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THE EXTERNAL QUALITY ASSURANCE SYSTEM OF THE WHO GLOBAL SALM-SURV.

YEAR 2006

ISBN: 978-87-92158-43-7

1. Introduction

In January 2000, WHO launched an international *Salmonella* surveillance and laboratory support project, the "WHO Global Salm-Surv" (WHO GSS) 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 GSS embraces 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 GSS 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 153 laboratories in 2006.
The EQAS is organized yearly by the National Food Institute (FOOD-DTU) in collaboration with Centers for Disease Control and Prevention (CDC) in Atlanta, USA; World Health Organization (WHO) in Geneva, Switzerland; and with Institute Pasteur (IP) in Paris, France.

The objective is to monitor the quality of the *Salmonella* serotyping and the antimicrobial susceptibility data produced and pin point areas which need attention in order to produce reliable data. The goal is having all laboratories perform *Salmonella* serotyping with a maximum of three errors (38 %) and susceptibility testing within the range of either of the following: a maximum of 5 % very major / major and 5 % minor errors, or a maximum of 10 % minor errors.

2. Materials and Methods

2.1 Participants

An invitation to participate in the External Quality Assurance System (EQAS) on serotyping and susceptibility testing of *Salmonella* and identification of *Campylobacter* and an unknown foodborne pathogen was announced through the WHO GSS list server in early spring 2006. Participation was free of charge but each laboratory was expected to cover expenses associated with their own analysis.

2.2 Strains

Eight strains of *Salmonella*, two strains of *Campylobacter* and an unknown foodborne pathogen (*Yersinia enterocolitica* O3) were selected for this trial among isolates from the National Food Institute's strain collection. Individual sets of the *Salmonella* and *Y. enterocolitica* strains were inoculated as agar stab cultures and the *Campylobacter* strains were lyophilised in glass vials. The serotype of each *Salmonella* strain was verified by the CDC and IP prior to distribution. In addition CDC verified the susceptibility patterns of the *Salmonella* strains. Furthermore, laboratories were provided with a lyophilised international reference strain for susceptibility testing; *E. coli* CCM 3954 ~ ATCC 25922 purchased at the Czech Collection of Micro-organisms (CCM); The Czech Republic.

2.3 Serotyping

Prior to the survey, each of the *Salmonella* strains was serotyped at the National Food Institute using antisera purchased from the Statens Serum Institute (SSI). Serotype was designated on the basis of O (somatic) and phase 1 and phase 2 H (flagellar) antigens according to scheme of Kaufmann-White (2001). For the purposes of this survey, the

serotype designation obtained by the National Food Institute was considered the “reference” or “intended response”.

2.4 Antimicrobials

Antimicrobial susceptibility testing (AST) on the *Salmonella* strains were performed at the National Food Institute and the obtained MIC values served as a reference standard. The following antimicrobials were used in the trial: ampicillin, AMP; amoxicillin + clavulanic acid, AUG; cefotaxime, CTX; cefpodoxime, POD; ceftazidime, CAZ; ceftiofur, XNL; chloramphenicol, CHL; ciprofloxacin, CIP / enrofloxacin, ENRO; gentamicin, GEN; nalidixic acid, NAL; streptomycin, STR; sulphonamides, SMX; tetracycline, TET; trimethoprim, TMP and trimethoprim + sulphonamides, SXT (App. 1).

MIC determination was performed using Sensititre systems from Trek diagnostics Ltd with the exception of cefotaxime, ceftazidime, and trimethoprim + sulphonamides. These exceptions were tested using E-test from AB-Biodisk. Guidelines and breakpoints were according to the Clinical and Laboratory Standards Institute (CLSI) document M07-A7 (2007) “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically”; Approved Standard - Seventh Edition, document M100-S16 (2006) “Performance Standards for Antimicrobial Susceptibility Testing”; Seventeenth Informational Supplement and document M31-A2 (2002) “Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated from Animals”; Approved Standard - Second Edition. Exceptions were the following antimicrobials where epidemiological cut-off values were used: ciprofloxacin, gentamicin (according to www.eucast.org) and streptomycin (according to FOOD-DTU) (App.2).

2.5 Distribution

The cultures and documents (App. 3a,b,c,d,e) downloaded to a diskette were enclosed in double pack containers (class UN 6,2) and sent to the selected laboratories according to the International Air Transport Association (IATA) regulations as “Biological Substance category B” classified UN3373. Prior to shipping each laboratory was informed about the dispatched parcels and the air way bill (AWB) number for tracking of the parcel and pick up at the airport. Import permit was necessary for shipping the parcels to a large number of countries.

2.6 Procedure

The laboratories were instructed to follow the protocol and subculture the strains prior to performing the method that was routinely used by their laboratory. The testing included serotyping and susceptibility testing of eight *Salmonella* strains, susceptibility testing of one quality control strain (*E. coli* CCM 3954 / ATCC 25922), identification of two *Campylobacter* strains and an unknown foodborne pathogen (*Yersinia enterocolitica* O3). Furthermore, labs were requested to save and maintain the ATCC reference strains for future proficiency tests according to App. 3d.

After completion of the tests, the laboratories were requested to enter the obtained results (identification of the *Campylobacter* and unknown sample, the serotype and / or serogroup, MIC values or zone-diameter in millimetres and the susceptibility categories of the *Salmonella* strains) into an electronic record sheet in the WHO GSS web based database through a secured individual login or alternatively send the record sheets from the enclosed protocol by fax to FOOD-DTU.

The *Salmonella* strains were categorised as resistant, intermediate or susceptible against the tested antimicrobials. All antimicrobials used should be interpreted individually even cephalosporins which normally are interpreted according to Approved Standard - Seventh Edition, document M100-S16 (2006) “Performance Standards for Antimicrobial Susceptibility Testing, Table 2A”. Laboratories were instructed to use the same antimicrobials and *Salmonella* antisera used in their daily routine methods. In addition, they were instructed to use the their own standard breakpoints for categorising the susceptibility data obtained.. All laboratories entered either the zone diameter or MIC value for the *E. coli* (ATCC 25922) reference strain. After submitting the data the laboratories were instructed to retrieve an instant generated individual report from the secured web site evaluating the submitted results. All deviations from the expected were reported along with suggestions of how to either solve or investigate the problem. Deviations of the antimicrobial susceptibility results were categorised as minor, major or very major. Minor deviations are defined as an intermediate result that was determined as susceptible, resistant or vice versa (i.e. I $\leftrightarrow$ S or I $\leftrightarrow$R). When a susceptible strain was classified as resistant it was regarded as a major deviation (i.e. S $\rightarrow$ R). When a resistant strain was classified as susceptible it was regarded as a very major deviation (i.e. R $\rightarrow$ S).

3. Results

A total of 167 laboratories in 83 countries responded, and were enrolled in the EQAS. When the deadline for submitting results was reached, 153 laboratories in 75 countries had uploaded data. The following countries provided data (also shown below in Figure 1):
Albania, Argentina, Australia, Barbados, Bolivia, Bosnia and Herzegovina, Brazil, Bulgaria, Cambodia, Cameroun, Canada, Central Africa Republic, Chile, China, Colombia, Costa Rica, Cote d'Ivoire, Croatia, Cyprus, Czech Republic, Democratic Republic of Congo, Denmark, Ecuador, Egypt, Estonia, Finland, France, Greece, Guatemala, Hungary, Iceland, India, Italy, Jamaica, Japan, Jordan, Korea, Kuwait, Lithuania, Macedonia, Madagascar, Malaysia, Malta, Mauritania, Mauritius, Mexico, Moldova, Morocco, New Zealand, Nigeria, North America (Colorado, Georgia, Idaho, Iowa, Maryland, Nevada and Vermont), Oman, Paraguay, Peru, Philippines, Poland, Russia, Serbia and Montenegro, Senegal, Scotland, Slovak Republic, Slovenia, Sri Lanka, South Africa, Sudan, Suriname, Thailand, Trinidad and Tobago, Tunisia, Turkey, Ukraine, Uruguay, Venezuela and Vietnam.

Figure 1. Participating countries.

In the description of results, arbitrary thresholds of quality limits have not been used. The susceptibility results are expressed purely as correct, minor, major or very major deviations as described above.

3.1 Methods used by EQAS-participants

The participating laboratories all used their routine methods for performing serotyping and AST.

Of the 153 laboratories responding, 131 (85.6 %) participated in some or the entire serotyping component of the program.

Of the 153 laboratories responding, 133 (86.9 %) submitted antimicrobial susceptibility results. 113 laboratories used disk diffusion, and 20 laboratories used MIC determination.

