptibility testing of *Salmonella* in participating laboratories. In 2003, the program was extended to include other foodborne pathogens as well, and the number of participants has increased from 44 laboratories in 2000, to 152 laboratories in 2003.

The EQAS is organized yearly by the Danish Institute for Food and Veterinary Research (DFVF) in collaboration with Centers for Disease Control and Prevention (CDC) in Atlanta, WHO in Geneva, and with Institute Pasteur in Paris.

## Materials and methods

The EQAS was announced at the WHO Global Salm-Surv list server, and all interested laboratories were encouraged to sign up. A total of 178 laboratories were enrolled. Participation is free of charge except for each laboratories own expense for the analysis.

Bacterial strains were selected and tested by DFVF, followed by verification of serotypes and resistance patterns at Institute Pasteur and CDC, respectively.

Bacterial strains were shipped according to the IATA regulations for shipping of Dangerous Goods classified as "UN2824 Infectious substances, affecting humans", as lyophilized or stab cultures. Test form and questionnaires for evaluation purpose and for general information were enclosed.

Laboratories were requested to subculture strains as soon as possible, to keep the strains stored at refrigerator temperature, and to enter test results within 2 months through a password protected Web database at the WHO Global Salm-Surv homepage.

The laboratories were requested to use the testing methods routinely used at their laboratory. The testing included serotyping and susceptibility testing of eight *Salmonella* strains, susceptibility testing of one quality control strain (*E. coli* ATCC 25922), identification to species level of two thermophilic *Campylobacter* strains, and identification/typing of one blinded sample (a non-toxin producing strain of *E. coli* O157).

The *Salmonella* strains represented different serogroups (Table 1) and antimicrobial susceptibility patterns (Table 4). The strains were tested to as many as possible of the following antimicrobials: Ampicillin (Amp), chloramphenicol (Chl), ciprofloxacin (Cip), gentamicin (Gen), kanamycin (Kan), nalidixic acid (Nal), streptomycin (Str), sulphonamide (Su), tetracycline (Tet), trimethoprim (Tmp) and the combination of trimethoprim/sulphonamide (T/S).

Immediately after data entry, an individual evaluation report with comments on deviating results was displayed on the screen. If a participant was not able to enter the results, it was done by DFVF.

## Results

### Participants

A total of 152 of 178 enrolled laboratories (85 %) submitted their test results. Bacterial strains were sent to 21 of the laboratories not submitting any results.

The 152 participating laboratories represented 78 countries: Albania, Argentina, Australia, Barbados, Bolivia, Bosnia and Herzegovina, Botswana, Brazil, Bulgaria, Cambodia, Cameroon, Canada, Central African Republic, Chile, China, Colombia, Costa Rica, Croatia, Cyprus, Czech, Denmark, Estonia, Finland, France, Gabon, Georgia, Germany, Greece, Honduras, Hungary, Iceland, India, Indonesia, Ireland, Israel, Italy, Ivory Coast, Jamaica, Japan, Jordan, Korea, Kuwait, Latvia, Lebanon, Lithuania, Madagascar, Malaysia, Malta, Morocco, Mauritius, Moldova, New Caledonia, New Zealand, Norway, Oman, Paraguay, Peru, Philippines, Poland, Romania, Scotland, Senegal, Serbia and Montenegro, Singapore, Slovakia, Slovenia, South Africa, Spain, Sri Lanka, Taiwan, Thailand, Trinidad and Tobago, Tunisia, Turkey, Uruguay, USA, Venezuela and Vietnam.

Of the 152 participating laboratories, 104 (68 %) also participated in the previous year, 2002.

### Serotyping

A total of 125 laboratories (82 %) performed at least one serotyping, and 10 laboratories (15 %) performed serogrouping or incomplete typing with no specification of the serovar. Of the 125 serotyping laboratories, 66 laboratories (53 %) serotyped all eight strains.

Of 840 serotyping results, 678 results (81 %) were correct. Table 1 presents the serotyping results for each strain with all deviations listed. Number of deviations ranges from 11 % for *Salmonella* Montevideo to 32 % and 35 % for *Salmonella* Vinohrady and *Salmonella* Cerro, respectively.

