# The External Quality Assurance System of the WHO Global Salm-Surv, Year 2007

World Health Organization

CDC
Centers for Disease Control and Prevention

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DTU Food
National Food Institute

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## 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 WHO Member States' capacity to detect and respond to *Salmonella* occurrences, as well as to initiate global surveillance of *Salmonella.* Today the WHO GSS embraces important foodborne pathogens other than *Salmonella*, especially *Campylobacter*, which also has become of great concern in various 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 and ascertain the performance of 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 157 laboratories in 2007.

The EQAS is organized annually by the National Food Institute (DTU Food), Copenhagen, Denmark in collaboration with Centers for Disease Control and Prevention (CDC) in Atlanta, USA; World Health Organization (WHO) in Geneva, Switzerland; and Institute Pasteur (IP) in Paris, France.

The objective is to monitor the quality of the *Salmonella* serotyping and the antimicrobial susceptibility data produced by Member States and pin point areas which need attention in order to produce reliable data. The goal is having all national reference laboratories perform *Salmonella* serotyping with a maximum of one error 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.

The technical advisory group for the WHO EQAS scheme consists of members of the WHO GSS steering committee.
The data of individual laboratories is only known to the laboratory in question, the EQAS Organizer (DTU Food) and the respective WHO GSS regional centre, but is otherwise confidential. All summary conclusions are made public.

## 2. Materials and Methods

### 2.1 Participants

Two pre-notifications were announced through the WHO GSS list server in early spring 2007 (App 1) The pre-notifications included invitations to participate in the EQAS on serotyping and susceptibility testing of *Salmonella* and identification of *Campylobacter* and an unknown foodborne pathogen. 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* were selected for this trial among isolates from the National Food Institute's strain collection. However, the unknown foodborne pathogen (*Vibrio parahaemolyticus*) was selected by IP. Individual sets of the *Salmonella* and *Vibrio parahaemolyticus* 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 which did not participate in 2006 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 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; gentamicin, GEN; nalidixic acid, NAL; streptomycin, STR; sulphonamides, SMX; tetracycline, TET; trimethoprim, TMP and trimethoprim + sulphonamides, SXT (App. 2).

MIC determination was performed utilizing 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, cefpodoxime (according to DTU Food) (App.3).

2.5 Distribution

The cultures and documents (App. 3) downloaded to a CD 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 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 (*Vibrio parahaemolyticus*). Furthermore, the laboratories were requested to save and maintain the ATCC reference strains for future proficiency tests according to App. 4.

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 DTU Food.

The *Salmonella* strains were categorised as resistant (R), intermediate (I) or susceptible (S) against the tested antimicrobials. All antimicrobials used should be interpreted individually with exception of cephalosporins which were 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 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 instantly 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 → S). In this report, the deviations to antimicrobial susceptibility testing are divided into two categories – critical deviations (major and very major deviations) and total deviation including also the minor deviations.

## 3. Results

A total of 198 laboratories responded to the pre-notification, and were enrolled in the EQAS. When the deadline for submitting results was reached, 156 laboratories in 76 countries had uploaded data. The following countries provided data (also shown below in Figure 1): Albania, Argentina, Australia, Barbados, Belarus, Bolivia, Bosnia and Herzegovina, Brazil, Bulgaria, Cambodia, Cameroun, Canada, Chile, China, Colombia, Costa Rica, Cote d'Ivoire, Croatia, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Finland, France, Germany, Georgia, Greece, India, Italy, Jamaica, Japan, Jordan, Korea, Lithuania, Luxembourg, Macedonia, Madagascar, Malaysia, Malta, Mauritius, Mexico, Moldova, Morocco, The Netherlands, New Zealand, Nicaragua, Nigeria, North America, Oman, Panama, Paraguay, Peru, Philippines, Poland, Russia, Saudi Arabia, Serbia, Senegal, Slovak Republic, Slovenia, Sri Lanka, South Africa, Suriname, Taiwan, Thailand, Trinidad and Tobago, Tunisia, Turkey, Ukraine, United Kingdom, 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, very major, critical and total deviations as described above.

3.1 Methods used by EQAS-participants

The participating laboratories were all requested to use their routine methods for performing serotyping and AST.

Of the 156 laboratories submitting results, 140 (90%) participated in some of or in the entire serotyping component of the program.

Of the 156 laboratories submitting results, 143 (92%) submitted antimicrobial susceptibility results. 119 laboratories used disk diffusion, and 24 laboratories used MIC determination. Information was given beforehand to the participants regarding the reference breakpoints used or breakpoint guidelines for interpretation of MIC determinations. However, no information was distributed concerning disk diffusion. In addition, the participants were informed how to interpret the resistance of cephalosporins.

Of the 142 laboratories submitting results, 95 (72%) and 99 (74%) performed identification of the two *Campylobacter* strains correctly and 86 laboratories (83%) of the unknown culture.

3.2 *Salmonella* serogrouping and serotyping

The percentage of laboratories that performed full serotyping on all eight strains decreased in 2007 compared to 2006, from 81% (105 laboratories) to 77% (109 laboratories). The proportion of correct serotype results increased in 2007, from 85% correct tests (n=808) in 2006 to 88% correct tests (n=920) in 2007 (Table 1).

| Year | Labs serotyped all eight strains | | Correct serotyping results | |
|---|---|---|---|---|
| | Number of labs | | Number of correct tests | |
| | n | % | n | % |
| 2000 | 34 | 92 | 164 | 76 |
| 2001 | 78 | 80 | 508 | 71 |
| 2002 | 80 | 81 | 664 | 90 |
| 2003 | 69 | 54 | 692 | 80 |
| 2004 | 78 | 60 | 701 | 81 |
| 2006 | 105 | 81 | 808 | 85 |
| 2007 | 109 | 77 | 920 | 88 |

Table 1. The overall performance of serotyping, 2007.

Table 2 illustrates the number of participating laboratories versus the number of correctly serotyped samples. In 2007, a total of 66 laboratories (47%) of 140 participating laboratories serotyped all eight strains correctly and further 29 laboratories (21%) had seven strains correctly serotyped. In total, 95 laboratories met the threshold for adequate performance serotyping in 2007. It was also the year where most laboratories met the threshold ever in the

history of the WHO GSS EQAS. In addition, none of the laboratories had all strains incorrect which also have been observed for the first time in 2007.

| Number of correct serotypes | EQAS 2000 | | EQAS 2001 | | EQAS 2002 | | EQAS 2003 | |
|---|---|---|---|---|---|---|---|---|
| | Number of labs | | Number of labs | | Number of labs | | Number of labs | |
| | n | % | n | % | n | % | n | % |
| 8 | 9 | 24 | 35 | 36 | 52 | 53 | 32 | 25 |
| 7 | 9 | 24 | 13 | 13 | 17 | 17 | 15 | 12 |
| 6 | 4 | 11 | 9 | 9 | 14 | 14 | 18 | 14 |
| 5 | 3 | 8 | 10 | 10 | 3 | 3 | 23 | 18 |
| 4 | 3 | 8 | 4 | 4 | 2 | 2 | 14 | 11 |
| 3 | 3 | 8 | 7 | 7 | 3 | 3 | 13 | 10 |
| 2 | 3 | 8 | 4 | 4 | 6 | 6 | 4 | 3 |
| 1 | 2 | 5 | 4 | 4 | 1 | 1 | 5 | 4 |
| 0 | 1 | 3 | 12 | 12 | 1 | 1 | 3 | 2 |
| In total | N=37 | 100% | N=98 | 100% | N=99 | 100% | N=127 | 100% |
| Number of correct serotypes | EQAS 2004 | | EQAS 2006 | | EQAS 2007 | | Overall EQAS 2000-2007 | |
| | Number of labs | | Number of labs | | Number of labs | | Number of labs | |
| | n | % | n | % | n | % | n | % |
| 8 | 41 | 32 | 42 | 32 | 66 | 47 | 277 | 38 |
| 7 | 14 | 11 | 35 | 27 | 29 | 21 | 105 | 15 |
| 6 | 17 | 13 | 19 | 15 | 13 | 9 | 84 | 12 |
| 5 | 16 | 12 | 12 | 9 | 11 | 8 | 78 | 11 |
| 4 | 11 | 9 | 7 | 5 | 7 | 5 | 48 | 7 |
| 3 | 10 | 8 | 5 | 4 | 6 | 4 | 41 | 6 |
| 2 | 10 | 8 | 3 | 2 | 2 | 1 | 38 | 5 |
| 1 | 5 | 4 | 4 | 3 | 6 | 4 | 27 | 4 |
| 0 | 5 | 4 | 3 | 2 | 0 | 0 | 25 | 3 |
| In total | N=129 | 100% | N=130 | 100% | N=140 | 100% | N=723 | 100% |

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

In table 3 the laboratories' performance in serotyping the strains correctly has been listed by region. In general, it seems like the region "Asia and the Middle East" has serotyped the strains less accurately in 2007 compared to the other regions. Five laboratories in this region serotyped 55% correctly of an average six strains. In the Oceanic region four laboratories serotyped all eight strains 100% correctly.

