The External Quality Assurance System of the WHO Global Salm-Surv, Year 2008

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1. Introduction

In January 2000, WHO launched an international effort to enhance *Salmonella* surveillance, including laboratory support for laboratory-based surveillance, the "WHO Global Salm-Surv" (WHO GSS). The focus of WHO GSS was to enhance WHO Member States' capacity to detect and respond to foodborne diseases outbreaks by conducting surveillance of *Salmonella.* Since its' inception, WHO GSS has expanded to include additional, important foodborne pathogens, especially *Campylobacter*. *Salmonella* and *Campylobacter* are among the most important foodborne pathogens. These organisms account for millions of cases of diarrhoeal disease and thousands of deaths per year and impact both developing and industrialized countries. Furthermore, there is growing concern over increasing antimicrobial resistance in *Salmonella* and infections with resistant *Salmonella* are associated with increased morbidity and mortality.

To support laboratories participating in WHO GSS, an External Quality Assurance System (EQAS) programme was established in 2000. The goal of this programme was to assess the quality of *Salmonella* serotyping and antimicrobial susceptibility data produced by Member States and to enhance the reliability of this data by identifying areas which could benefit from additional support. The program was expanded in 2003 to include other foodborne pathogens, and the number of participants submitting data to one or more components of the EQAS has increased from 44 laboratories in 2000, to 187 laboratories in 2008. WHO GSS has set a goal of having all national reference laboratories perform *Salmonella* serotyping with a maximum error rate of 13% and susceptibility testing with a maximum error rate of 10 % (either < 5% very major / major errors and <5 % minor errors, or < 10% minor errors).

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; Public Health Agency of Canada (PHAC); and Institute Pasteur (IP) in Paris, France. The technical advisory group for the WHO EQAS programme consists of members of the WHO GSS Steering Committee.
Individual laboratory data is kept confidential and is only known to participating laboratory, the EQAS Organizer (DTU Food) and the respective WHO GSS regional centre. All summary conclusions are made public.

2. Materials and Methods

2.1 Participants

A pre-notification announcement of the 2008 EQAS was made through the WHO GSS list server March 25, 2008 and a reminder was sent April 3, 2008 (App 1). The pre-notification was available in English, Spanish, Portuguese, French, Chinese and Russian. The pre-notification included invitations to participate in the 2008 EQAS programme for serotyping and susceptibility testing of *Salmonella*, identification of *Campylobacter*, and identification of an unknown foodborne pathogen. In addition, countries in Latin America were offered the opportunity to participate in an EQAS pilot programme for serotyping and susceptibility testing of *Shigella* spp. 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* and two strains of *Campylobacter* were selected for the 2008 EQAS from the National Food Institute's strain collection. The unknown foodborne pathogen (*Enterobacter sakazakii*) was selected by Institut Pasteur (IP) and the four *Shigella* isolates were selected by Public Health Agency of Canada (PHAC). Individual sets of the *Salmonella, Shigella* and *Enterobacter sakazakii* strains were inoculated as agar stab cultures and the *Campylobacter* strains were lyophilised in glass vials. The serotype of each *Salmonella* strain was designated on the basis of O (somatic) and phase 1 and phase 2 H (flagellar) antigens according to scheme of Kaufmann-White (2007). *Salmonella* serotype was determined by DTU-Food and verified by the CDC and IP prior to distribution. In addition, CDC verified the susceptibility patterns of the *Salmonella* and *Shigella* strains. All of the *Shigella* isolates were serotyped by PHAC and the National *Salmonella* and *Shigella* Center (NSSC), National Institute of Health, Thailand

verified antimicrobial susceptibility and serotype, respectively. All results were later confirmed at DTU-Food.