Table 2 shows the number of laboratories with respectively 0, 1, 2,...,8 correct serotypings in 2003 compared to previous years. Of 125 laboratories serotyping, 32 labs (26 %) correctly serotyped all strains, and further 33 laboratories (26 %) had six or seven strains correctly serotyped. The number of laboratories correctly identifying all eight serotypes increased significantly from 2001 to 2002 ( $p=0.0437$ ), but was followed by a significant decrease from 2002 to 2003 ( $p<0.0001$ )\*. Subsequently, a significant increase in number of laboratories with 5 ( $p=0.0002$ ), 4 ( $p=0.0051$ ) and 3 ( $p=0.0458$ ) correctly typed serotypes was seen in 2003 compared to 2002.

*S. Paratyphi* B (strain 4.3) was correctly serotyped by 88 laboratories, and the variety Java (*d*-tartrate positive *S. Paratyphi* B) was reported by 37 of these (42 %). In cases where only the serovar (*Paratyphi* B) was reported, results were not recorded as deviations.

\* Logistic regression analysis was performed. Dependent variable was defined as number of laboratories with certain number of correct answers out of total number of participating laboratories. Independent variable was an interaction term between number of correct answers and year of participation.

**Table 1.** List of *Salmonella* serotypes sent to the participants and number and list of deviations.

Strain	Correct serotype		No. of labs serotyping	% deviations	Deviating results (frequency indicated in bracket if more than once)
WHO 4.1	Montevideo	6,7,14:gm[p]s:[1,2,7]	119	10.9	Menston (3), Montevideo II, Oakland, Oranienburg, Riggil, Rissen, Sanjuan, Schwarzengrund, S. enterica subsp salamae, Salmonella II, II
WHO 4.2	Schwarzengrund	1,4,12,27:d:1,7	120	14.2	Stanley (4), Ayinde (2), Ahmadi, Duisburg, Eppendorf, Kambala, Brezany, Typhimurium, Makumira, Mons, Montevideo, Sarajane, Southampton
WHO 4.3	Paratyphi B d-tartrate positive (variety Java)	1,4,[5],12:b:1,2 d-tartrate positive	116	21.6	Abony (4), Typhimurium (3), Derby (2), Saintpaul (2), Schleissheim (2), Uppsala (2), Brandenburg, Chartres II, Fortune, Hato, San Diego, Lagos, Agona, Wagania, Indiana, Onarimon
WHO 4.4	Panama	1,9,12:lv:1,5	120	13.3	Javiana (2), Lawndale (2), Dublin, Enteritidis, London var 15+, Paratyphi C, Victoria, Goettingen, Sarajane, Panamá/Kapemba, Kapemba, Irumu, Italiana, Itami/Javiana
WHO 4.5	Cerro	6,14,18:z4z23:[1,5]	90	34.4	Aarhus (7), Bousso (5), IV (3), Arapahoe, Virchow, Usumbura, Tallahassee, Siegburg, Memphis, Corvallis, Chichiri, Chailey, Cerro/Aarhus, Arizonae IIIa, Blukwa, IIIa, S. Enterica subsp. Salamae
WHO 4.6	Havana	1,13,23:fg[s]: -	96	18.8	Raus (6), Agbeni, Worthington, Tschangu, Tees Rissen, Berta, Bron, Chagoua, NewYork/Okatie, NewYork, Okatie, Poona
WHO 4.7	Vinohrady	28:mt:[enz15]	75	30.7	Abadina (4), Morillons (4), Croft (2), S. enterica subsp salamae (2), Hatfield, Tennessee, Techimani, Southbank, Othmarschen, Panama, Pomona, Nitra, I
WHO 4.8	Singapore	6,7:k:enx	112	13.4	Thompson (5), Braenderup, Escanaba, Kastrup, Ljubljana, Norwich, Paratyphi C, Rissen, Singapore/Escanaba, II, I,