| Region: | Year: | Number of laboratories (n) | Number of strains serotyped (n) | Percent strains correctly serotyped (%) |
|---|---|---|---|---|
| Africa | 2001 | 6 | 37 | 73.0 |
| | 2002 | 9 | 62 | 87.1 |
| | 2003 | 11 | 70 | 71.4 |
| | 2004 | 9 | 51 | 62.7 |
| | 2006 | 16 | 95 | 71.6 |
| | 2007 | 11 | 73 | 80.8 |
| Asia & Middle East | 2001 | 10 | 60 | 50.0 |
| | 2002 | 5 | 30 | 83.3 |
| | 2003 | 5 | 35 | 54.3 |
| | 2004 | 5 | 33 | 54.5 |
| | 2006 | 5 | 35 | 74.3 |
| | 2007 | 5 | 40 | 55.0 |
| Caribbean** | 2001 | 0 | 0 | 0 |
| | 2002 | 0 | 0 | 0 |
| | 2003 | 3 | 18 | 61.1 |
| | 2004 | 2 | 8 | 87.5 |
| | 2006 | 3 | 14 | 78.6 |
| | 2007 | 2 | 9 | 77.8 |
| China | 2001 | 4 | 32 | 96.9 |
| | 2002 | 3 | 24 | 100.0 |
| | 2003 | 8 | 60 | 75.0 |
| | 2004 | 7 | 46 | 78.3 |
| | 2006 | 6 | 48 | 85.4 |
| | 2007 | 10 | 80 | 91.3 |
| Europe | 2001 | 43 | 323 | 80.5 |
| | 2002 | 50 | 384 | 90.0 |
| | 2003 | 60 | 401 | 84.8 |
| | 2004 | 57 | 392 | 84.7 |
| | 2006 | 52 | 403 | 86.4 |
| | 2007 | 54 | 415 | 89.4 |
| North America | 2001 | 4 | 32 | 87.5 |
| | 2002 | 2 | 16 | 100.0 |
| | 2003 | 6 | 41 | 95.1 |
| | 2004 | 8 | 55 | 81.8 |
| | 2006 | 10 | 80 | 96.3 |
| | 2007 | 12 | 94 | 97.9 |
| Oceanic | 2001 | 4 | 30 | 100.0 |
| | 2002 | 6 | 43 | 93.0 |
| | 2003 | 6 | 46 | 93.5 |
| | 2004 | 5 | 38 | 97.4 |
| | 2006 | 5 | 37 | 94.6 |
| | 2007 | 4 | 32 | 100.0 |
| Russia | 2001 | 1 | 8 | 12.5 |
| | 2002 | 1 | 8 | 62.5 |
| | 2003 | 1 | 7 | 14.3 |
| | 2004 | 4 | 26 | 69.2 |
| | 2006 | 5 | 40 | 80.0 |
| | 2007 | 8 | 51 | 80.4 |
| Latin America* | 2001 | 11 | 78 | 57.7 |
| | 2002 | 11 | 82 | 87.8 |
| | 2003 | 13 | 83 | 75.9 |
| | 2004 | 15 | 88 | 79.5 |
| | 2006 | 13 | 84 | 84.5 |
| | 2007 | 15 | 107 | 88.8 |
| Southeast Asia | 2001 | 15 | 113 | 54.0 |
| | 2002 | 12 | 90 | 92.2 |
| | 2003 | 15 | 100 | 81.0 |
| | 2004 | 17 | 130 | 81.5 |
| | 2006 | 15 | 117 | 84.6 |
| | 2007 | 19 | 140 | 91.4 |

Table 3. The number of laboratories which correctly serotyped the strains by region.

*: Include Cuba, South - and Central – America. **: Include English and French speaking countries and Surinam.

The majority of the laboratories (n=135) serotyped the internal quality control strain (used in 2000, 2001, 2004, 2006) WHO 7.2 correctly leading to a deviation rate of only 3.6%. Table 4 illustrates the laboratories' ability to serotype the internal quality strain correctly. Furthermore, this ability seems to be somehow consistent in the years it has been used. This level is very satisfactory with most laboratories testing this strain and with the best result ever.

| Year | Labs serotyped Enteritidis correctly | |
|---|---|---|
| | Number of labs | |
| | n | % |
| 2000 | 34 | 92% |
| 2001 | 64 | 84% |
| 2004 | 113 | 95% |
| 2006 | 116 | 94% |
| 2007 | 135 | 96% |

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

The overall performance of the serogrouping is satisfactory as the percentages of the deviations are very low for all the test strains ranging from 0.7% (WHO 7.1) to 8.7% (WHO 7.7) with an average of 2,9% (Table 5). Strain WHO 7.7 seems to cause some problems determining the serogroup. The strain was a Poona (I 13,22:z:1,6) which was only serogrouped by 115 laboratories, the lowest this year. The laboratories found the following deviations O:4, O:7, O:8, O:9 and O:11.
Strain WHO 7.1 (Concord; I 6.7:l,v:1,2) was tested by 136 laboratories and resulted only in one deviation: (O:9)

The deviations regarding the serotyping results ranged between 3.6% – 19.2%. Strain WHO 7.1 accounted for the highest percentage of deviations, whereas the remaining seven strains all had less than 14.5% incorrect results.
Of the eight strains, two contained a G-complex and two other stains an E and a L complex. Only one stains contained a less common O-antigen (O:13)
A number of laboratories have difficulties detecting the flagella phase in the strains. Many laboratories have entered to the database serotypes which only differed from the expected serotype on the phase two flagellar antigen. In addition, laboratories were observed to have similar problems detecting the complexes.

| Strain | Correct serotype | | No. of labs: serogrouping | Deviations (%) | Deviating results | No. of labs: serotyping | Deviations (%) | Deviating results |
|---|---|---|---|---|---|---|---|---|
| **WHO7.1** | Concord | 6,7:l,v:1,2 | 136 | 0.7 | O:9 (1) | 125 | 19.2 | Mkamba (5), Potsdam (4), Bonn, Panama, Colorado, Virchow, Richmond, Kortrijk, Langeveld, Thompson, Orkland, Nessziona, Gabon, Ohio, Wil, Stathcona, Salmonella ssp |
| **WHO7.2** | Enteritidis | 9,12:g,m:- | 134 | 2.9 | O:6,14 (1)<br>O:7 (2)<br>O:9,12 (1) | 140 | 3.6 | Postdam, Rissen, Blegdam, Dublin, Warragul, |
| **WHO7.3** | Livingstone | 6,7,d:l,w | 133 | 2.3 | O:6,7 (1)<br>O:8 (1)<br>O:9 (1) | 128 | 10.9 | Kambole (3), Gabon (2), Paratyphi C (2), Herston, Isangi, Typhi, Nievkerk, Gombe, Ohio, Kisii |
| **WHO7.4** | Montevideo | 6,7:g,m,s:- | 135 | 2.2 | O:6,7 (1)<br>O:8 (2) | 131 | 6.9 | II (3), Eboko, Chincol, Rissen, Menston, Othmarschen |
| **WHO7.5** | Mbandaka | 6,7,14:z10:e,n,z15 | 134 | 2.2 | O:6,7 (1)<br>O:6,14 (1)<br>O:8 (1) | 131 | 14.5 | Breanderup (6), Djugu (2), Aequatoria, Larose, Gombe, Papuana, Denver, Kaduna, Georgia, Montevideo, Glostrup, Lockleaze, Kastrup |
| **WHO7.6** | Elisabethville | 3,10:r:1,7 | 129 | 3.0 | O:1,3,19 (2)<br>O:7 (1)<br>O:8 (1) | 130 | 10.0 | Weltevreden (5), Simi (2), Seegefeld, Montevideo, Give, Westhampton, Salmonella ssp (2) |
| **WHO7.7** | Poona | 13,22:z:1,6 | 115 | 8.7 | O:4 (1)<br>O:7 (3)<br>O:8 (4)<br>O:9 (1)<br>O:11 (2) | 121 | 14.0 | Bristol (2), Farmsen (2), Saugi, Derby, II, Kuru, Manhattan, Nyanza, Borbeck, Montevideo, Gabon, Durban, Marburg. Salmonella ssp (2) |
| **WHO7.8** | Isangi | 6,7:d:1,5 | 134 | 1.5 | O:8 (2) | 136 | 13.2 | Kisii (4), Kambole (2), Livingstone (2), Wil (2), Paratyphi C (2), Herston, Manhattan, Poitiers, Choleraesuis, Nieukerk, Salmonella ssp |

Table 5. List of *Salmonella* serogroups, serotypes and deviations, 2007

## 3.3 Antimicrobial susceptibility testing of *Salmonella*.

A total of 12,976 antimicrobial susceptibility tests were performed in 2007 (Table 6). Of these, 93% were in agreement with the expected results (App.2). A total of 6% minor, 2% major and 1% very major deviations were observed.

| Year | Number of laboratories participating in each EQAS iteration | Average number of antimicrobial agents tested by participating laboratories | Percentage correct test results | Percentage minor deviations (S to I or to R switch) | Percentage major deviations (S to R switch) | Percentage very major deviations (R to S switch) | Percentages critical deviations R to S and S to R switch) | Percentages Total deviations |
|---|---|---|---|---|---|---|---|---|
| 2000 | 44 | 9.1 | 92 | 4 | 4 | 0 | 4 | 8 |
| 2001 | 108 | 8.9 | 91 | 6 | 2 | 1 | 3 | 9 |
| 2002 | 119 | 8.9 | 91 | 6 | 2 | 1 | 3 | 9 |
| 2003 | 147 | 9.3 | 92 | 4 | 2 | 2 | 4 | 8 |
| 2003* | 147 | 8.1 | 93 | 4 | 3 | 0 | 3 | 7 |
| 2004 | 152 | 10.2 | 93 | 4 | 2 | 1 | 3 | 7 |
| 2006 | 143 | 11.2 | 88 | 8 | 3 | 1 | 4 | 12 |
| 2007 | 143 | 10.8 | 93 | 4 | 2 | 1 | 3 | 7 |
| Overall* | 129 | 9.6 | 91 | 6 | 2 | 1 | 3 | 9 |

Table 6. The number of susceptibility test performed from 2000 to 2007.