No specific information was given beforehand to the participants regarding either reference breakpoints used or breakpoint guidelines for interpretation of the AST results. In addition, the participants were not informed how to interpret the resistance of cephalosporins.

Of the 153 laboratories responding, 95 (62.1 %) performed identification of the two *Campylobacter* strains and 144 laboratories (94.1 %) of the unknown culture.

3.2 *Salmonella* serogrouping and serotyping

The number of laboratories that performed full serotyping on all eight strains increased significantly in 2006 compared with 2004, from 74 (58 %) to 108 (83 %). Furthermore, correct serotype results increased as well in 2006, from 701 correct tests (81 %) in 2004 to 813 correct tests (86 %) in 2006. The percentage of laboratories attempting to serotype all eight strains is the highest since this WHO GSS EQAS started, and not since 2002 has the percentage of correct serotype results been so high (Table 1).

Year	Labs serotyped all eight strains		Correct serotyping results	
	Number of labs		Number of correct tests	
	n	%	n	%
2000	34	77	164	76
2001	64	62	498	80
2002	78	80	648	90
2003	66	53	678	81
2004	74	58	701	81
2006	**108**	**83**	**813**	**86**

Table 1. The overall performance of serotyping, 2006.

Table 2 shows the number of participating laboratories versus the number of correctly serotyped samples. In 2006 a total of 42 laboratories (32 %) of 131 participating laboratories serotyped all eight strains correctly and further 35 laboratories (27 %) had seven strains correctly serotyped.

Number of correct serotypes	EQAS 2000		EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004		**EQAS 2006**	
	Number of labs		Number of labs		Number of labs		Number of labs		Number of labs		**Number of labs**	
	n	%	n	%	n	%	n	%	n	%	**n**	**%**
8	9	26	32	37	50	52	32	25	41	32	**42**	**32**
7	9	26	13	15	17	18	15	12	14	11	**35**	**27**
6	3	9	9	10	14	14	18	14	16	13	**22**	**17**
5	3	9	10	11	3	3	23	18	16	12	**12**	**9**
4	3	9	4	5	2	2	14	11	11	9	**7**	**5**
3	2	6	7	8	3	3	12	10	10	8	**5**	**4**
2	3	9	4	5	6	6	3	2	10	8	**3**	**2**
1	1	3	4	5	1	1	5	4	5	4	**3**	**3**
0	1	3	4	5	1	1	3	2	4	3	**2**	**2**
In total	N=34	100 %	N=87	100 %	N=87	100 %	N=125	100 %	N=127	100 %	**N=131**	**100 %**

Table 2. The laboratories' ability to correctly serotype zero to eight strains.

In Figure 2 the laboratories' performance in serotyping the eight strains correctly has been listed by region. It seems that performance challenges in some regions may be due to the fact that a disproportionate number of laboratories in the region have more deviations from the expected eight serotypes than other laboratories. This is more profound in the regions of South America, Southeast Asia, Africa and the Caribbean than the other regions. It is illustrated in Figure 3 that almost all regions have laboratories which perform satisfactorily even if the region in a general perspective performs less well. This is an important observation as one of the key objectives in the WHO GSS programme is to gain reliable serotyping results in order to detect emerging of new serotypes and determine the prevalence of various serotypes in different parts of the world.

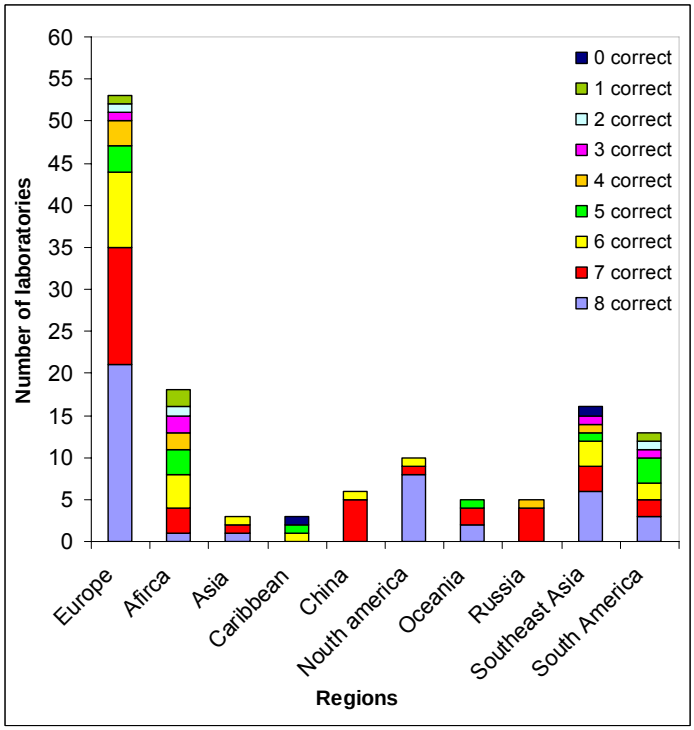

Figure 2. The number of laboratories which correctly serotyped the eight strains by region.

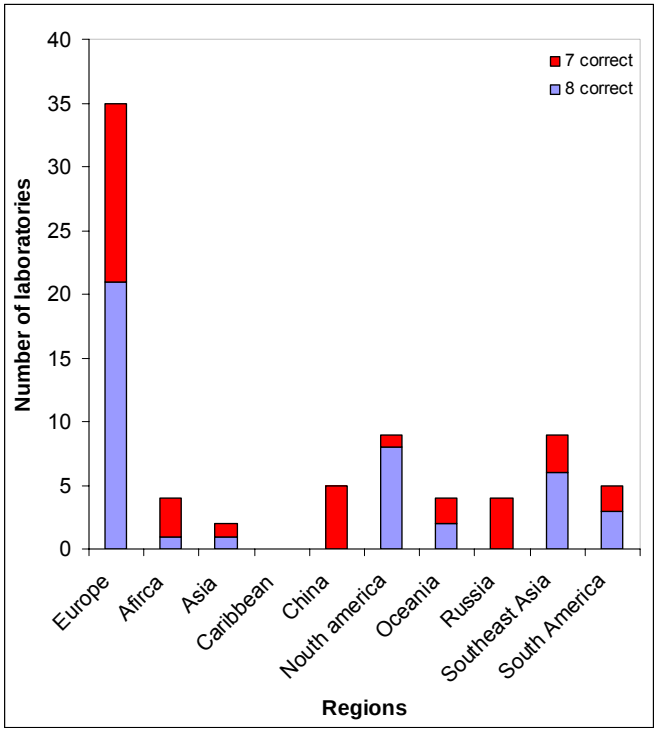

Figure 3. The number of laboratories which serotyped up to seven strains correctly by region.

The number of serogroup deviations ranges from 0.8 % (WHO 6.2, 6.3 and 6.5) to 7.4 % (WHO 6.7) (Table 3). Strain WHO 6.8 also seems to cause some problems for determining the serogroup (6.6 %). This is not surprising as both strains belong to the same serogroup (O:3,10). Five of the laboratories which had deviations regarding the two strains listed them as serogroup O:1,3,19. The overall performance of the serogrouping is satisfactory as the percentages of the deviations are very low for strain WHO 6.1 – 6.6 (<2.3 %).

The range of deviations is wide regarding the serotyping results (6.5 – 41.0 %). Strain WHO 6.1 accounts for the highest percentage of deviations, whereas the remaining seven strains all have less than 16.5 %. A total of 38 (31.1 %) laboratories serotyped strain WHO 6.1 incorrectly as Typhimurium. Strain WHO 6.1 is a monophasic Typhimurium – a Typhimurium which has lost the second flagella phase due to a deletion in the phase 2 flagellin gene. The correct formula for this strain is I: 1,4,12:i:-.

A number of laboratories ranging from five (4.5 %) (WHO 6.7) to 10 (9.1 %) (WHO 6.5) have difficulties detecting the second flagella phase in strains WHO 6.2, 6.3, 6.5, 6.7 and 6.8. The laboratories have chosen serotypes which only differed from the expected serotype on the

second flagella phase. In addition, 11 laboratories (10.1 %) were observed to have similar problems detecting the G-complex in strain WHO 6.4. The majority of the laboratories serotyped the internal quality control strain (used in 2000, 2001 and 2004) WHO 6.6 correctly with only a deviation rate of 6.5 %. Table 4 illustrates the laboratories' ability to serotype the internal quality strain correctly. Furthermore, this ability seems to be somehow stable in the years where it has been used. In both 2004 and 2006, 94% of laboratories have serotyped the strain correctly. This level is very satisfactory considering the laboratories' lower performance in serotyping in general (Table 1 and 2).