**Table 2.** Number of correct serotypings in relation to number of laboratories, EQAS 2003 compared to previous years.

Number of correct serotypes	EQAS 2001		EQAS 2002		EQAS 2003	
	No of labs		No of labs		No of labs	
	n	%	n	%	N	%
8	32	37	50	52	32	26
7	13	15	17	18	15	12
6	9	10	14	14	18	14
5	10	11	3	3	23	18
4	4	5	2	2	14	11
3	7	8	3	3	12	10
2	4	5	6	6	3	2
1	4	5	1	1	5	4
0	4	5	1	1	3	2
In total	87	100 %	97	100 %	125	100 %

### Antimicrobial susceptibility testing

A total of 151 laboratories reported their susceptibility data. Of these, 136 laboratories performed disk diffusion and 15 laboratories performed MIC-determinations. Two laboratories reported both methods. Results of the method used for diagnostic purpose at the laboratories are included here.

The results of antimicrobial susceptibility testing of eight *Salmonella* strains were categorised as resistant (R), intermediate (I) or susceptible (S) according to the breakpoints normally used in the laboratories. The expected resistance patterns for the strains are listed in Table 3. The results (percentage of R/I/S) for each strain and antimicrobial are presented in Table 4, where figures in bold indicate the expected interpretation, and grey cells indicate where < 90 % of the results hit correct interpretation.

**Table 3.** Expected resistance for the *Salmonella* strains EQAS 2003.

Strain	Expected resistance	Strain	Expected resistance
WHO 4.1	Str <sup>IS</sup> Su <sup>R</sup> Tet <sup>R</sup> Tmp <sup>R</sup> T/S <sup>R</sup>	WHO 4.5	Sensitive to all tested antimicrobials
WHO 4.2	Str <sup>R</sup> Su <sup>R</sup> Tet <sup>R</sup> Tmp <sup>R</sup> T/S <sup>R</sup>	WHO 4.6	Sensitive to all tested antimicrobials
WHO 4.3	Amp <sup>R</sup> Nal <sup>R</sup> Str <sup>I</sup> Su <sup>R</sup> Tmp <sup>R</sup> T/S <sup>R</sup>	WHO 4.7	Sensitive to all tested antimicrobials
WHO 4.4	Amp <sup>R</sup> Chl <sup>R</sup> Gen <sup>RI</sup> Kan <sup>R</sup> Nal <sup>R</sup> Str <sup>R</sup> Su <sup>R</sup> Tet <sup>R</sup> Tmp <sup>R</sup> T/S <sup>R</sup>	WHO 4.8	Chl <sup>R</sup> Kan <sup>R</sup> Su <sup>R</sup> Tet <sup>R</sup> Tmp <sup>R</sup> T/S <sup>R</sup>

**Table 4.** Susceptibility test results (% R/I/S) of the *Salmonella* strains in 151 laboratories.

Strain	Amp	Chl	Cip	Gen	Kan	Nal	Str	Su	Tet	Tmp	T/S
4.1	2/3/95	0/1/99	0/0/100	1/0/99	2/6/92	1/6/93	35/42/23	100/0/0	97/0/3	100/0/0	97/0/3
4.2	2/3/94	1/1/98	0/0/100	1/0/99	3/3/94	1/4/94	91/5/4	98/1/1	98/1/1	99/0/1	97/1/2
4.3	99/0/1	1/1/98	1/5/94	2/1/97	2/3/95	99/0/1	72/21/6	99/0/1	6/14/80	99/0/1	99/0/1
4.4	77/0/23	79/0/21	1/1/98	64/9/27	80/1/19	99/0/1	83/6/12	84/1/15	79/6/16	83/0/17	77/0/23
4.5	1/2/97	1/0/99	0/0/100	1/0/99	2/5/93	2/1/98	2/15/83	6/2/92	4/8/89	0/0/100	2/1/98
4.6	0/3/97	0/1/99	0/0/100	1/1/97	3/4/93	1/3/96	3/19/79	8/3/89	6/11/83	0/0/100	2/2/97
4.7	3/4/93	1/1/97	0/0/100	2/1/97	3/7/90	1/5/94	6/22/72	8/6/87	7/15/78	1/0/99	2/2/96
4.8	2/3/94	99/1/1	0/0/100	1/1/97	98/0/2	1/4/96	10/31/59	99/0/1	99/1/1	100/0/0	100/0/0