Furthermore, laboratories which did not participate in 2007 EQAS 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 Antimicrobials

Antimicrobial susceptibility testing (AST) on the *Salmonella* strains were performed at the DTU-Food and the obtained results served as a reference standard. The following antimicrobials were used in the 2008 EQAS: ampicillin, AMP; cefotaxime, CTX; ceftazidime, CAZ; ceftriaxone, CRO; chloramphenicol, CHL; ciprofloxacin, CIP; gentamicin, GEN; nalidixic acid, NAL; streptomycin, STR; sulphonamides, SMX; tetracycline, TET; trimethoprim, TMP and trimethoprim + sulphonamides, SXT. In addition, it was also possible to confirm the presence of ESBL producing isolates by using the antimicrobials CTX and CAZ in combination with the inhibitor clavulanic acid (App. 2).
Minimum Inhibitory Concentration (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 (2006) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically"; Approved Standard - Seventh Edition, document M100-S18 (2008) "Performance Standards for Antimicrobial Susceptibility Testing"; Seventeenth Informational Supplement and document M31-A3 (2008) "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 DTU Food) (App.3).

2.4 Distribution

The cultures 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 most laboratories were informed about the dispatched parcels and the air way bill (AWB) number for tracking of the parcel and pick up at the airport. Import

permits were necessary for shipping the parcels to a large number of countries. Many of the parcels were shipped as "overpack" through international hubs which offered to support the costs of further distributing the parcels. Helen Tabor from PHAC; Canada, Matt Mikoleit from CDC; United States, Aroon Bangtrakulnonth from NSSC; Thailand, Enrique Perez from Health Surveillance, Disease Prevention and Control; Brazil, Francois Xavier Weill from IP; France, Rita Tolli from Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Italy and Ma Yue from National Institute For The Control Pharmaceutical And Biological Products, China shipped to all Canadian, North American, Thai, Latin American, Francophone African, Italian and Chinese institutes, respectively. The first parcel was dispatched from DTU-Food August 29, 2008 and November 24, 2008 – requested by a Belgian participant.

2.5 Procedure

The laboratories were instructed to download the protocol and documents (App. 3) available in English only from http://www.antimicrobialresistance.dk/. In addition, they were requested to subculture the strains prior to performing the method routinely used by their laboratory. The testing included serotyping and susceptibility testing of eight *Salmonella* and four *Shigella* (Latin America only) strains, susceptibility testing of one quality control strain (*E. coli* CCM 3954 / ATCC 25922), identification of two *Campylobacter* strains and identification of an unknown foodborne pathogen (*Enterobacter sakazakii*). Furthermore, the laboratories were requested to save and maintain the ATCC reference strain for future proficiency tests according to App. 4ab.

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* and *Shigella* 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 database was activated September 1, 2008 and closed March 31, 2009.

The *Salmonella* and *Shigella* strains were categorised as resistant (R), intermediate (I) or susceptible (S) against the tested antimicrobials. Antimicrobials were intended to be interpreted individually with exception of cephalosporins which were interpreted according to CLSI Approved Standard - Seventh Edition, document M100-S18 (2008) "Performance Standards for Antimicrobial Susceptibility Testing, Table 2A". Laboratories were instructed to use the same antimicrobials and *Salmonella / Shigella* 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 were requested to enter 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 secure web site. 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). 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 199 laboratories responded to the pre-notification, and were enrolled in the EQAS. When the deadline for submitting results was reached, 187 laboratories in 89 countries had uploaded data. The following countries provided data to at least one of the EQAS components (also shown below in Figure 1):

Albania, Algeria, Argentina, Australia, Barbados, Belarus, Belgium, Bolivia, Bosnia and Herzegovina, Brazil, Brunei, Bulgaria, Cambodia, Cameroon, Canada, Central African Republic, Chile, China, Colombia, Costa Rica, Cuba, Croatia, Cyprus, Czech Republic, Democratic Republic of Congo, Denmark, Ecuador, Egypt, Estonia, Ethiopia, Finland, France, Ghana, Georgia, Greece, Honduras, Hungary, India, Ireland, Israel, Italy, Ivory Coast, Jamaica, Japan, Jordan, Korea, Lithuania, Luxembourg, Macedonia, Madagascar, Malaysia, Malta, Mauritius, Mexico, Moldova, Morocco, Namibia, Nepal, The Netherlands, New Zealand, Nicaragua, Oman, Panama, Paraguay, Peru, Philippines, Poland, Russia, Saudi Arabia, Serbia, Senegal, Slovak Republic, Slovenia, Sri Lanka, South Africa, Sudan, Suriname, Taiwan, Thailand, Trinidad and Tobago, Tunisia, Turkey, United Kingdom, United States of America, Uruguay, Venezuela, Vietnam, Yemen and Zambia.