Strain	Correct serotype		No. of labs: serogrouping	Deviations (%)	Deviating results	No. of labs: serotyping	Deviations (%)	Deviating results number of laboratories
	Name	Formular						
WHO6.1	Group O:4 monophasic	1,4,12:i:-	128	2.3 %	O:2 (1) O:9,46 (1) O:13 (1)	122	41.0 %	Typhimurium (38), Farsta (3), Gloucester (2), Tsevie (1), Lagos (1), Agama (1), Paratyphi B (1), Mathura (1), Tumodi (1), Bongori (1)
WHO6.2	Saintpaul	1,4,12:e,h:1,2	131	0.8 %	O:1,3,19 (1)	119	11.8 %	Sandiego (6), Chester (2), Reading (1), Bardo (1), Typhimurium (1), Paratyphi B (1), Chartres (1), I 4,12:-:- (1)
WHO6.3	Virchow	6,7:r:1,2	131	0.8 %	O:8 (1)	121	9.9 %	Nigeria (3), I 6,7:r:- (3), Ngili (1), Bsilla (1), Papuana (1), Lomita (1), Infantis (1)
WHO6.4	Rissen	6,7:f,g:-	129	2.3 %	O:4 (1) O:6,14 (1) O:9 (1)	118	10.2 %	Montevideo (5), Othmarschen (2), Eingedi (2), Derby (1), Blegdam (1)Oranienburg (1)
WHO6.5	Reading	4,5,12:e,h:1,5	131	0.8 %	O:9 (1)	121	16.5 %	Saintpaul (6), Sandiego (2), Chester (2), Hato (1) Enteritidis (1), Paratyphi B (1), Bradford (1), Mono (1), Derby (1), I 4,5,12:-:- (1) Typhimurium (1), Eppendorf (1)
WHO6.6	Enteritidis	9,12:g,m:-	128	2.3 %	O:7 (1) O:9,46 (2)	124	6.5 %	Gallinarum (2), Monetvideo (1), Typhi (1), Berta (1), Suberu (1), Bournemount (1), London (1)
WHO6.7	London	3,10:l,v:1,6	121	7.4 %	O:4 (2) O:8 (2) O:1,3,19 (5)	111	9.9 %	Give (2), Amherstiana (2), Amsterdam (1), Clackamas (1), Sinstorf (1), Birmingham (1), Stockholm (1), Ruzizi (1), Nchanga (1)
WHO6.8	Give	3,10:l,v:1,7	122	6.6 %	O:4 (1) O:7 (1) O:9 (1) O:1,3,19 (5)	114	9.6 %	London (4), Nchanga (2), Kortrijk (1), Bredeney (1), Stormont (1), Mokola (1)

Table 3. List of *Salmonella* serogroups, serotypes and deviations, 2006

Year	Labs serotyped Enteritidis correctly	
	Number of labs	
	n	%
2000	37	89%
2001	74	86%
2004	121	94%
2006	**124**	**94%**

Table 4. The laboratories' performance of the internal quality strain.

3.3 Antimicrobial susceptibility testing of *Salmonella*.

A total of 12,902 antimicrobial susceptibility tests were performed (Table 5) in 2006. Of these, only 88.7 % were in agreement with the expected results (App.1). A total of 7.0 % minor and 4.3 % major deviations (susceptible – resistant switch) were observed. This is the lowest score recorded since the beginning of the WHO EQAS.

Year	All testings performed	Percentage correct results	Percentage minor deviations (S_I or I_R switch)	Percentage major deviations (S_R 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
2004	12.381	93.0	4.5	2.5
2006	**12.902**	**88.7**	**7.0**	**4.3**

Table 5. The number of susceptibility test performed from 2000 to 2004.
*: Data from 2003 is exclusive one strain which may have lost resistance due to transport or storage stress

Major difficulty was observed for the strain WHO 6.2 where several laboratories did not have the following antimicrobials in agreement: AUG, POD, XNL, CHL, CIP, GEN, SXT and TET (Table 6).

Strain	AUG	AMP	CTX	POD	CAZ	XNL	CHL	CIP	GEN	NAL	STR	SMX	SXT	TET	TMP
WHO S-6.1	24/24/**52**	**99**/0/1	4/1/**95**	3/5/92	2/2/**96**	4/4/**92**	**78**/6/16	0/1/**99**	2/1/**97**	5/25/**70**	**96**/3/1	**100**/0/0	7/4/**89**	**95**/1/4	3/0/**97**
WHO S-6.2	55/**42**/3	**99**/1/0	1/1/**98**	3/**10**/87	2/2/**96**	3/10/**87**	8/**22**/70	8/18/**74**	**65**/25/10	**99**/1/0	**90**/5/5	**99**/0/1	3/8/**89**	30/27/**43**	3/1/**96**
WHO S-6.3	21/8/**71**	**98**/1/1	47/45/**8**	**100**/0/0	21/5/**74**	**80**/13/7	1/3/**96**	3/9/**88**	2/2/**96**	**97**/2/1	8/16/**76**	10/1/**89**	2/1/**97**	**96**/2/2	1/1/**98**
WHO S-6.4	20/12/**68**	**99**/1/0	**88**/11/1	**100**/0/0	27/10/**63**	**97**/3/0	**98**/1/1	0/1/**99**	2/2/**96**	3/2/**95**	37/**40**/23	**93**/0/7	**95**/0/5	**77**/3/20	**99**/0/1
WHO S-6.5	2/0/**98**	4/0/**96**	2/1/**97**	3/0/**97**	0/3/**97**	0/3/**97**	1/2/**97**	0/2/**98**	2/2/**96**	2/0/**98**	3/5/**92**	10/5/**85**	2/1/**97**	6/17/**77**	0/0/**100**
WHO S-6.6	1/3/**97**	6/6/**88**	1/2/**97**	3/5/**92**	3/2/**95**	3/7/**90**	0/3/**97**	0/1/**99**	**95**/1/4	2/2/**96**	**87**/2/11	**96**/0/4	3/2/**95**	8/27/**65**	1/1/**98**
WHO S-6.7	3/0/**97**	2/3/**95**	1/1/**98**	0/3/**97**	2/1/**97**	4/7/**89**	1/1/**98**	1/1/**98**	1/2/**97**	2/3/**95**	10/26/**64**	8/5/**87**	2/2/**96**	2/2/**96**	0/3/**97**
WHO S-6.8	1/0/**99**	2/1/**97**	1/1/**98**	0/5/**95**	1/1/**98**	0/7/**93**	1/4/**95**	1/1/**98**	2/1/**97**	2/1/**97**	5/13/**82**	5/3/**92**	1/1/**98**	2/8/**90**	0/3/**97**

Table 6. Susceptibility test results (% R/I/S) of the *Salmonella* strains tested in 2006
Numbers in bold: % with expected interpretation. Grey cell: < 90 % of laboratories determined correct interpretation.

In Table 7 the percentage of major deviations per antimicrobial is shown. Many of the antimicrobials seem to pose a problem for many laboratories and in general the percentages of the deviations are higher compared to previous years. Especially, AUG (8.8 %), CTX (7.2 %), CAZ (7.3 %), STR (5.3 %), SMX (5.6 %) and TET (9.0 %) seem to cause problems.

Antimicrobial	EQAS 2000		EQAS 2001		EQAS 2002	
	Total no of determinations	% major deviations	Total no of determinations	% major deviations	Total no of determinations	% major deviations
AMP	343	6.1	793	4.0	918	2.9
CHL	343	3.8	785	1.8	911	1.8
CIP	334	1.2	784	0.6	911	0.5
GEN	343	5.0	792	1.1	905	2.8
KAN	312	4.5	595	2.0	680	1.5
NAL	328	1.8	697	1.4	893	2.1
STR	312	3.5	643	7.0	734	4.2
SMX	248	4.8	412	4.4	503	3.6
TET	335	6.0	775	6.7	869	3.3
TMP	295	2.7	398	1.5	507	3.0
SXT			728	2.1	731	2.3
Antimicrobial	EQAS 2003*		EQAS 2004		EQAS 2006	
	Total no of determinations	% major deviations	Total no of determinations	% major deviations	Total no of determinations	% major deviations
AMP	1.005	1.6	1.178	3.2	1.100	1.9
CHL	982	0.7	1.159	1.7	1.068	2.7
CIP	981	0.4	1.162	0.3	1.118	1.5
GEN	979	1.6	1.201	2.0	1.086	3.0
KAN	732	2.3	-	-	-	-
NAL	933	1.1	1.130	1.2	1.043	2.1
STR	761	4.3	947	1.3	904	5.3
SMX	615	3.6	734	5.3	657	5.6
TET	981	4.0	1.122	4.8	1.062	9.0
TMP	582	0.5	729	1.9	615	1.1
SXT	922	0.5	1.051	2.8	1.004	3.0
AUG	-	-	973	5.5	958	8.8
CTX	-	-	995	0.4	964	7.2
POD	-	-	-	-	313	0.6
CAZ	-	-	-	-	777	7.3
XNL	-	-	-	-	233	2.6

Table 7. Number of tests performed and percentage of major deviations (susceptible – resistant switch) for each antimicrobial 2000 – 2006.