Numbers in bold: % with expected interpretation. Grey cell: < 90 % of results hit correct interpretation

As seen in Table 4, it is reasonable to believe that the multiple resistant strain *S. Panama* (strain 4.4) has lost most of the resistance due to transport or storage stress. The loss of resistance was reported from numerous participants from different parts of the world and could not be attributed to a specific batch of strains or to a specific batch of shipping. The resistance markers in question are known often to be located on large plasmids, which can be lost due to environmental stress such as prolonged transportation, improperly storage conditions or repeated subcultivating. It was therefore decided not to include the susceptibility testing results of the strain in this report, except for Table 5 where data for 2003 is presented with and without the strain in order to visualize the bias caused by

the strain. If including strain 4.4, the apparent loss of resistance would cause 254 extra deviations and constitute 34 % of the total number of deviations!

In total 9,473 antimicrobial susceptibility tests were performed (Table 5). Of these, 94.7 % (8,969) were in agreement with the expected results, 3.5 % were minor deviations and 1.8 % were major deviations. Results were regarded as deviations if they were incorrectly interpreted as resistant, intermediate or sensitive. I-S or I-R deviations were called minor deviations, while S-R or R-S deviations were called major.

The percentage of correct results, and the percentage of minor and major deviations in 2003 compared to previous years are presented in Table 5. Compared to 2002, a significant higher proportion of susceptibility tests were correct ( $p=0.0000$ ), as well as a significant reduction in the proportion of minor ( $p=0.0000$ ) and major ( $p=0.0015$ ) mistakes was observed in 2003. Percentage of correct results for the individual antimicrobial is presented in Table 6 where also percentage of major deviations is shown.

**Table 5.** Susceptibility testing results from 2000 to 2003

Year	All testings performed	Percentage correct results	Percentage minor deviations (S-I or I-R switch)	Percentage major deviations (R-S switch)
2000	3,151	91.7	4.5	3.8
2001	7,409	91.2	5.8	3.0
2002	8,554	91.2	6.4	2.5
2003	10,827	93.0	3.3	3.7
2003*	9,473	94.7	3.5	1.8

\* Excluding strain 4.4 which may have lost its resistance due to transport or storage stress

**Table 6.** Number of tests performed and percentage of major deviations for each antimicrobial.

Anti-microbial	EQAS 2001		EQAS 2002		EQAS 2003*	
	Total no. of determinations	% major deviations	Total no. of determinations	% major deviations	Total no. of determinations	% major deviations
Amp	793	4.0	918	2.9	1,005	1.6
Chl	785	1.8	911	1.8	982	0.7
Cip	784	0.6	911	0.5	981	0.4
Gen	792	1.1	905	2.8	979	1.6
Kan	595	2.0	680	1.5	732	2.3
Nal	697	1.4	893	2.1	933	1.1
Str	643	7.0	734	4.2	761	4.3
Su	412	4.4	503	3.6	615	3.6
Tet	775	6.7	869	3.3	981	4.0
Tmp	398	1.5	507	3.0	582	0.5
T/S	728	2.1	731	2.3	922	0.5

\* Excluding strain 4.4 which may have lost its resistance due to transport or storage stress

Major deviations can be further divided into very major (measuring sensitive when resistant) or just major deviations (measuring resistant when sensitive). All in all 30 laboratories (20 %) had no deviations at all (excl. strain 4.4). A total of 332 minor deviations, 134 major deviations and 38 very major deviations were observed (excl. strain 4.4). Of the 121 labs having deviations, 48 labs had only minor deviations and 25 labs had very major deviations. Five laboratories were responsible for 72 of the 169 major and very major deviations.