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

Of the 187 laboratories submitting results, 170 (91%) of the laboratories participated in the *Salmonella* serogrouping component whereas 151 (81%) participated in some of or in the entire *Salmonella* serotyping element of the program. Thirty laboratories were invited to participate in the *Shigella* pilot of the four isolates. Of these laboratories, 15 (50%) submitted *Shigella* serogroup results (speciation) whereas nine laboratories further analysed the isolates resulting in a serotype.

Of the 187 laboratories submitting results, 168 (90%) submitted antimicrobial susceptibility results for *Salmonella*. 147 (88%) of these laboratories submitted results for the quality control (QC) strain *E. coli* ATCC 25922. The majority of laboratories (n=114) used disk diffusion, a MIC method was utilised by a smaller number of laboratories (n=33). The antimicrobial susceptibility testing of *Shigella* was performed by 14 laboratories.

Information on which MIC breakpoints have been used for interpretation of the expected values was disseminated to all participants. However, this did not include equivalent breakpoints for disk diffusion. In addition, all participants were instructed in how to interpret resistance to 3rd generation cephalosporins and fluoroquinolones.

Of the 154 laboratories that requested to participate in the *Campylobacter* module, 105 (68%) reported results for both *Campylobacter* strains.

The unknown culture was correctly identified by 92% of the 128 participating laboratories.

3.2 *Salmonella* serogrouping and serotyping

The percentage of laboratories that reported complete serotype results on all eight strains in 2008 decreased for the second year in a row to 66% (n=100). This is a decline from 81% (n=105) in 2006 and 78% (n=109) in 2007. The proportion of correct serotype results decreased in 2008, from 88% correct tests (n=920) in 2007 to 83% correct tests (n=888) in 2008 (Table 1).

Table 2 illustrates the number of participating laboratories versus the number of correctly serotyped samples. In 2008, 50 of 151 (33%) participating laboratories serotyped all eight strains correctly and an additional 36 (24%) laboratories correctly serotyped seven of the eight strains. In total for 2008, 86 (57%) participating laboratories met the threshold for adequate performance of *Salmonella* serotyping in 2008.

This is a decrease compared to 2007 where 68% met the quality threshold. Additionally, 13 % of laboratories correctly identified two or fewer strains, nearly a two-fold increase from 2007 (5%).

Table 3 summarises serotyping performance by region. As compared to 2007, a decline in the accuracy of serotyping results was observed in many of the regions. The accuracy of EQAS serotyping submitted by laboratories in China increased from 91.3% in 2007 to 94.4% in 2008. Between 2007 and 2008, China was also the region which experienced the largest influx of EQAS participants. During this time, the number of participating laboratories increased, from 10 in 2007 to 15 in 2008. The largest decrease in accuracy was observed in the African region where 49.3% had serotyped the isolates correctly compared to 80.8% in 2007. Compared to 2007, similar trends were observed in other regions such as Southeast Asia, and Latin America where declines of: 9.8%, and 17.1%, respectively.