No specific strain caused major difficulties to the antimicrobials tested (Table 7).

| Strain | AUG | AMP | CTX | POD | CAZ | XNL | CHL | CIP | GEN | NAL | STR | SMX | SXT | TET | TMP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WHO S-7.1 | 26/28/**61** | **139**/0/1 | **111**/0/3 | **45**/1/1 | **103**/0/1 | **32**/0/0 | **133**/2/3 | 1/0/**137** | **136**/0/4 | 3/0/**134** | **106**/1/4 | **84**/0/1 | 4/2/**116** | **126**/2/3 | 0/0/**73** |
| WHO S-7.2 | 3/2/**109** | 9/7/**121** | 1/0/**113** | 5/**5**/38 | 1/0/**103** | 0/2/**30** | 3/4/**129** | 1/0/**137** | **135**/1/2 | 1/1/**134** | **98**/5/7 | **82**/0/3 | 4/1/**115** | 9/23/**98** | 2/0/**71** |
| WHO S-7.3 | 2/0/**111** | 9/3/**128** | 1/0/**113** | 1/1/**47** | 0/1/**103** | 0/3/**29** | **132**/2/5 | 1/0/**136** | 3/3/**133** | 2/0/**135** | **104**/3/4 | **83**/0/2 | **118**/1/3 | **125**/2/3 | **71**/1/1 |
| WHO S-7.4 | 17/11/**85** | **137**/0/3 | 2/1/**112** | 3/1/**45** | 1/0/**103** | 0/2/**30** | 2/0/**137** | 1/4/**133** | **131**/3/6 | 3/4/**129** | **105**/1/5 | **83**/0/1 | 4/1/**117** | 8/13/**110** | 1/0/**71** |
| WHO S-7.5 | 3/1/**109** | 9/3/**128** | 0/1/**113** | 3/0/**46** | 1/0/**103** | 0/2/**30** | 1/0/**137** | 1/1/**136** | 4/1/**135** | 2/1/**134** | **62**/34/13 | **83**/0/1 | **116**/1/4 | **129**/1/1 | **71**/0/2 |
| WHO S-7.6 | 2/0/**111** | 5/4/**130** | 1/0/**113** | 2/0/**47** | 0/0/**103** | 0/2/**30** | 1/0/**137** | 1/0/**136** | 3/2/**133** | 2/2/**132** | 12/**44**/50 | 8/2/**75** | 4/0/**117** | 6/6/**119** | 2/0/**71** |
| WHO S-7.7 | 2/2/**109** | 9/0/**130** | 0/3/**112** | 2/0/**47** | 0/0/**103** | 0/2/**31** | 1/1/**136** | 1/1/**135** | 2/2/**134** | 2/0/**134** | 8/**35**/66 | 12/1/**72** | 2/0/**119** | 8/8/**116** | 0/1/**72** |
| WHO S-7.8 | 2/2/**110** | 9/1/**129** | 1/2/**111** | 3/1/**45** | 0/1/**103** | 0/2/**31** | 1/2/**136** | 1/0/**137** | 4/1/**133** | 3/11/**123** | 3/8/**97** | 7/3/**75** | 2/0/**120** | 5/13/**113** | 0/1/**72** |

Table 7. Susceptibility test results (no. R/I/S) of the *Salmonella* strains tested in 2007
*Numbers in bold: % with expected interpretation. Grey cell: < 90% of laboratories determined correct interpretation.*

In tables 7 and 8, major deviations per antimicrobial are illustrated. Some of the antimicrobials in particular seem to pose a problem for many laboratories. Especially, AUG (6%), POD (4%), STR (4%), SMX (5%) and TET (4%) seem to cause "critical deviations". The same antimicrobials with exception of SMX also result in major "total deviations" (Table 8).

In table 9, 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.

Twenty-three laboratories tested the reference strain using the MIC determinations and 102 laboratories used the disk diffusion method.
No mistakes were recorded when using MIC determinations with exception of a few antimicrobials e.g CIP (n=3), CTX (n=3), GEN (n=1) and SMX (n=2).
All antimicrobials resulted in deviations submitted by most laboratories using disk diffusion with exception to FFN. Participating laboratories seems to have major problems to the following antimicribials: AMP (n=14), CIP (n=12), CHL (n=17), CTX (n=15), SMX (n=12) and SXT (n=14).

| Antimicrobial | EQAS 2000 (N=44) | | | EQAS 2001 (N=108) | | | EQAS 2002 (N=119) | | | EQAS 2003* (N=147) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total no of determinations | % critical deviations | % total deviations | Total no of determinations | % critical deviations | % total deviations | Total no of determinations | % critical deviations | % total deviations | Total no of determinations | % critical deviations | % total deviations |
| Ampicillin | 343 | 6 | 8 | 822 | 4 | 7 | 918 | 2 | 3 | 1019 | 2 | 4 |
| Chloramphenicol | 343 | 4 | 7 | 814 | 2 | 3 | 903 | 2 | 3 | 996 | 1 | 2 |
| Ciprofloxacin | 334 | 1 | 6 | 813 | 1 | 4 | 911 | 0 | 2 | 995 | 0 | 1 |
| Gentamicin | 343 | 4 | 5 | 821 | 2 | 4 | 905 | 2 | 16 | 993 | 2 | 2 |
| Kanamycin | 312 | 4 | 16 | 623 | 2 | 7 | 680 | 2 | 10 | 738 | 2 | 6 |
| Nalidixic acid | 328 | 1 | 4 | 726 | 2 | 8 | 885 | 2 | 4 | 947 | 1 | 4 |
| Sulfamethoxazole | 248 | 3 | 5 | 431 | 6 | 9 | 495 | 4 | 4 | 615 | 4 | 5 |
| Streptomycin | 312 | 4 | 12 | 679 | 7 | 27 | 718 | 4 | 34 | 768 | 9 | 39 |
| Sulphonamides + Trimethoprim | - | - | - | 757 | 2 | 5 | 724 | 7 | 10 | 929 | 2 | 2 |
| Tetracycline | 335 | 6 | 13 | 804 | 7 | 18 | 861 | 3 | 7 | 995 | 4 | 11 |
| Trimethoprim | 295 | 1 | 1 | 416 | 1 | 2 | 499 | 3 | 3 | 582 | 1 | 1 |
| Overall | 3193 | 3 | 8 | 7706 | 3 | 9 | 8499 | 3 | 9 | 9577 | 3 | 7 |

| Antimicrobial | EQAS 2004 (N=152) | | | EQAS 2006 (N=143) | | | EQAS 2007 (N=143) | | | Overall EQAS 2000 -2007* (N=856) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total no of determinations | % critical deviations | % total deviations | Total no of determinations | % critical deviations | % total deviations | Total no of determinations | % critical deviations | % total deviations | Total no of determinations | % critical deviations | % total deviations |
| Ampicillin | 1178 | 3 | 5 | 1092 | 2 | 3 | 1114 | 5 | 7 | 6486 | 3 | 5 |
| Amoxicillin / Clavulanic acid | 973 | 6 | 12 | 950 | 9 | 22 | 908 | 6 | 17 | 2831 | 7 | 17 |
| Ceftazidime | - | - | - | 769 | 7 | 11 | 830 | 1 | 1 | 1599 | 4 | 6 |
| Chloramphenicol | 1159 | 2 | 2 | 1060 | 3 | 15 | 1105 | 0 | 6 | 6380 | 2 | 5 |
| Ciprofloxacin | 1162 | 0 | 1 | 1110 | 2 | 6 | 1101 | 1 | 1 | 6426 | 1 | 3 |
| Cefotaxime | 995 | 0 | 14 | 956 | 7 | 15 | 914 | 1 | 2 | 2865 | 3 | 10 |
| Gentamicin | 1201 | 2 | 3 | 1078 | 3 | 7 | 1111 | 3 | 4 | 6452 | 3 | 6 |
| Kanamycin | - | - | - | - | - | - | - | - | - | 2468 | 3 | 10 |
| Nalidixic acid | 1130 | 1 | 4 | 1035 | 2 | 6 | 1092 | 2 | 3 | 6143 | 2 | 5 |
| Cefpodoxime | - | - | - | 305 | 1 | 26 | 389 | 4 | 16 | 694 | 3 | 21 |
| Sulfamethoxazole | 734 | 5 | 8 | 649 | 6 | 7 | 678 | 5 | 6 | 3850 | 5 | 6 |
| Streptomycin | 947 | 1 | 21 | 896 | 5 | 22 | 875 | 4 | 26 | 5195 | 5 | 26 |
| Sulphonamides + Trimethoprim | 1051 | 3 | 4 | 996 | 3 | 5 | 971 | 3 | 3 | 5428 | 3 | 5 |
| Tetracycline | 1122 | 5 | 11 | 1054 | 9 | 20 | 1047 | 4 | 11 | 6218 | 5 | 13 |
| Trimethoprim | 729 | 2 | 2 | 607 | 1 | 2 | 583 | 1 | 2 | 3711 | 1 | 2 |
| Ceftiofur | - | - | - | 225 | 2 | 9 | 258 | 0 | 6 | 483 | 1 | 8 |
| OVERALL | 12381 | 3 | 7 | 12782 | 4 | 12 | 12976 | 3 | 7 | 67229 | 3 | 9 |

Table 8. Number of tests performed and percentage of major deviations for each antimicrobial 2000 – 2007.