If testing is correctly standardized and performed in accordance to the guidelines given by the CLSI, the results for the *E. coli* ATCC 25922 quality control strain are supposed to be inside the quality control (QC) ranges given by CLSI.

Of 151 laboratories performing susceptibility testing, 141 laboratories (93.4%) reported QC data. In 63 of these laboratories (45 %), all results for the *E. coli* QC strain were correct. Of these, 32 laboratories tested the strain to all antimicrobials. For the remaining laboratories a mean of 2.4 tests were out of range. A total of 1,320 tests for QC were performed, and of these, 14 % (187) were outside QC range. QC range and number of laboratories outside range compared to previous years are shown in Table 7.

**Table 7.** Results outside the QC range given by CLSI for *E. coli* ATCC 25922.

Anti-microbial	QC range <sup>1</sup> <i>E. coli</i> ATCC 25922		Laboratories <u>outside</u> QC range		
	MIC (ug/ml)	Disks (mm)	EQAS 2001	EQAS 2002	EQAS 2003
			% of labs (N) <sup>3</sup>	% of labs (N) <sup>3</sup>	% of labs (N) <sup>3</sup>
<b>Amp</b>	2-8	16-22	19 (97)	16 (109)	14 (140)
<b>Chl</b>	2-8	21-27	20 (97)	15 (107)	22 (137)
<b>Cip</b>	.004-.016	30-40	14 (97)	14 (108)	9 (138)
<b>Gen</b>	0.25-1	19-26	12 (99)	12 (108)	9 (138)
<b>Kan</b>	1-4	17-25	14 (87)	11 (79)	12 (103)
<b>Nal</b>	1-4	22-28	14 (74)	14 (102)	16 (132)
<b>Str</b>	4-16 <sup>2</sup>	12-20	12 (81)	11 (82)	9 (105)
<b>Su</b>	8-32	15-23	34 (53)	26 (57)	17 (82)
<b>Tet</b>	0.5-2	18-25	22 (96)	13 (102)	19 (137)
<b>Tmp</b>	0.5-2	21-28	22 (50)	11 (66)	14 (79)
<b>T/S</b>	≤0.5/9.5	23-29	14 (90)	12 (102)	14 (129)

<sup>1</sup> NCCLS standard, Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing; 12th Informational suppl. NCCLS document M100-S12, Wayne, Pennsylvania.

<sup>2</sup> QC range developed by the manufacturer of Sensititre®

<sup>3</sup> The number of laboratories performing the test

### **Identification of *Campylobacter* and blank sample**

A total of 97 laboratories (64 %) submitted results on *Campylobacter* identification (one strain of *C. jejuni* and one strain of *C. coli*). All in all 85 % of the species identifications were correct.

*C. jejuni* was successfully recovered by 92 laboratories. Eighty laboratories (87 %) performed correct species identification. Deviating results were *C. coli* (9 laboratories) and *C. lari* (3 laboratories). *C. coli* was also successfully recovered by 92 laboratories. Seventy-seven laboratories (83 %) performed correct species identification. Deviating results were *C. jejuni* (7 laboratories), *C. lari* (4 laboratories) and *C. upsaliensis* (4 laboratories).

A total of 115 laboratories (76 %) submitted results on identification of the blinded bacterial sample, *E. coli* serotype O157. Only one deviating result was reported (*Pseudomonas putida*). Further typing was reported by four laboratories, in all cases with indication of correct serotype.

### **Evaluation of the EQAS by participating laboratories**

The evaluation of the EQAS program was based on a response of 96 laboratories to a questionnaire. Written materials (announcement, welcoming letter, reporting form and individual evaluation reports) were evaluated as very good (48 %), good (14 %), satisfactory (12 %), poor or very poor (0%). Organisation of the EQAS, information describing EQAS and fulfilment of expectations for the participants were evaluated as very good (40 %), good (51 %), satisfactory (9 %), poor or very poor (0 %). The interactive web database was evaluated as very good (40 %), good (41 %), satisfactory (17 %), poor (2 %) and very poor (0 %). In addition, 26 % of the laboratories found it important and 74 % found it very important to participate in the EQAS. None of the laboratories found it irrelevant or not important.