In Table 8, deviations are defined as values that exceed the interval limits of the quality control strain. The table illustrates the proportion of laboratories which have submitted exceeding values of the QC interval of reference strain *E. coli* ATCC 25922 using both disk diffusion and MIC determinations.

Of the 20 laboratories tested the reference strain using the MIC determinations and 113 laboratories used the disk diffusion method.

No mistakes were recorded to XNL and ENRO using MIC determinations. Deviations submitted by more that two laboratories using MIC determination were observed to CTX, GEN, NAL, SMX, and SXT.

The following antimicrobials resulted in deviation submitted by most laboratories using disk diffusion: CTX (n:21), CHL (n:21), NAL (n:21), SXT (n:20), SMX (n:19) and AMP (n:18).

Antimicrobials	QC range *E.coli* ATCC 25922		Laboratories outside QC range			
			EQAS 2006 MIC		EQAS 2006 Disks	
	MIC (ug/ml)	Disks (mm)	% of labs	n	% of labs	n
AUG	2-8	18-24	6	17	10	99
AMP	2-8	16-22	5	20	16	113
CTX	0.03-0.12	29-35	30	10	20	105
POD	0.25-1	23-28	25	4	11	35
CAZ	0.06-0.5	25-32	20	10	14	86
XNL	0.25-1	26-31	0	9	30	23
CHL	2-8	21-27	13	16	19	110
CIP	0.004-0.016	30-40	11	19	8	108
ENRO	0.008-0.03	32-40	0	1	67	18
GEN	0.25-1	19-26	17	18	14	113
NAL	1-4	22-28	19	16	20	106
STR	4-16	12-20	14	14	10	92
SMX	8-32	15-23	33	9	29	65
SXT	0-0.5	23-29	19	16	19	106
TET	0.5-2	18-25	12	17	12	108
TMP	0.5-2	21-28	13	8	17	66

Table 8. Range of obtained values for *E. coli* ATCC 25922 by disk diffusion and MIC determinations.

3.4 Identification of *Campylobacter* strains and the unknown culture

C. jejuni in strain #1 was successfully recovered by 86 laboratories. Ninety percent of the laboratories performed correct species identification. *C. coli* was successfully recovered by 94 laboratories but only 66% of the laboratories performed correct species identification (Table 9).

Year	Number. of participants	Correct species	Strain number	Number of submitted results	% correct identification	Deviating results
2003	97	C.jejeni	Strain # 1	92	87 %	*C. coli (n:9)* *C. lari (n:3)*
2003	97	C.coli	Strain # 2	92	83 %	*C. jejuni (n:7)* *C. lari (n:4)* *C. upsaliensis (n:4)*
2004	109	C.lari	Strain # 1	95	80 %	*C .coli (n:11)* *C .jejuni (n:8)*
2004	109	C.jejuni	Strain # 2	107	87 %	*C. coli (n:8)* *C. lari (n:4)* *C. upsaliensis (n :2)*
2006	99	C.jejeni	Strain # 1	86	90 %	*C. lari (n:3)* *C.coli (n:3)* *C. upsaliensis (n:3)*
2006	99	C.coli	Strain # 2	94	66 %	*C. lari (n:19)* *C. jejuni (n:11)* *C. upsaliensis (n:2)*

Table 9. Laboratories which successfully identified *Campylobacter*.

A total of 134 laboratories submitted identification results for the unknown bacterial sample, *Yersinia enterocolitica* O3. Only ten deviating results were reported (4x *Shigella*, 2x *E.coli*, 1x *Bacillus*, 1x *Enterobacter*, 1x *Klebsiella*, 1x *Acinetobacter*).

Further typing was reported by 66 % laboratories, in all cases with indication of correct serotype (Table 10).

Year	Participating labs	Correct identification of the blank sample
	Number of labs	%
2003	**115**	**99% E.coli O157**
2004	**121**	**94% Shigella** **74% S.flexineri**
2006	**134**	**93% Yersinia** **89% Y. enterocolitica** **66% Y. enterocolitica O3**

Table 10. Laboratories which successfully identified *Yersinia*.

4. Discussion

4.1 *Salmonella* serogrouping and serotyping.

In 2006 we observed a significant increase in the number of laboratories which were able to serotype all eight strains and an increase in the total number of correctly serotyped isolates (Table 1). We believe the reason behind this increase was the the selection of *Salmonella* strains from serogroups shared by globally predominant serovars. In addition, full serotyping

could be performed using commonly available antisera. In previous years (2003-2004) laboratories needed less common antisera to fully serotype all EQAS strains.

This was launched to survey the barriers of the laboratories.
The conclusion that the 2006 survey was less difficult is supported by Table 2. The table illustrates that app. 80 % of the 132 laboratories were able to obtain a correct serotype of six out of the eight strains. This has not been recorded since 2002 where only 87 laboratories participated.
We also believe that the WHO GSS laboratory training programme's focus on serotyping might have had an impact on the quality of the serotyping. Thus 94 % of the laboratories serotyped the internal control strain correctly which is the highest score observed (Table 4). Furthermore, one of the tasks in the WHO GSS laboratory sub-committee and one of the objectives for the WHO GSS regional centres have been to provide participants with information on where to purchase high quality antisera and even to support some with antisera.
Despite the increased improvement some laboratories still face problems in serotyping correctly. Considering that 94 % of all laboratories had the internal control strain correctly serotyped, Figure 1 shows that some regions still suffer from the lack of reliable antisera. A large proportion of the laboratories which do not manage to serotype many of the strains correctly are found in the regions of Africa, Caribbean, Southeast Asia and the South Americas. Many countries in these regions have fewer resources available for the laboratories, and some have problems importing the needed antisera. Unfortunately, WHO GSS does not have funds to support all of these countries. However, WHO GSS does have an advocacy component in the training programme in order to teach the laboratories to apply for sustainable funds in their home countries. Even if some regions have problems, it is still possible to obtain reliable serotyping data from almost all regions (Figure 3). This is an important observation as the WHO GSS wants to be able to rely on the data uploaded to the WHO GSS country database with regards to serotype prevalence.
The problems in obtaining the correct serotype have mainly been due to the difficulties detecting the second flagella phase (WHO 6.2, 6.3, 6.5, 6.7 and 6.8). It is hard to believe that this should be a result of a lack of antisera as the laboratories select other serovars which only differ from the expected antigenic formula on the second flagella phase according to

Kaufmann-White serotyping scheme. For instance, in strain WHO 6.2 the expected serotype was *Salmonella* Saintpaul (I 4,5,12:e,h:1,2). Six laboratories reported *Salmonella* Sandiego which share the same O and phase 1 H antigen, (I 4,[5],12:e,h:e,n,z15), two laboratories chose *Salmonella* Chester (I 4,[5],12:e,h:e,n,z), one laboratory Reading (I 4,[5],12:e,h:1,5) and another laboratory Chartres (I 4,12:e,hl,w). We believe the problem may be due to lack of availability of appropriate media and to some extend poor quality antisera. In order to detect the second flagella phase one needs to phase invert the strain by point inoculating it onto Swarm agar containing the corresponding first phase concentrated flagella antisera. Very few companies sell Swarm agar, so many laboratories have to produce the agar themselves. This might cause problems as the composition is not well-known and the final product might have weaknesses in allowing the strain to swarm on the surface. In addition, if poor quality antisera or absorbed antisera in an inappropriate order have been used the chance of observing incorrect clumping might be higher than laboratories using high quality antisera from a certified supplier using quality assurance procedures in the production of the antisera..
38 laboratories identified strain WHO 6.1 as *Salmonella* Typhimurium, the intended response for WHO 6.1 was I 1,4,12:i:-. This strain has a deletion in the phase 2 flagellin gene rendering it monophasic, the deletion is irreversible.
The G-complex in strain WHO 6.4 caused some problems as well. This indicates that the laboratories do not have access to all of the factor antisera contained in the G-complex as many have chosen serovars which only differ from the intended response by G complex single factors. We have seen similar problems in previous years (e.g. in 2004 with E complex serology of *Salmonella* Chester).