Table 9. Range of obtained values for *E. coli* ATCC 25922 by disk diffusion and MIC determinations.

| Antimicrobial | Interval of the quality control strain[1] | | EQAS 2000 (N=44) | | EQAS 2001 (N=107) | | EQAS 2002 (N=114) | | EQAS 2003 (N=144) | | EQAS 2004 (N=140) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIC (ug/ml) | Disks (mm) | % of labs | N[3] | % of labs | N[3] | % of labs | N[3] | % of labs | N[3] | % of labs | N[3] |
| **Amcillin** | 2-8 | 16-22 | 27 | 37 | 19 | 97 | 16 | 109 | 14 | 140 | 10 | 132 |
| **Amoxicillin / Clavulanic acid** | 2-8 | 8-24 | - | - | - | - | - | - | - | - | 13 | 117 |
| **Ceftazidime** | 0.06-0.5 | 25-32 | - | - | - | - | - | - | - | - | - | - |
| **Chloramphenicol** | 2-8 | 21-27 | 37 | 38 | 20 | 97 | 15 | 107 | 22 | 137 | 13 | 128 |
| **Ciprofloxacin** | 0.004-0.016 | 30-40 | 20 | 35 | 14 | 97 | 14 | 108 | 9 | 138 | 8 | 132 |
| **Cefotaxime** | 0.03-0.12 | 29-35 | - | - | - | - | - | - | - | - | 18 | 111 |
| **Enrofloxacin** | 0.008-0.03 | 32-40 | - | - | - | - | - | - | - | - | - | - |
| **Gentamicin** | 0.25-1 | 19-26 | 23 | 39 | 12 | 99 | 12 | 108 | 9 | 138 | 10 | 134 |
| **Kanamycin** | 1-4 | 17-25 | 19 | 36 | 14 | 87 | 11 | 79 | 12 | 103 | - | - |
| **Nalidixic acid** | 1-4 | 22-28 | 35 | 37 | 14 | 74 | 14 | 102 | 16 | 132 | 9 | 126 |
| **Cefpodoxime** | 0.25-1 | 23-28 | - | - | - | - | - | - | - | - | - | - |
| **Sulfamethoxazole** | 8-32 | 15-23 | 53 | 19 | 34 | 53 | 26 | 57 | 17 | 82 | 16 | 84 |
| **Streptomycin** | 4-16[2] | 12-20 | 22 | 36 | 12 | 81 | 11 | 82 | 9 | 105 | 6 | 110 |
| **Sulphonamides / Trimethoprim** | ≤0.5/9.5 | 23-29 | - | - | 14 | 90 | 12 | 102 | 14 | 129 | 11 | 120 |
| **Tetracyclin** | 0.5-2 | 18-25 | 42 | 42 | 22 | 96 | 13 | 102 | 19 | 137 | 13 | 129 |
| **Trimethoprim** | 0.5-2 | 21-28 | 30 | 31 | 22 | 50 | 11 | 66 | 14 | 79 | 9 | 87 |
| **Ceftiofur** | 0.25-1 | 26-31 | - | - | - | - | - | - | - | - | - | - |

| Antimicrobial | EQAS 2006 (N=137) | | | | | | EQAS 2007 (N=126) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | All | | MIC | | Disk | | All | | MIC (N=23) | | Disk (N=102) | |
| | % of labs | N[3] | % Of labs | N[3] | % of labs | N[3] | % of labs | N[3] | % of labs | N[3] | % of labs | N[3] |
| **Amcillin** | 14 | 133 | 5 | 20 | 16 | 113 | 11 | 124 | 0 | 23 | 14 | 101 |
| **Amoxicillin / Clavulanic acid** | 9 | 116 | 6 | 17 | 10 | 99 | 8 | 102 | 0 | 17 | 9 | 85 |
| **Ceftazidime** | 15 | 96 | 20 | 10 | 14 | 86 | 9 | 92 | 0 | 8 | 10 | 84 |
| **Chloramphenicol** | 18 | 126 | 13 | 16 | 19 | 110 | 14 | 123 | 0 | 21 | 17 | 102 |
| **Ciprofloxacin** | 8 | 127 | 11 | 19 | 8 | 108 | 12 | 121 | 13 | 23 | 12 | 98 |
| **Cefotaxime** | 21 | 115 | 30 | 10 | 20 | 105 | 16 | 104 | 30 | 10 | 15 | 94 |
| **Ceftiofur** | 22 | 32 | 0 | 9 | 30 | 23 | 11 | 35 | 0 | 12 | 17 | 23 |
| **Enrofloxacin** | 63 | 19 | 0 | 1 | 67 | 18 | - | - | - | - | - | - |
| **Florfenicol** | - | - | - | - | - | - | 0 | 13 | 0 | 5 | 0 | 8 |
| **Gentamicin** | 14 | 131 | 17 | 18 | 14 | 113 | 6 | 124 | 5 | 22 | 7 | 102 |
| **Nalidixic acid** | 20 | 122 | 19 | 16 | 20 | 106 | 7 | 120 | 0 | 21 | 8 | 99 |
| **Cefpodoxime** | 12 | 39 | 25 | 4 | 11 | 35 | 9 | 47 | 0 | 6 | 10 | 41 |
| **Sulfamethoxazole** | 29 | 74 | 33 | 9 | 29 | 65 | 22 | 64 | 15 | 13 | 24 | 51 |
| **Streptomycin** | 11 | 106 | 14 | 14 | 10 | 92 | 6 | 97 | 0 | 15 | 7 | 82 |
| **Sulphonamides / Trimethoprim** | 19 | 122 | 19 | 16 | 19 | 106 | 13 | 107 | 0 | 14 | 15 | 93 |
| **Tetracyclin** | 12 | 125 | 12 | 17 | 12 | 108 | 7 | 117 | 0 | 20 | 8 | 97 |
| **Trimethoprim** | 17 | 74 | 13 | 8 | 17 | 66 | 10 | 67 | O | 9 | 12 | 58 |

3.4 Identification of *Campylobacter* strains and the unknown culture

Strain #1 (*C. lari)* was successfully recovered by 95 laboratories and 72% of the laboratories performed correct species identification. Strain #2 (*C. coli)* was also successfully recovered by almost the same number of laboratories (n=99) and 74% of the laboratories performed correct species identification (Table 10). The numbers of deviation for strain #1 were equally distributed among *C. jejuni, C. upsaliensis* and *C. coli.* whereas for strain #2 most deviations were identified as *C. jejuni.*

| Year | Number. of participanting laboratories | Correct species | Strain number | Number of submitted results | % correct identification | Deviating results |
|---|---|---|---|---|---|---|
| 2003 | 97 | *C. jejuni* | Strain # 1 | 92 | 87% | *C. coli (n:9)*<br>*C. lari (n:3)* |
| 2003 | 97 | *C. coli* | Strain # 2 | 92 | 83% | *C. jejuni (n:7)*<br>*C. lari (n:4)*<br>*C. upsaliensis (n:4)* |
| 2004 | 109 | *C. lari* | Strain # 1 | 95 | 80% | *C. coli (n:11)*<br>*C. jejuni (n:8)* |
| 2004 | 109 | *C. jejuni* | Strain # 2 | 107 | 87% | *C. coli (n:8)*<br>*C. lari (n:4)*<br>*C. upsaliensis (n :2)* |
| 2006 | 99 | *C. jejuni* | Strain # 1 | 86 | 90% | *C. lari (n:3)*<br>*C. coli (n:3)*<br>*C. upsaliensis (n:3)* |
| 2006 | 99 | *C. coli* | Strain # 2 | 94 | 66% | *C. lari (n:19)*<br>*C. jejuni (n:11)*<br>*C. upsaliensis (n:2)* |
| 2007 | 142 | *C. lari* | Strain # 1 | 95 | 72% | *C. jejuni (n:10)*<br>*C. coli (n:9)*<br>*C. upsaliensis (n:7)* |
| 2007 | 142 | *C. coli* | Strain # 2 | 99 | 74% | *C. lari (n:3)*<br>*C. jejuni (n:20)*<br>*C. upsaliensis (n:2)* |

Table 10. Laboratories which successfully identified *Campylobacter.*

A total of 86 laboratories submitted identification results for the unknown bacterial sample, *Vibrio parahaemolyticus* which was a significant decrease compared to 2006 where 134 laboratories submitted results. Fourteen laboratories reported deviating results (*Yersinia enterocolitica* (n=2), *Shigella dysenteriae* type A2, *Psedomonas paucimobilis, Hafnia alvei, Shigella ssp, Staph. epidermidis, Salmonella* Poona, *Moraxella lacunata, Cellulomonas ssp, Salmonella* London, *Echantillon blanc, Enterobacter cloacae* and *Staph ssp*.). (Table 11)

| Year | Participating labs | Correct identification of the blank sample |
|---|---|---|
| | Number of labs | % |
| 2003 | **115** | **99% E. coli O157** |
| 2004 | **121** | **94% Shigella**<br>**74% S. flexineri** |
| 2006 | **134** | **93% Yersinia**<br>**89% Y. enterocolitica**<br>**66% Y. enterocolitica O3** |
| 2007 | **86** | **83% Vibrio parahaemolyticus** |

Table 11. Laboratories which successfully identified *Yersinia*.