### **Discussion**

In order to identify the barriers for serotyping, the level of difficulty in serotyping was considerably increased in 2003 compared to the previous year. Thus, an extended spectrum of antisera and performance of additional biochemical testing was needed to perform complete serotyping for some of the strains. For example, *S. Cerro* (strain 4.5) is readily mistaken for *S. Aarhus*, because they only differ in phase 2 H-antigen, and *S. Vinohrady* (strain 4.7) belong to a very rare serogroup (O:28). Furthermore, for *S. Paratyphi B* (strain 4.3) the variety Java can only be determined by additional biochemical testing, as an extra challenge.

The increased level of difficulty was obviously reflected by the fact that only 26 % of the laboratories were able to serotype all eight *Salmonella* correctly, compared to 52 % in 2002. The high number of deviations for strain 4.5 and 4.7, the fact that fewer results were submitted for these strains (Table 1), together with comments from many of the participants when submitting results strongly suggests that many of the laboratories simply lack the antisera needed for complete serotyping.

The apparent improvement in global capacity to serotype observed in 2002, where 90 % of all serotyping results were correct compared to 80 % in 2001, was mainly believed to be a consequence of providing participants in WHO Global Salm-Surv training courses with small amounts of high-quality antisera. In 2003, the percentage of correct serotypings was back at the same level as



in 2001. When considering lack of antisera as the number one barrier for serotyping, the observed decrease in 2003 could be expected in light of the large number of participants from resource-limited countries of which only 45 % has attended any WHO Global Salm-Surv training course. In addition, only 50 % of the participants have reported that they serve as a national reference laboratory for *Salmonella*, and so could be expected to have the antisera - and the skills - for serotyping.

Also basic understanding of the Kaufmann-White serotyping scheme, and of *Salmonella* taxonomy appears to play a role, as seen by some of the deviations listed in Table 1.

For antimicrobial susceptibility testing there was a significant improvement compared to previous years. A total of 94,7 % of all testings was correct, and the percentage of both minor and major deviations has declined significantly (Table 5). Still, a total of 80 % of the laboratories had at least one deviation, and a total of 48 % of the laboratories had major or very major deviations (R-S switches). A few laboratories were responsible for almost half of the major and very major deviations.

As last year, deviations were especially frequent for testing of the aminoglycosides streptomycin and kanamycin, and for tetracycline and sulphonamides. Testing of these antimicrobials is highly influenced by variations in media conditions such as cationic concentration, acidity and agar depth. Also misreading of sulphonamide- and trimethoprim results because of the delayed bacterial response to these antimicrobials may have influenced the outcome.

When performing antimicrobial susceptibility testing, it is extremely important to include reference strains for internal quality control (QC). The QC results revealed that 14 % of the performed tests with the *E. coli* QC strain were outside the QC range given by CLSI indicating that the methods were not adequately standardized for more than half (55 %) of the participating laboratories. This also goes in line with the fact, that almost half of the laboratories had important deviations.

These results indicate that number one barrier for antimicrobial susceptibility testing is inadequate standardization of methods, but also use of expired disks, improper storage or repeated subculturing of strains with loss of resistance genes, are plausible causes of incorrect testing. The reason for leaving out results for strain 4.4 is mentioned earlier in this report.

In conclusion, the results indicate a strong need for antisera at high quality and affordable prices, a further need for training the skills in *Salmonella* serotyping and finally a further need for strengthen the awareness of performing internal QC to identify the barriers for antimicrobial susceptibility testing in each individual laboratory and to learn how to intervene if results are out of control.

We were pleased to experience that many of the laboratories were able to participate on the new part of the EQAS (identification of *Campylobacter* and one blinded sample) and that the results revealed good skills in especially *E. coli* identification. It is possible that some of the laboratories with incorrect *Campylobacter* identifications, accidentally made a switch, since they reported just the opposite than expected for the two strains. Only a few laboratories performed further typing of the *E. coli* strain. It is likely that the rest of the laboratories were not aware of this additional testing.