4.2 Antimicrobial susceptibility testing of *Salmonella*

Over-all, the percentage of correct susceptibility testing of *Salmonella* was 88.7 % with 4.3 % major deviations (Table 5). This is far from satisfactory considering that laboratories performed better in the previous years.
We believe that several issues have contributed to this decrease in performance. The laboratories did not receive a breakpoint guideline to interpret their obtained results. By experience we know that many laboratories use a large range of different breakpoints to interpret their results why some variation was expected. In addition, no guideline or indication on how to interpret the cephalosporins was disseminated, thus some laboratories followed the

CLSI guidelines which indicate that all cephalosporins should be interpreted resistant if one is interpreted resistant, regardless of the value detected from the results. Unfortunately, the database system used in this EQAS was designed to evaluate each antimicrobial individually regardless of the CLSI guideline why some laboratories had deviations to cefotaxime and ceftazidime in strains WHO 6.3 and 6.4. It has not been possible to correct the errors related to this issue, why some laboratories without purpose have been designated errors.
This indicates a need for harmonisation of the susceptibility testing and the EQAS system. However, it is also important to determine the additional factors which caused the errors. The factors could be either: demanding test strains, difficult reading of the antimicrobials, lack of attention to the QC results or it could be the methodology.
The major problems observed with strain WHO 6.2 might have been caused by mixed clones (Table 6). Some laboratories reported resistant tetracycline colonies why it is reasonable to believe that some of the other deviations observed with this strain could have been caused by the same mixed clones as resistant genes often are located on the same plasmid.
AUG, CTX, CAZ, SMX, STR and TET all yielded a high degree of unsatisfactory results. Problems associated with AUG are often due to a breakpoint phenomenon where many strains have a value close to breakpoints why some chose to read the strains as intermediate and others as resistant.
AUG caused deviations in four cases (WHO 6.1 -6.4). Only in WHO 6.1 and 6.2 it is reasonable to assume that the problem was caused by breakpoint related issues. In the two remaining cases the problem causing app. 20 % of the laboratories to report the strains as susceptible are not clear.
Streptomycin often poses a challenge in susceptibility testing, as many strains are borderliners and balance between resistance / intermediate or intermediate / susceptible e.g. WHO 6.3, 6.4 and 6.7.
Surprisingly, TET accounted for 9.0 % deviations. We believe this could be a matter of inconsistent breakpoints used for interpretation. Normally, TET should not cause any problems as it is a rather easy antimicrobial to test but it should be noted that CLSI recently have changed the breakpoints for TET.
When performing antimicrobial susceptibility testing, it is extremely important to include reference strains for internal quality control (QC). The QC results revealed that a total of 19.3 % of the performed tests with the *E. coli* ATCC 25922 were outside the QC range given by

CLSI. These results indicate that the number one barrier for antimicrobial susceptibility testing is inadequate standardization of methods, but also the use of expired disks, improper storage or repeated sub-culturing of strains leading to loss of resistance genes, are plausible causes of incorrect testing.

4.3 Identification of *Campylobacter* strains and the unknown culture

Several laboratories had problems identifying strain #2 - *C. coli* (34 %). Nineteen laboratories have incorrectly identified strain #2 as *C. lari*. It is possible that the strain did not exhibit indoxyl acetate hydrolysis well, a finding that could lead to false-negative results. On the contrary, 11 laboratories identified strain #2 (the *C. coli* strain) as *C. jejuni*. *C. jejuni* hydrolyze hippurate whereas *C. coli* are hippurate hydrolysis-negative. This result is more difficult to explain as we believe it is hard to misread the blue dye in a positive conventional test. The reason might also be that the quality of the reagent was poor and not tested against known strains when produced. Two laboratories reported *C. upsalensis.*

Surprisingly, 93 % of the 134 laboratories identified the unknown sample containing *Yersinia* but even more surprisingly, 66 % were able to detect the O3 somatic phase. It was believed that many laboratories did not have the means for purchase the reagents to detect the somatic phase.

5. Conclusion

The serotyping results indicate a need for further training of the skills in *Salmonella* serotyping. Future training efforts should be aimed at enhancing the capability to detect the second flagella phase and disseminating protocols for preparing high quality swarm agar plates. Detection of the second flagella phase is one of more profound barriers for obtaining a satisfactory serotyping result.

Harmonising the methodology and providing adequate guidelines for antimicrobial susceptibility testing is crucial for improving the results. Clearly, there is a need to disseminate the latest breakpoint guidelines, to strengthen awareness of performing and interpreting internal QC, as well as to identify the barriers for antimicrobial susceptibility testing in each individual laboratory. In addition, it is very important to emphasise to use the QC results obtained in optimising and adjusting the methodology as many laboratories seems to report values exceeding the QC ranges.

We were pleased to see that many of the laboratories were able to identify *Campylobacter* and the unknown isolate - *Yersinia*. The results revealed good skills in especially identification of *Yersinia*.

Reference.

1. Popoff and Le Minor**,** 2001.8th ed. Popoff,M.Y.,Le Minor, L., 2001. Antigenic formulas of the Salmonella serovars. WHO Collaborating Centre for Refrence and Research on Salmonella.

Appendix 1; Strain collection and reference values in MIC.

Strain	AMP	AUG	CHL	CIP/ENRO	POD	XNL	CAZ	CTX	GEN	NAL	STR	SMX	TET	TMP	SXT
WHO S-6,1	**R (>32)**	S (8/4)	**R (>64)**	S (0,06)	**I (1)**	S (2)	S (0,5)	S (<=0,25)	S (<=1)	S (8)	**R (>64)**	**R (>1024)**	**R (>32)**	S (<=4)	S (0,25)
WHO S-6,2	**R (>32)**	**I (16/8)**	**I (16)**	S (1)	**I (1)**	S (2)	S (1)	S (<=0,25)	**R (16)**	**R (>64)**	**R (>64)**	**R (>1024)**	S (4)	S (<=4)	S (0,5)
WHO S-6,3	**R (>32)**	S (8/4)	S (4)	S (0,25)	**R (>4)**	**R (>8)**	S (0,5)	S (8)	S (<=1)	**R (>64)**	S (8)	S (<=64)	**R (32)**	S (<=4)	S (<=0,12)
WHO S-6,4	**R (>32)**	S (8/4)	**R (>64)**	S (<=0,03)	**R (>4)**	**R (>8)**	S (2)	**R (48)**	S (<=1)	S (<=4)	**I (16)**	**R (>1024)**	**R (>32)**	**R (>32)**	**R (>4)**
WHO S-6,5	S (<=1)	S (<=2/1)	S (<=2)	S (<=0,03)	S (0,25)	S (1)	S (<=0,25)	S (<=0,25)	S (<=1)	S (<=4)	S (<=4)	S (<=64)	S (<=2)	S (<=4)	S (<=0,12)
WHO S-6,6	S (4)	S (<=2/1)	S (8)	S (<=0,03)	**I (1)**	S (2)	S (0,5)	S (<=0,25)	**R (32)**	S (<=4)	**R (>64)**	**R (>1024)**	S (<=2)	S (<=4)	S (<=0,12)
WHO S-6,7	S (<=1)	S (<=2/1)	S (4)	S (<=0,03)	S (0,25)	S (<=0,5)	S (<=0,25)	S (<=0,25)	S (<=1)	S (<=4)	S (8)	S (<=64)	S (<=2)	S (<=4)	S (<=0,12)
WHO S-6,8	S (<=1)	S (<=2/1)	S (4)	S (<=0,03)	S (0,25)	S (<=0,5)	S (<=0,25)	S (<=0,25)	S (<=1)	S (<=4)	S (8)	S (<=64)	S (<=2)	S (<=4)	S (<=0,12)

AMP, ampicillin; AUG, amoxicillin + clavulanic acid; CTX, cefotaxime; POD, cefpodoxime; CAZ, ceftazidime; XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin / ENRO, enrofloxacin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; SMX, sulphonamides; TET, tetracycline; TMP, trimethoprim; and SXT, trimethoprim + sulphonamides

R: Resistance (in gray and bold), I: Intermediate (in bold), S:Sensitive (colourless).

Appendix 2; Reference MIC breakpoints for *Salmonella*.