## 4. Discussion

4.1 *Salmonella* serogrouping and serotyping.

In 2007, we observed a decrease in the number of laboratories which were able to serotype all eight strains but an increase in the total number of correctly serotyped isolates (Table 1). We believe the reason behind this result was caused by the selection of *Salmonella* strains of globally predominant serovars. The *Salmonella* isolates were selected based on the most common regional serovars originated from human, food and veterinary sources and listed in WHO GSS country data bank (CDB). The data was presented as a poster at the International Conference on Emerging Infectious Diseases, Atlanta, USA in 2006 by Musto et al. (2006). In previous years (2003-2004) laboratories needed less common antisera to fully serotype all of the EQAS strains whereas in 2007 most laboratories should have the antisera needed in stock. This conclusion was supported by the fact that 2007 was the year where most laboratories managed to perform serotyping within the quality threshold.

We believe that the WHO GSS laboratory training programme's focus on serotyping may have had an impact on the quality of the serotyping. In addition, a WHO GSS training course on production of high quality antisera was conducted only two years ago by IP. This effort and the focus in general to provide and find suppliers of high quality antisera might also have contributed to the relative high success in performing serotyping this year.

Ninety-six percent of the laboratories serotyped the internal control strain ((WHO 7.2) correctly which is the highest score observed to date (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 has been to provide participants with information on where to purchase high quality antisera and even support some laboratories with obtaining antisera.
Considering that 96% of all laboratories had the internal control strain correctly serotyped, Table 3 shows that some regions still suffer from the lack of reliable antisera. A large proportion of the laboratories who do not manage to serotype many of the strains correctly are found in the regions of Africa (81%), Russia (80%) and the Central Asia and Middle East (55%). Many countries in these regions have fewer resources available for the laboratories, and some have problems importing the needed antisera. Even if some regions have problems, it is still possible to obtain reliable serotyping data from almost all regions (Table 3). This is an important observation as the WHO GSS wants to be able to rely on the data uploaded to the CDB with regards to serotype prevalence.

The problems in obtaining the correct serotype have mainly been due to the difficulties detecting the phase two flagellar antigen but also the somatic phase. It is unlikely 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 one of the phases according to Kaufmann-White serotyping scheme. This observation supports the idea that the main barrier for obtaining a reliable serotyping result is the lack of quality antisera. It is obvious that some antisera cause more problems than others. In strain WHO 7.1 and WHO 7.3, it seems as H:w / H:v and H:2 and O: 7 accounts for the majority of the deviations. The G-complex in strain WHO 7.2 and WHO 7.4 along with O:12 and O:7 causes the deviations for these two isolates. The H:z10 really makes it difficult in strain WHO 7.5 where almost all of the deviations belong to O:6.7 and H:e,n,z15. In WHO 7.6 and WHO 7.8, it is the H:7 and H:5 which the laboratories tend to mistype. In WHO 7.7, it is clearly the somatic phase which account for the problems as the more uncommon O:13, 22 seras are needed.

We believe the problem may be due to lack of availability of appropriate quality antisera. Poor quality antisera or absorbed antisera in an inappropriate order might have been used and 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.

4.2 Antimicrobial susceptibility testing of *Salmonella*

Over-all, the percentage of correct susceptibility testing of *Salmonella* was 93% with 3% critical deviations (Table 6). This is considered to be satisfactory compared with the previous year. Despite of this success too many of the laboratories seem to have values exceeding the QC range.

When performing antimicrobial susceptibility testing, it is essential to include reference strains for internal quality control. When appropriately utilized, the reference strain will provide quality control for both the method and the reagents. If results for the quality control strain are not within the expected parameters, results for the test organisms should not be reported. A high number of laboratories reported results outside the quality control range and especially those who use disk diffusion. Results like this typically arise from inadequate standardization of methodologies or improper storage of disks. For these laboratories, deviations in antimicrobial susceptibility testing can likely be remedied by improving quality control practices. We recommend dispensing different volumes of the test suspension onto the Müller Hinton II agar plates to estimate the volume needed to have all zone diameters of the antimicrobials within the QC ranges if utilizing a cotton swab consistently results in low QC performance.

We believe that several issues have contributed to the overall increase in performance this year. The laboratories received a breakpoint guideline to interpret their obtained MIC results. In addition, guidelines 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.

Almost all of the laboratories had tested strain WHO 7.1 resistant to CTX, CAZ and XNL indicating the strain was ESBL producing. The strain contained the encoding gene $bla_{\text{CTX-M-15}}$.

Susceptibility testing is particularly difficult for certain antimicrobial agents. A high percentage of deviations were observed with: AMP, AUG, POD, SMX, STR, SXT and TET. Problems associated with AUG are often due to a “breakpoint phenomenon” where many strains have values close to the breakpoint causing some to read the strains as intermediate and others as resistant. In addition, beta-lactamase producing strains may have a reduced susceptibility to amoxicillin / clavulanic acid that is sometimes difficult to interpret. Streptomycin often poses a challenge in susceptibility testing as many strains have zone diameters or MICs near the breakpoint. Some laboratories have wanted to discuss the breakpoint of STR and DTU Food will in the near future estimate if the breakpoint should be altered. Tetracycline usually causes deviations but accounted only for 4% in 2007 which is still deemed too high.

Sulfamethoxazole deviations may have been caused by a high content of thymidine and thymine in the media or difficulty in the interpretation of sulphonamide results. Excessive levels of thymidine or thymine have been shown to antagonize the effects of sulphonamides and trimethoprim. Additionally, while most antimicrobials produce clear, definitive zones of inhibition, it is not uncommon to observe light growth near the sulphonamide break point. As such, it is recommended that sulphonamide zone diameters be measured from the point of 80% inhibition, not the point of complete inhibition typically utilized for other classes.

Regional data demonstrate important differences in antimicrobial susceptibility results. Particular focus is required for Africa, Central Asia and the Middle East. The laboratories' continuous participation in the WHO-GSS EQAS in these regions is low and only a few training courses have been conducted by WHO GSS in these regions so far. In addition, unpublished data from the survey conducted in this year indicates that the availability of reagents for many laboratories in developing countries poses a challenge as resources are limited.

Overall, the results indicate 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 discordant results. The factors could be either: demanding strains (difficult to identify, or susceptibility close to breakpoints), difficult reading of the antimicrobial disk diffusion zones or end points of MICs, lack of attention to the QC results, or the methodology. Additionally, transcription errors or random human errors not flagged by in-house quality management system may have occurred.

4.3 Identification of *Campylobacter* strains and the unknown culture

Many of the laboratories had problems with the *Campylobacter* strains due to the fact that they were not viable. We have this year used another procedure to lyophilise the vials and it did not seem to pay off for future EQAS's. We intend to use the previous utilized method to lyophilise the vial contents.

We observed that the laboratories again this year had problems identifying the *C. coli* isolate (74%). It is a minor improvement compared to 2006 (66%) but far from the previous results in 2003 where 83% of participating laboratories identified it correctly. It is surprising that 20 laboratories determine the isolate *C. jejuni* as this is hippurate positive compared to *C. coli*.

Twenty-six laboratories have incorrectly identified strain #1 as either *C. jejuni, C. coli or C. upsaliensis*. 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, 10 laboratories identified it as *C. jejuni* which as mentioned above hydrolyse hippurate.

The unknown isolate was shipped in an inappropriate media for this species which is why it in many cases was not viable on arrival. Eighty-three percent of the 86 laboratories identified the unknown sample containing *Vibrio parahaemolyticus.*

## 5. Conclusion

The serotyping results indicate a continuous need for improving skills in *Salmonella* serotyping. Future training efforts should be aimed at enhancing the capability to detect the flagella phases and disseminating protocols for preparing high quality swarm agar plates. Detection of the phase two flagellar antigen is one of the 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 the use of QC results obtained in optimising and adjusting the methodology as many laboratories seem 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 – *Vibrio parahaemolyticus* despite the problem with the viability of the strains.

## 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 Reference and Research on *Salmonella*, Institute Pasteur, Paris, France.
2. Musto J, Lo Fo Wong D, Wegener HC and WHO GSS members, 2006. World Health Organization Global Salm-Surv – understanding worldwide Salmonella distribution. International Conference on Emerging Infectious Diseases, March 19-22, 2006, Atlanta, Georgia, USA, p. 193-194.

# APPENDIX 1

**Fra:** Discussion group involved in Salmonella surveillance [mailto:GLOBALSALM-SURV@LISTSERV.CDC.GOV]
**På vegne af** Robinson, Cherae' L. (CDC/CCID/NCZVED) (CTR)
**Sendt:** 23. februar 2007 21:29
**Til:** GLOBALSALM-SURV@LISTSERV.CDC.GOV
**Emne:** Signing up for EQAS 2007

**WHO Global Salm-Surv Electronic Discussion Group**
**English Version**
**Message #2007- 4**
**Subject: Signing up for EQAS 2007**

Greetings and Happy New Year, WHO Global Salm-Surv Members,
WHO Global Salm-Surv strives to increase the quality of laboratory-based surveillance of *Salmonella* and other foodborne pathogens. We have just closed the year 2006 WHO Global Salm-Surv External Quality Assurance System (EQAS), and we are now pleased to announce the launch of EQAS 2007.

**WHY PARTICIPATE IN EQAS?**
EQAS provides the opportunity for proficiency testing. Proficiency testing is considered an important tool for the production of reliable laboratory results of consistently good quality.

**WHAT IS OFFERED IN EQAS?**
EQAS offers serogrouping, serotyping and antimicrobial susceptibility testing of eight *Salmonella* isolates, species identification of two *Campylobacter* isolates and identification of one blank bacterial sample.

**WHO SHOULD PARTICIPATE IN EQAS 2007?**
All national or regional reference laboratories performing work on *Salmonella* and *Campylobacter,* interested in participating in a quality assurance program, are invited to participate in EQAS.

We expect that all national or regional reference laboratories that have participated in WHO Global Salm-Surv Training Courses will participate in EQAS.

The list of participants will be evaluated by the WHO GSS Regional Centres in cooperation with the EQAS coordinator. Laboratories which signed up and received strains in year 2006 but **did not submit** any data should explain the reason for this in order to participate in 2007.

**COST FOR PARTICIPATING IN EQAS**
Participation is free of charge. Never the less, we anticipate that laboratories which are capable of paying for shipping the parcel intend to do so. It is possible for laboratories which have an agreement with FedEx and where FedEx serve the country regarding dangerous goods (UN3373) to forward us the import account number. It will save us time and resources.

**SIGNING UP FOR THE EQAS 2007**
**This link will take you to a page where you can sign up for the EQAS 2007:**
**http://thor.dfvf.dk/signup**
You will be asked to fill in the following information:
- Name of institute, department, laboratory and contact person
- Complete mailing address for shipping (not post-office box number)

- Telephone, fax, e-mail
- FedEx import account number if such one is available
- Level of participation in EQAS 2007
- Level of reference function in your country

**If you experience any problems when you sign up electronically, please try again in a few days and contact the EQAS coordinator Rene Hendriksen by e-mail (rsh@food.dtu.dk) or fax ( 7234 6001).**

**SHIPPING AND TIMELINE TO RECEIVE ISOLATES AND PROTOCOLS**

**Shipping of the bacterial isolates will be taken care of by numerous institutes because of the increasing number of participants unless you provide us with a FedEx import account number. You will receive a welcome letter through e-mail with further information. The welcome letter will tell you the name of the institute that is going to send isolates to YOUR laboratory.**

**Please remember to provide the coordinator with a valid import permission in order to minimize delay in shipping the isolates to your laboratory.** It is very important already in this stage to apply for an import permit at your ministry. Every year the final deadline is passed by several months due delayed import permissions and we will try to avoid this in this year. **Please apply for a permit to receive the following "Biological Substance Category B": eight *Salmonella* strains, two *Campylobacter*, one *E.coli* and a blank sample between August and September 2007.**

**The isolates will be shipped in August - September 2007.** Protocols and passwords for entering the results will be provided by e-mail.

**TIMELINE FOR RESULTS TO BE TURNED INTO DFVF**

Results must be returned to the National Food Institute, FOOD-DTU (former Danish Institute for Food and Veterinary Research, DFVF) by 1st of January 2007. When you enter your results via a password protected website, an evaluation report of your results will be generated immediately. Full anonymity is ensured; only FOOD-DTU and the WHO Global Salm-Surv Regional Centre in your region will be given access to your results.

**Deadline for Signing up to participate in EQAS: April the 1st, 2007**

# APPENDIX 2

| WHO Strain no: | Sero-group: | Serovar | Ampicillin, AMP | Amoxicillin + Clavulanic acid, | Chloramphenicol, CHL | Ciprofloxacin, CIP | Cefpodoxime, POD | Ceftiofur, XNL | Ceftazidime, CAZ |
|---|---|---|---|---|---|---|---|---|---|
| WHO S-7,1 | O:7 | Concord | R ( > 32 ) | S ( 8/4 ) | R ( > 64 ) | S ( 0.03 ) | R ( > 4 ) | R ( > 8 ) | R (> 256) |
| WHO S-7,2 | O:9 | Enteritidis | S ( 4 ) | S ( <=2/1 ) | S ( 8 ) | S ( 0.03 ) | I ( 1 ) | S ( 2 ) | S (1) |
| WHO S-7,3 | O:7 | Livingstone | S ( < 2 ) | S ( < 2/1 ) | R ( > 64 ) | S ( 0.03 ) | S ( < 0.25 ) | S ( < 1 ) | S (1) |
| WHO S-7,4 | O:7 | Montevideo | R ( > 32 ) | S ( < 8/4 ) | S ( < 4 ) | S ( 0.03 ) | S ( < 0.25 ) | S ( < 0.5 ) | S (0,25) |
| WHO S-7,5 | O:7 | Mbandaka | S (<1) | S ( < 2/1 ) | S ( < 8 ) | S ( 0.03 ) | S ( < 0.5 ) | S ( < 1 ) | S (1) |
| WHO S-7,7 | O:13 | Poona | S (<1) | S ( < 2/1 ) | S ( < 4 ) | S ( 0.03 ) | S ( < 0.25 ) | S ( < 0.5 ) | S (0,25) |
| WHO S-7,8 | O:7 | Isangi | S ( < 2 ) | S ( < 2/1 ) | S ( < 8 ) | S ( 0.03 ) | S ( < 0.5 ) | S ( < 1 ) | S (0,5) |
| WHO S-7,6 | O:3,10 | Elisabethville | S (<1) | S ( < 2/1 ) | S ( <4 ) | S ( 0.03 ) | S ( < 0.25 ) | S ( < 1 ) | S (0,25) |

| WHO Strain no: | Cefotaxime, CTX | Gentamicin, GEN | Nalidixan, NAL | Streptomycin, STR | Sulfonamid, SMX | Tetracyclin, TET | Trimethoprim, TMP | Sulfonamid + trimethoprim, | ESBL gener: |
|---|---|---|---|---|---|---|---|---|---|
| WHO S-7,1 | R (> 256) | R ( > 32 ) | S ( < 4 ) | R ( > 64 ) | R ( > 1024 ) | R ( > 32 ) | S ( < 4 ) | S (0,25) | SHV-12, TEM-1, CTX-M15/28 |
| WHO S-7,2 | S (1) | R ( > 32 ) | S ( <= 4 ) | R ( > 64 ) | R ( > 1024 ) | S ( < 2 ) | S ( < 4 ) | S (0,125) | |
| WHO S-7,3 | S (0,25) | S ( < 1 ) | S ( < 4 ) | R ( 64 ) | R ( > 1024 ) | R ( > 32 ) | R ( > 32 ) | R (> 32 ) | |
| WHO S-7,4 | S (0,064) | R ( > 32 ) | S ( < 4 ) | R ( > 64 ) | R ( > 1024 ) | S ( < 2 ) | S ( < 4 ) | S (0,25) | |
| WHO S-7,5 | S (0,25) | S ( < 1 ) | S ( < 4 ) | R ( 32 ) | R ( > 1024 ) | R ( > 32 ) | R ( > 32 ) | R ( > 32 ) | |
| WHO S-7,7 | S (0,064) | S ( < 1) | S ( < 4 ) | I ( 16 ) | S ( < 64 ) | S ( < 2 ) | S ( < 4 ) | S (0,125) | |
| WHO S-7,8 | S (0,25) | S ( < 1 ) | S ( < 8 ) | S ( < 4 ) | S ( < 64 ) | S ( < 2 ) | S ( < 4 ) | S (0,25) | |
| WHO S-7,6 | S (0,125) | S ( < 1 ) | S ( < 4 ) | I ( 16 ) | S ( < 64 ) | S ( < 2 ) | S ( < 4 ) | S (0,125) | |

WHO C-7,1 *Campylobacter lari*
WHO C-7,2 *Campylobacter coli*

WHO B-7,1 *Vibrio parahaemolyticus*

National Food Institute

WHO Global Salm-Surv
Building Capacity for the Surveillance and Detection of Foodborne and other infectious Enteric Diseases
www.who.int/salmsurv

# PROTOCOL

For serotyping and susceptibility testing of *Salmonella* and identification of other human pathogens



## 1 INTRODUCTION

In 2000, the WHO Global Salm-Surv network launched an External Quality Assurance System (EQAS). The EQAS is organized by the National Food Institute, Technical University of Denmark (FOOD-DTU), in collaboration with partners and Regional Sites in the WHO GSS.