Antimicrobial \ Laboratories		Ref value (CLSI)
Ampicillin / Amoxicillin	Sensitiv	≤8
	Intermediate	16
	Resistance	≥32
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Amoxicillin + Clavulanic acid	Sensitiv	≤8
	Intermediate	16
	Resistance	≥32
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Cefotaxime	Sensitiv	≤8
	Intermediate	16-32
	Resistance	≥64
Antimicrobial \ Laboratories		**Ref value (FOOD-DTU)**
Cefpodoxime	Sensitiv	≤0,5
	Intermediate	1
	Resistance	≥2
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Ceftazidime	Sensitiv	≤8
	Intermediate	16
	Resistance	≥32
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Ceftiofur	Sensitiv	≤2
	Intermediate	4
	Resistance	≥8
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Chloramphenicol	Sensitiv	≤8
	Intermediate	16
	Resistance	≥32
Antimicrobial \ Laboratories		**Ref value (EUCAST)**
Ciprofloxacin / Enrofloxacin	Sensitiv	<0,125
	Intermediate	-
	Resistance	≥0,125
Antimicrobial \ Laboratories		**Ref value (EUCAST)**
Gentamicin	Sensitiv	≤2
	Intermediate	4
	Resistance	≥8
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Nalidixic acid	Sensitiv	≤16
	Intermediate	-
	Resistance	≥32
Antimicrobial \ Laboratories		**Ref value (FOOD-DTU)**
Streptomycin	Sensitiv	≤8
	Intermediate	16
	Resistance	≥32
Antimicrobial \ Laboratories		**Ref value**
Sulphonamides	Sensitiv	≤256
	Intermediate	-
	Resistance	≥512
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Sulphonamides + Trimethoprim	Sensitiv	≤2/38
	Intermediate	-
	Resistance	≥4/76
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Tetracycline	Sensitiv	≤4
	Intermediate	8
	Resistance	≥16
Antimicrobial \ Laboratories		**Ref value (CLSI)**
Trimethoprim	Sensitiv	≤8
	Intermediate	-
	Resistance	≥16

WHO Global Salm-Surv
External Quality Assurance System (EQAS) 2006

Appendix 3a.: Documents

PROTOCOL

Serotyping and susceptibility testing of *Salmonella* & identification of other human pathogens

Introduction

In 2000, the global WHO Global *Salmonella* Surveillance network launched an External Quality Assurance System (EQAS). The EQAS is organized by the Danish Institute for Food and Veterinary Research (DFVF) in collaboration with partners and Regional Sites in WHO GSS.

As previous years the WHO EQAS 2006 includes serotyping and susceptibility testing of eight *Salmonella* strains, susceptibility testing of one *E. coli* ATCC strain for Quality Control, identification of two thermophilic *Campylobacter* isolates and of one "blank" bacterial isolate.

This year we have included an original CERTIFIED culture of the *E. coli* reference strain ATCC 25922 (=CCM 3954) for internal Quality Control. This original certified strain is a gift from WHO GSS, free of charge. Please take proper care of the strain. Handle it and maintain it as suggested in the enclosed manual. Use it for future internal quality control for susceptibility testing in your laboratory. The strain will not be included next year.

All testing should be done by the methods routinely used in your laboratory. If your laboratory do not serogroup/serotype, or do not test *Campylobacter*, you may omit that part of the EQAS.

Objectives

The main objective of the EQAS is to support laboratories to assess and if necessary improve the quality of serotyping and susceptibility testing of enteric human pathogens, especially *Salmonella*. Furthermore, to assess and improve the comparability of surveillance data on *Salmonella* serotypes and antimicrobial susceptibility reported by different laboratories.

Outline of the EQAS 2006

Receipt and storage of strains

In May and June around 150 laboratories from all parts of the world receive a parcel containing eight *Salmonella* strains, one reference strain *E. coli* ATCC 25922 for Quality Control, two *Campylobacter* strains and one blank bacterial isolate. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains among the selected material. **Please confirm receiving the parcel to Dr. Anne Mette Seyfarth (contact info below).**

The strains are shipped as stab cultures or lyophilized cultures. Please keep strains refrigerated. On arrival, the cultures must be subcultured and ensured proper storage conditions until testing. A suggested procedure for reconstitution of lyophilized *Campylobacter* is presented below.

Serotyping of *Salmonella*

Eight *Salmonella* strains (S-6.1,….,S-6.8) should be serotyped by the method routinely used in the laboratory. If you do not have all the antisera please go as far as you can. Please report the

serogroup, since also serogrouping results will be evaluated this year. If you do not serotype in your laboratory, you may omit serotyping.

Susceptibility testing of *Salmonella* and *E. coli* ATCC 25922 for Quality Control
Eight *Salmonella* strains and the *E. coli* reference strain should be susceptibility tested towards as many as possible of the following antimicrobials by the methods routinely used:
Amoxicillin+clavulanic acid, ampicillin, cefpodoxime, ceftiofur, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin or enrofloxacin, gentamicin, nalidixic acid, streptomycin, sulphonamide, tetracycline, trimethoprim, trimethoprim+sulphonamide.

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

Identification of *Campylobacter* and the blank isolate
Two thermophilic *Campylobacter* isolates (C-6.1 and C-6.2) should be identified to species level. The blank isolate (B-6.1) should be identified to species level and further typed if relevant. As mentioned, you may omit this part of the EQAS if your lab does not perform such testing.

Suggested procedure for reconstitution of the lyophilized Campylobacter strains:

1) Open, take out some of the material and dissolve in 1/2 ml broth. Leave it for 10 minutes. Spread 1 loop or 1 swab of the solution on blood agar. Incubate microaerophilic for 24-48 h at 37ºC or 42ºC.

2) Take rest of the broth (with the dissolved material) and incubate microaerophilic as mentioned above with parafilm on top. After incubation spread on blood agar and incubate microaerophilic again.

3) If you don't succeed with 1) or 2) take rest of the lyophilized material, and shake it directly onto blood agar. Add a little saline, and spread properly with a triangle or hockey stick. Incubate microaerophilic as mentioned above

Reporting of the results

Fill in your results in the enclosed test form. Please enter your results into the interactive web database on the WHO Global Salm-Surv homepage www.who.int/salmsurv/en. A description of how to enter your data is presented below. You can find your username and password in the letter following the parcel. **Please submit results by latest September 1.**

Immediately after entering your results in the web database, an evaluation report is generated. A description of how to enter results follows on next page. If you do not have access to the Internet, please return results by fax or mail. Finally, a summary report with all results will be performed and made available. Individual results will be anonymously and only passed on to the official GSS Regional Centre in your region.

……

We are looking forward to receiving your results. If you have any questions or concerns, please contact:

Anne Mette Seyfarth ams@dfvf.dk Phone: (+45) 7234 6346 or Rene Hendriksen rsh@dfvf.dk Phone: (+45) 7234 6288

Danish Institute for Food and Veterinary Research (DFVF)
Fax (+45) 7234 6341, Bülowsvej 27, DK-1790 Copenhagen V, Denmark

WHO Global Salm-Surv
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How to enter the results into the WHO GSS web-database

1) You enter the WHO Global Salm-Surv web page (http://www.who.int/salmsurv/en), then
   - click on "GSS Activities"
   - click on the link "http://www.who.int/entity/salmsurv/activities/GSS_EQAS/en"
   - click on "Data entry for the year 2006"
   - write your username and password **in low letters** and click on "Login".
     You can find your username and password in the letter following your parcel.

2) In the next page "Materials and methods" you navigate with Tab-key and mouse
   - fill in the brand of antisera.
   - fill in the method for susceptibility testing.
   - enter the brand of accessories, e.g. Oxoid.
   - fill in whether you followed the CLSI guidelines and breakpoints or not.
   - fill in whether your institute serves as a national reference laboratory
   - In the field for comments: Please indicate the breakpoints you used if not CLSI.
   - *click on "Save and go to next page"* **REMEMBER TO SAVE LIKE THIS IN EACH PAGE !!**

3) In the data entry pages "Salmonella strains 1-8", you
   - **SELECT** the serogroup (O-group) from the pop-up list, **DO NOT WRITE**
   - **Wait a few seconds – the page will automatically reload, so that the pop-up in the field "Serotyp" only contains serotypes belonging to the chosen serogroup.**
   - **SELECT** the serotype from the pop-up list – **DO NOT WRITE**
   - enter the antigenic formula (e.g. 1,4,5,12:i:1,2)
   - enter the zonediameters in mm or MIC values in µg/ml.
   - enter the interpretation as R, I or S.
   - fill in comments if relevant e.g. which antisera you miss for complete serotyping
   - *click on "Save and go to next page"*

IMPORTANT: Please leave the field empty, do not write anything if you did not serotype or susceptibility test.