As in previous years the WHO EQAS 2007 includes serotyping and susceptibility testing of eight *Salmonella* strains, susceptibility testing of one *E. coli* reference strain for quality control (ATCC 25922 (CCM 3954)), identification of two thermophilic *Campylobacter* isolates and identification of one 'unknown' bacterial isolate.

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

For new participants of the EQAS who have not already received the mentioned reference strain, this is included in the parcel. The reference strain will not be included in the years to come. The reference strain is an original CERTIFIED culture and is free of charge. Please take proper care of the strain. Handle and maintain it as suggested in the enclosed manual. Please use it for future internal quality control for susceptibility testing in your laboratory.

## 2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of 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.

## 3 OUTLINE OF THE EQAS 2007

### 3.1 Shipping, receipt and storage of strains

In August/September 2007 around 180 laboratories from all parts of the world will receive a parcel containing eight *Salmonella* strains, two *Campylobacter* strains and one 'unknown' bacterial isolate. The reference strain will be included for participants who have not previously received this. 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 by the enclosed confirmation form**

The reference strain and the *Campylobacter* strains are shipped lyophilised, and the *Salmonella* strains, as well as the 'unknown' isolate are stab cultures. On arrival, the stab cultures must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of lyophilized strains is presented below.

### 3.2 Serotyping of *Salmonella*

The eight *Salmonella* strains 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, and please report the serogroup, since also serogrouping results will be evaluated. When reporting serogroups, please use terms according to Kaufman-White (Popoff and Le Minor, 2001. 8th ed. Popoff, M.U., Le Minor, L., 2001. Antigenic formulas of the Salmonella serovars. WHO Collaborating Centre for Reference and Research on Salmonella), eg. 'O4' and *not* 'group B'.

Please fill in the information on the brand of antisera used in the typing of strains.

If you do not serotype in your laboratory, you may omit serotyping.

### 3.3 Susceptibility testing of *Salmonella* and *E. coli* ATCC 25922

The eight *Salmonella* strains and the *E. coli* reference strain should be susceptibility tested towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods routinely used in the laboratory.

### 3.3.1 Susceptibility testing of *Salmonella*

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

In this EQAS the breakpoints used as a key to interpreting MIC results are a mixture of reference values from CLSI, EUCAST and FOOD-DTU (see list below). This allows three categories of characterisation – resistant, intermediate or sensitive. Interpretations in concordance with the expected value will be categorised as 'correct', whereas deviations from the expected interpretation are categorizes as 'minor' (I ↔ S or I ↔ R), 'major' (S interpreted as R) or 'very major' (R interpreted as S).

As to the breakpoints that you routinely use in your laboratories to determine the susceptibility category we ask you to fill in these breakpoints in the database (or in the test form below).

| **Antimicrobials** | **Reference value, MIC (µg/mL)** | | |
|---|---|---|---|
| | **Sensitive** | **Intermediate** | **Resistant** |
| Ampicillin, AMP* | ≤8 | 16 | ≥32 |
| Amoxicillin + clavulanic acid, AUG* | ≤8 | 16 | ≥32 |
| Cefotaxime, CTX* | ≤8 | 16-32 | ≥64 |
| Cefpodoxime, POD*** | ≤0,5 | 1 | ≥2 |
| Ceftazidime, CAZ* | ≤8 | 16 | ≥32 |
| Ceftiofur, XNL* | ≤2 | 4 | ≥8 |
| Chloramphenicol, CHL* | ≤8 | 16 | ≥32 |
| Ciprofloxacin, CIP** | <0,125 | - | ≥0,125 |
| Gentamicin, GEN** | ≤2 | 4 | ≥8 |
| Nalidixic acid, NAL* | ≤16 | - | ≥32 |
| Streptomycin, STR*** | ≤8 | 16 | ≥32 |
| Sulfonamides, SMX* | ≤256 | - | ≥512 |
| Tetracycline, TET* | ≤4 | 8 | ≥16 |
| Trimethoprim, TMP* | ≤8 | - | ≥16 |
| Trimethoprim + sulfamethoxazole, TMP+SMX, SXT* | ≤2/38 | - | ≥4/76 |

*CLSI **EUCAST ***FOOD-DTU

For ciprofloxacin, please note that a low breakpoint has been used to determine resistance category. Considering the expected results of this EQAS, microorganisms are considered resistant to ciprofloxacin when showing reduced susceptibility to this antimicrobial.

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

All strains categorized reduced susceptibility against cefotaxime (CTX) or ceftazidime (CAZ) (MIC > 1 and MIC > 1 respectively) or resistance against ceftiofur (XNL) (MIC > 8) could be confirmed by confirmatory tests for ESBL production.

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

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

### 3.4 Identification of *Campylobacter* and the unknown isolate

The two thermophilic *Campylobacter* isolates should be identified to species level. The 'unknown' isolate 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.

#### 3.4.1 Suggested procedure for reconstitution of lyophilised strains

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

a) Open the ampoule. Take out some of the material and dissolve it in 0,5 ml appropriate broth. Leave it for 10 minutes. Inoculate the solution on a non selective agar plate (*E. coli*) or on a blood agar plate (*Campylobacter*) using either a 1 µl loop or a cotton swab. Incubate at 35ºC in ambient air for 16-18 h (*E. coli*) or microaerophilic for 24-48 h at 37ºC or 42ºC (*Campylobacter*).

b) Incubate the remaining culture/broth in the vial/ampoule as mentioned above (seal the vial/ampoule with parafilm if necessary). After incubation re-inoculate the culture using either a 1 µl loop or a cotton swab on none selective agar or blood agar as described above and incubate.

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

## 4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the enclosed test form and enter your results into the interactive web database. Please read the detailed description below before entering your results. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print an evaluation report of your results. **Please submit results by latest January 1st, 2008**. If you do not have access to the Internet or if you experience difficulties entering the data, please return results by fax or mail to the National Food Institute.

All results will summarized in a report which will be made available to all participants. Individual results will be anonymous and will only be 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:**

Mr. Rene Hendriksen

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## 5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

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

In general you navigate in the database with the Tab-key and mouse, and at any time a click on the WHO logo takes you back to the main menu.

1) Enter the WHO Global Salm-Surv web page (http://www.who.int/salmsurv/en), then
   a. Click on ‘GSS Activities’
   b. Click on the link ‘http://www.who.int/entity/salmsurv/activities/GSS_EQAS/en’
   c. Click on ‘Data entry for the year 2007’
   d. Write your username and password in lower case letters and click on ‘Login’.
      In the letter following your parcel you can find your username and password.
      Your username and password will be the same in future trials.

2) Click on 'Materials and methods'
    a. Fill in the brand of antisera (Very important as we would like to compare results with the brand of the antisera)
    b. Fill in the method used for susceptibility testing
    c. Enter the brand of accessories, e.g. Oxoid
    d. Fill in whether your institute serves as a national reference laboratory
    e. Click on 'Save and go to next page' - REMEMBER TO SAVE EACH PAGE LIKE THIS!

3) In the data entry page 'Routinely used breakpoints'
    a. Fill in the breakpoints that you routinely use in your laboratory to determine the susceptibility category. Remember to use the **operator keys** in order to show – equal to, less than, less or equal to, greater than or greater or equal to.

4) In the data entry pages '*Salmonella* strains 1-8', you
    a. 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 "Serotype" only contains serotypes belonging to the chosen serogroup.
    b. SELECT the serotype from the pop-up list – DO NOT WRITE – wait a few seconds and you can enter the antigenic formula (e.g. 1,4,5,12:i:1,2)
    c. Enter the zonediameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to, etc.
    d. Enter the interpretation as R, I or S
    e. If you have performed confirmatory tests for ESBL producing strains, please choose the test result from the pick list
    f. Fill in comments if relevant e.g. which antisera you miss for complete serotyping
    g. Click on 'Save and go to next page'

**If you have not performed these tests please leave the fields empty**

5) In the data entry page '*E. coli* reference strain':
    a. Enter the zonediameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to, etc.
    b. Click on 'Save and go to next page'

6) In the page 'Identification of *Campylobacter* and unknown sample':
    a. Choose the correct *Campylobacter* species from the pick list
    b. Fill in the species and type of the unknown bacterial isolate, and fill in the method used
    c. Click on 'Save and go to next page'

**If you have not performed these tests please leave the fields empty**

7) The next page is a menu, from where you can review the input pages or approve your input *and finally see and print the evaluated results*
    a. Go through the input pages make corrections if necessary. Remember to click on 'save and go to next page' if you make any corrections.

b. Approve your input. Be sure that you have filled in all the results before approval, as YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database, but allows you to see the evaluated results.
c. As soon as you have approved your input, an evaluation report will show. You can print each page, if you want to. You may have to choose a smaller text size to print the whole screen on one piece of paper. In the Internet Explorer (or the Internet program you may have), you click on 'view', 'text size' and e.g. 'smallest'.

8) 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'.

**End of entering your data – thank you very much!**

National Food Institute

# SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

## 1.1 Purpose

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has published a guideline for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test results.

## 1.2 References

M100-S17, January 2007 (Performance Standards for Antimicrobial Susceptibility Testing)

M07-A6, January 2003 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Approved Standard)

## 1.3 Definition of Terms

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

Reference Stock Culture: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

Working Stock Cultures: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

Subcultures (Passages): A subculture is the transfer of established growth to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time

## 1.4 Important Considerations

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC or CCM
- CLSI requires that QC is performed either on the same day or weekly (only after 30 day QC validation)
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides

- Periodically perform colony counts to check the inoculum preparation procedure
- Ideally, test values should be in the middle of the acceptable range
- Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems

### 1.5 Storage of Reference Strains

Preparation of stock cultures

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

Working cultures

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

### 1.6 Frequency of Testing

Weekly vs. daily testing

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

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

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

Corrective Actions

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

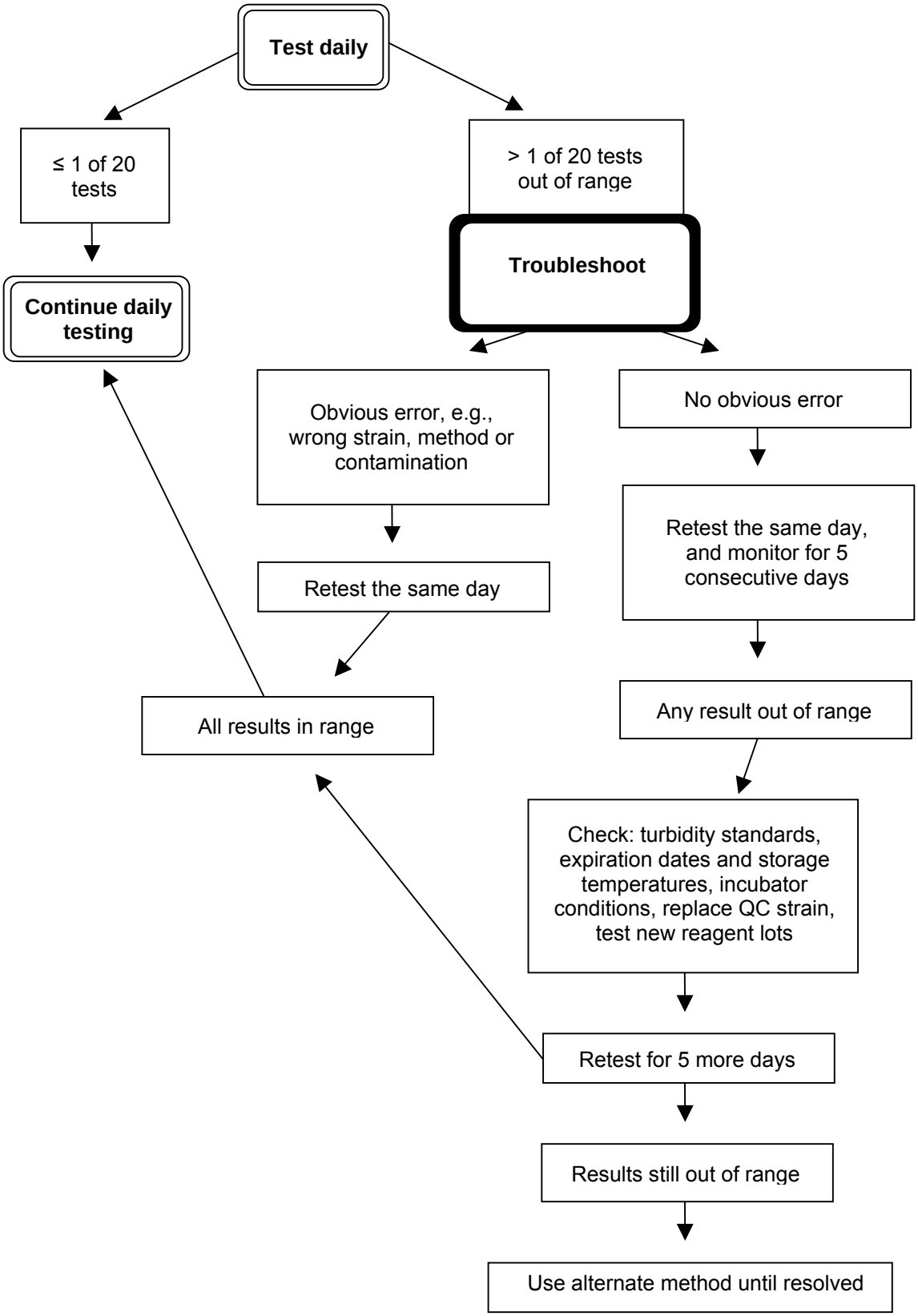
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

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

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

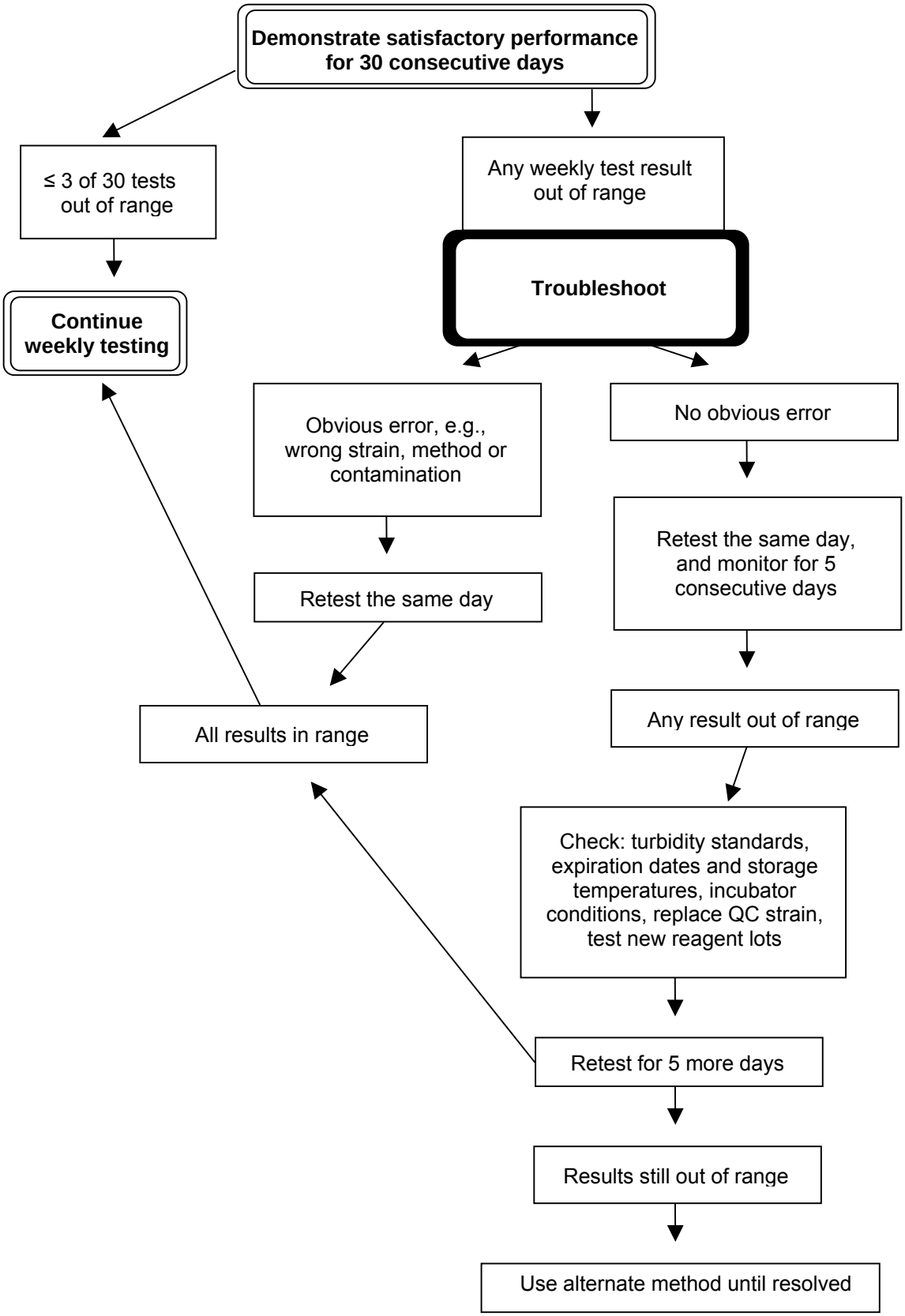
Repeat the 30 days validation before resuming weekly testing.

## 2 DAILY MIC QC CHART




Modified from CLSI M7-A6, page 35

## 3 WEEKLY MIC QC CHART




Modified from CLSI M7-A6, page 36

# INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Manual from Czech Collection of Microorganisms (CCM)
Masaryk University
Tvrdého 14
602 00 BRNO
Czech Republic

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

a. Check the number of the culture on the label inside the ampoule
b. Make a file cut on the ampoule near the middle of the plug
c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end
d. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule
e. Remove the pointed end of the ampoule into disinfectant
f. Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media
g. Incubate the inoculated medium at appropriate conditions for several days
h. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

Please note that:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue
- Cultures may need at least one subculturing before they can be optimally used in experiments
- Unopened ampoules should be kept in a dark and cool place!

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