4) In the data entry page "*E. coli* reference strain":
   - enter the zonediameters in mm or MIC values in µg/ml.
   - click on "Save and go to next page"

5) In the page "Identification of *Campylobacter* and blank sample":
   - choose the correct *Campylobacter* species from the pick list.
   - fill in the species and type of the blank bacterial isolate, and fill in the method used.
   - *click on "Save and go to next page"*

   If you haven't performed these testing, please leave the fields empty.

6) The next page is a menu, from where you can review the input pages or **approve your input**:

   - Go through the input pages, make corrections if necessary. Click on "Save and go to next page" if you make corrections.

   - Approve your input. **Be sure that you have filled in all your results, as you can only approve once. The approval blocks your data entry in the database, but allows you to see the evaluated results.**

   - As soon as you have approved your input, an individual evaluation report will show up. You can print each page, if you want to.

7) When you have seen all pages in the report, you will find a new menu. You can choose "Top menu", "Review evaluated results" or "Go to Global Salm-Surv homepage".

WHO Global Salm-Surv
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End of entering your data, thank you very much !

QUESTIONNAIRE

General questions about non-typhoid Salmonella

1. Annual numbers of *Salmonella* isolates processed in your laboratory?

	Annual average numbers	Origin of sample					
		Humans	Food	Animals	Feed	Environ-ment	unknown
1. Isolated in your lab							
2. Isolates obtained from other labs							

2. Percentage of *Salmonella* further typed

Serogrouped: _____%

Serotyped: _____%

Phage typed *S.* Typhimurium and *S.* Enteritidis: _____%

Phage typed other *Salmonellas:* _____%

3. Frequency of *Salmonella* serotyping at your laboratory

- □ once a week
- □ twice a month
- □ once a month
- □ more frequently, specify .....
- □ less frequently, specify……

4. Method used for serotyping

- □ slide agglutination
- □ micro well agglutination
- □ killed antigen
- □ live antigen
- □ a combination of the above or other, please specify .....……..

5. Source of sera

- □ we produce our own sera
- □ we buy commercially available sera. Manufacturer: …….....

Comments:

QUESTIONNAIRE

6. Frequency of antimicrobial susceptibility testing of *Salmonella* in your laboratory

- □ every day
- □ once a week
- □ twice a month
- □ once a month
- □ less frequently, specify .....

7. Annual number of *Salmonella* isolates susceptibility tested in your laboratory

.....

8. Method routinely used for antimicrobial susceptibility testing

□ NCCLS or other guidelines ? Please specify.

.....

.....

.....

□ Disks/tablets/strips/microtitreMIC/agardilutionMIC and source/brand:

……

□ Substrate used (e.g. Mueller-Hinton).....

□ Quality Control Strains used (e.g. E. coli ATCC 25922) .....

……

…….....

9. Have you or other persons from your institute participated in a Global Salm-Surv training course ?

No____ Yes____

QUESTIONNAIRE

General questions about thermophilic Campylobacter

1. Annual numbers of *Campylobacter* isolates processed in your laboratory

	Annual average numbers	Origin of sample					
		Humans	Food	Animals	Feed	Environ-Ment	Unknown
1. Isolated in your lab							
2. Isolates obtained from other labs							

2. Frequency of identification of *Campylobacter* in your laboratory

□ every day □ once a week
□ once a month □ other, please specify…….....…….....

3. Percentage of *Campylobacter* identified to species level: _____ %

Comments: ……..…….…….…….……..

4. Method used for identification

……

……

……

5. Annual number of *Campylobacters* susceptibility tested in your laboratory

.....……..…….

6. Frequency of susceptibility testing of *Campylobacter*

□ every day □ once a week
□ once a month □ other, please specify
.....…….....

7. Method used for antimicrobial susceptibility testing

.....

QUESTIONNAIRE

We are committed to improve the quality and usefulness of the EQAS. Therefore, we kindly ask you to take a moment to complete the questionnaire below, so we may learn how to improve the EQAS.

QUALITY OF THE EQAS

1) Your opinion of the letters etc. you have received during the EQAS 2004

	Very poor	Poor	Satisfactory	Good	Very good
A The listserver announcements					
B The EQAS welcome letter					
C The EQAS protocol and test form					
D The evaluation report obtained from the web database					

Give your comments, proposals etc. here:

_____

_____

2) Your opinion of how the EQAS 2004 was performed

	Very poor	Poor	Satisfactory	Good	Very good
A The organisation of the EQAS					
B The information about the EQAS					
C How did it meet your expectations					

Give your comments, expectations and suggestions here:

_____

_____

THE INTERACTIVE WEB DATABASE

3) Did you enter your results in the interactive web database through the Global Salm-Surv homepage? □ yes
□ no If not specify why:_____

4) Do you have any comments on the data you have entered?

_____

_____

QUESTIONNAIRE

5) Which problems did you meet when you entered data into the interactive web database?

_____

_____

6) What is your overall impression of the interactive web database
___ very poor ___ poor ___ satisfactory ___ good ___ very good

Specify why: _____

7) How could the interactive web database be improved?

_____

_____

THE USEFULNESS OF THE EQAS

8) How do you consider the importance of participating the EQAS program for you?

___ Irrelevant ___ not important ___ important ___ very important

Specify why: _____

_____

9) Would you like to participate in the EQAS 2005?
- □ yes
- □ maybe
- □ no If not specify why:_____

10) What would you like to see changed in EQAS 2005 (procedures, species, number of strains, antimirobials etc.):

Thank you very much for taking your time to fill in this questionnaire.

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Appendix 3b.: Documents

TEST FORM

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

Date of arrival of your parcel: _____

Period of testing: From date _____ to date _____

Storage conditions before and under testing: _____

Date for submitting results: _____

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TEST FORM

Serotyping and susceptibility testing of Salmonella

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.1	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.2	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

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TEST FORM

Serotyping and susceptibility testing of Salmonella

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.3	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.4	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

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TEST FORM

Serotyping and susceptibility testing of Salmonella

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.5	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.6	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

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TEST FORM

Serotyping and susceptibility testing of Salmonella

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.7	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

Strain	Serogroup/Serotype	Antimicrobial	Zonediam (mm) or MIC-value (ug/ml)	S/I/R
S-6.8	_____ *Serogroup* _____ *Serotype* _____ *Antigenic formula*	Ampicillin		
		Amoxicillin+clavulanic acid		
		Cefotaxime		
		Cefpodoxime		
		Ceftazidime		
		Ceftiofur		
		Chloramphenicol		
		Ciprofloxacin or enrofloxacin		
		Gentamicin		
		Nalidixic acid		
		Streptomycin		
		Sulphonamides		
		Trimethoprim		
		Trimethoprim+sulphonamide		
		Tetracycline		

TEST FORM

Susceptibility testing of the *E. coli* ATCC 25922 for Quality Control

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

TEST FORM

Identification of two thermophilic *Campylobacter* (mark with "X")

STRAIN	*C. jejuni*	*C. coli*	*C. upsaliensis*	*C. lari*
C-6.1				
C-6.2				

Method used in brief:

Identification of the blank isolate labelled "Enterobacteriaceae sp."

B-6.1	Species:_____ Serotype, if done: _____

Method used in brief:

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Appendix 3c.: Documents
Instructions for Opening and Reviving of Freeze-dried Cultures

Manual from CCM for the *E. coli* Quality Control strain

Czech Collection of Microorganisms
Masaryk University
Tvrdého 14
602 00 BRNO
Czech Republic

Freeze-dried 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.

1. **Check the number of the culture on the label inside the ampoule**
2. **Make a file cut on the ampoule near the middle of the plug**
3. **Disinfect the ampoule with alcohol-dampened gauze/ or alcohol-dampened cotton wool from just below the plug to the pointed end.**
4. **Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule**
5. **Remove the pointed end of the ampoule into disinfectant**
6. **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.**
7. **Incubate the inoculated medium at appropriate conditions for several days**
8. **Auclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding.**

Notes: 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 !

Appendix 3d.: Documents
Subculture and Maintenance of Quality Control Strains

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.

REFERENCES

- **M100-S15**, January 2005 (Performance Standards for Antimicrobial Susceptibility Testing)
- **M07-A6**, January 2003 (Methods for Dilution Antimicrobial that Grow Aerobically; Approved Standard)

DEFINITION OF TERMS

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

2. 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.

3. 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.

4. 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.

IMPORTANT CONSIDERATIONS

- Do not use disc diffusion strains for MIC methods.
- 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

STORAGE OF REFERENCE STRAINS

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

2. **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.

FREQUENCY OF TESTING

1. **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.

2. **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 day validation before resuming weekly testing.

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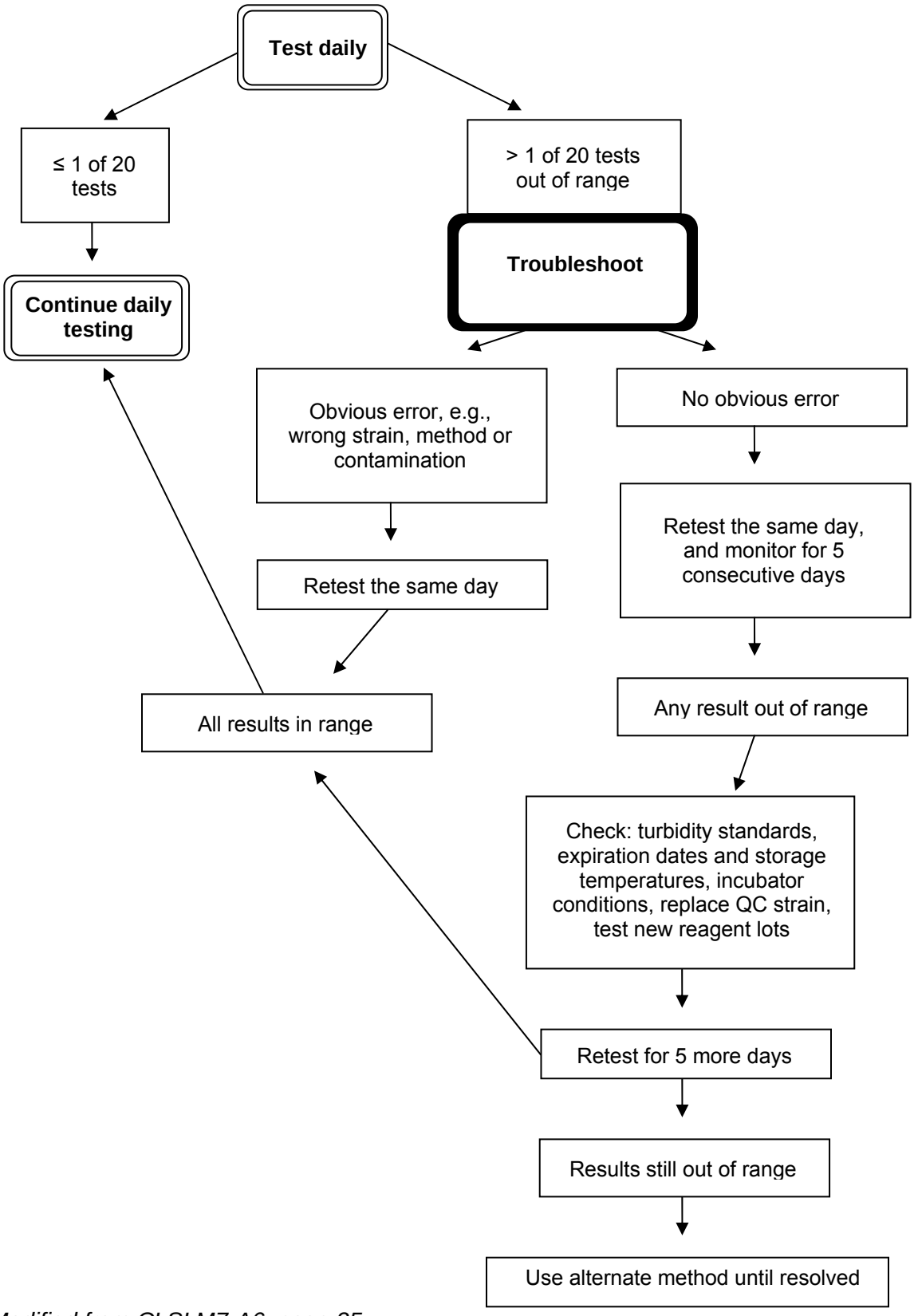
Daily MIC QC Chart



Modified from CLSI M7-A6, page 35

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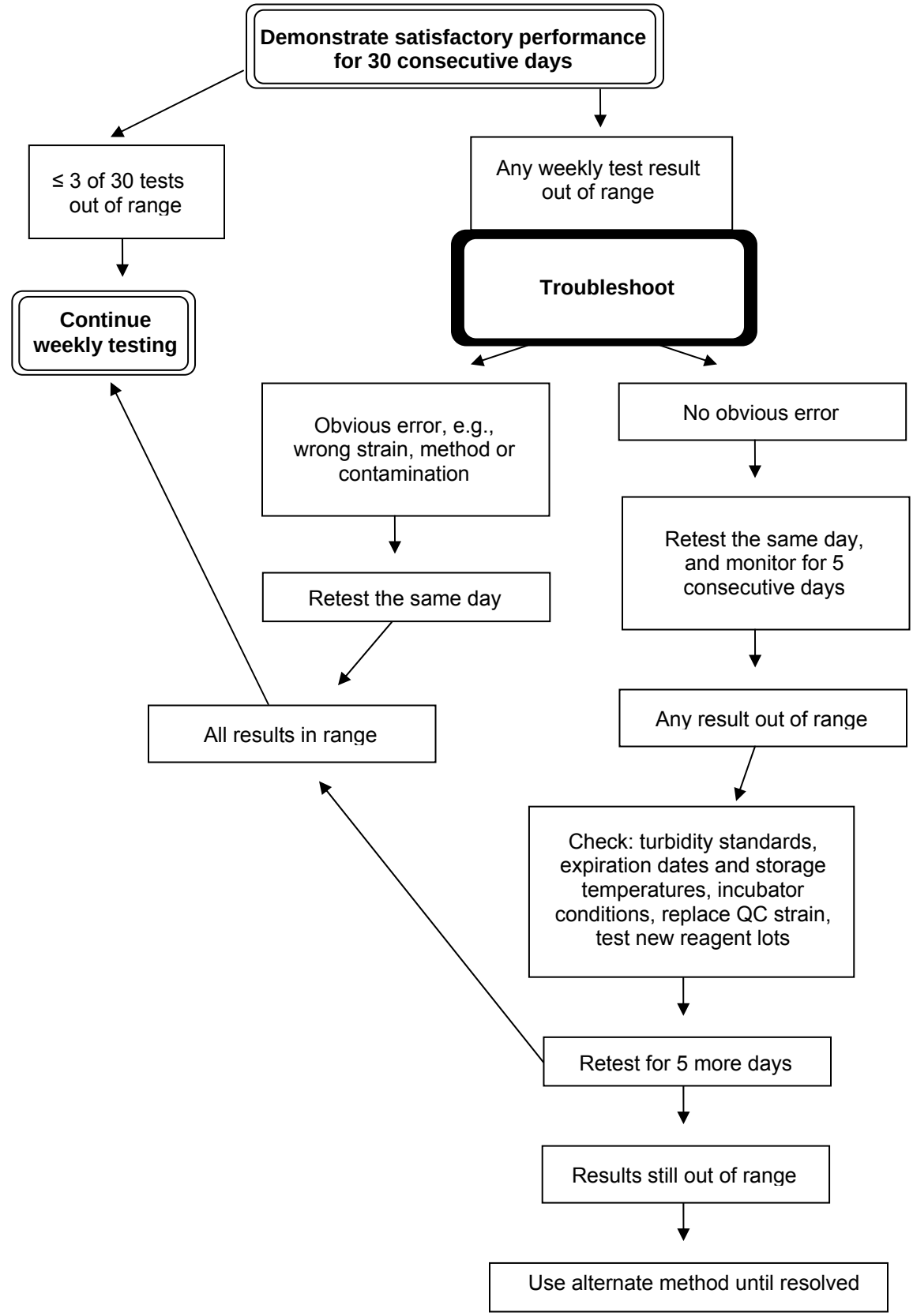
Weekly MIC QC Chart



Modified from CLSI M7-A6, page 36

Appendix 3e: Documents

Dear EQAS 2006 participant !

Please find enclosed the bacterial strains for the EQAS 2006 together with the following documents:

1 Protocol for 2006
1 Test form for 2006
1 Manual for Opening and Reviving of Freeze-dried Cultures
1 Manual for Subculture and Maintenance of Quality Control Strains

In the protocol you will find detailed description of all the testing and a description of how to enter your results into the interactive web database. For the dataentry you need a username and a password. Please keep this document. Your username and password will not appear in other documents.

Your username:

Your password:

Thank you for signing up ! We are looking forward to this sixth round of the GSS WHO EQAS.

For further information, please don't hesitate to contact:

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