The overall performance of *Salmonella* serogrouping is satisfactory as the percentages of deviations are very low for all the test strains, ranging from one deviation (0.6%) (WHO 8.1) to 11 deviations 6.4% (WHO 8.6) with an average of 2.9% (Table 5). Two strains (WHO 8.5 and WHO 8.6) seem to have been problematic. The strain WHO 8.5 was a *Salmonella* Meleagridis (I 3,10:e.h:l,w) which was serotyped by 169 laboratories. The laboratories found the following deviations O:4 (n=2), O:8 (n=2), O:9 (n=2) and O:1,3,19 (n=4). Strain WHO 8.6 (*Salmonella* Blockley; I 6,8:k:1,5) was tested by 172 laboratories and resulted 11 deviations: O:2 (n=1), O:4 (n=2), O:7 (n=6), O:9 (n=1) and O:6,14 (n=1).

The majority of the laboratories (n=139) serotyped the internal quality control strain (used in 2000, 2001, 2004, 2006, 2007) WHO 8.3 correctly (96%) leading to a low deviation rate of only 4.1%. Table 4 illustrates the laboratories' ability to serotype the internal quality strain correctly. Furthermore, this ability seems to be consistent in the years it has been used despite the increase of participating laboratories.

The deviations of the serotyping results ranged between 4.1% – 34.1%. The majority of the strains resulted in a high proportion of deviations with four strains having more than 20% of deviations. Only the *Salmonella* Enteritidis isolate was serotyped satisfactorily with 4.1% deviations.

3.3 Antimicrobial susceptibility testing of *Salmonella*

A total of 13,858 antimicrobial susceptibility tests were performed in 2008 by 168 participating laboratories. Of these, 91% were in agreement with the expected results (Table 6). A total of 4% minor, 2% major and 3% very major deviations were observed.

Strain WHO 8.2 and WHO 8.6 caused major difficulties to the antimicrobials tested with four and five antimicrobials causing a high degree of deviations, respectively (Table 7). In addition, streptomycin (STR) and tetracycline (TET) seem to be the hardest antimicrobials to test correctly.

Tables 7 and 8, summarise major deviations by antimicrobial. Some antimicrobials in particular seem to be particularly problematic for many laboratories. A disproportionately large number of critical deviations were observed for: CIP (19%), STR (7%) and TET (6%). The same antimicrobials with exception of CIP also result in very high numbers of "total deviations" (Table 8). The number of critical and total deviations observed overall in 2008, was 5% and 9%, respectively.

In the 2008 EQAS trial, we have included confirmatory ESBL testing of the eight *Salmonella* isolates. Isolate WHO S-8.6 was an ESBL producing *Salmonella* strain harbouring the $bla_{\text{TEM-52}}$. Sixty-five and 68 of the participants conducted confirmatory testing utilising ceftazidime / cefotaxime and ceftazidime with clavulanic acid / cefotaxime with clavulanic acid. The participants had 97% and 91% correct results in the tests for ceftazidime and cefotaxime, respectively (Table 9). Although the other seven test strains were non–ESBL producers, some laboratories reported ESBL testing results for these isolates, with up to one laboratory reporting an incorrect result.

The overall percentage of deviations varied by region with a high percent of both critical and total deviations in the Central Asia & Middle Eastern Region (7.4% / 13.9%) and Africa (9.7% / 16.2%) (Table 10).

In Table 11ab, 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. Thirty-three laboratories tested the reference strain using the MIC determinations and 114 laboratories used the disk diffusion method in 2008 (Table 11b).

No laboratory utilising an MIC method, reported deviations to: AMP, CHL, GEN, NAL and TET. The percentages of laboratories which had values exceeding the quality control intervals ranged from 5% (CAZ) to 13% (TMP).
In contrast, when tested by disk diffusion, all antimicrobials resulted in at least one deviation. Participating laboratories appeared to have more difficulty with specific antimicrobials: AMP (16%), CTX (15%), SMX (15%) and SXT (14%). Of note, this is virtually the same list of antimicrobials which caused the majority of deviations in 2007.

3.4 Identification of *Campylobacter* strains

Strains #1 and #2 were both *Campylobacter lari* which was successfully recovered by 105 laboratories. The laboratories performed correct species identification in 63% and 60% of the cases for strain #1 and #2, respectively (Table 12). Incorrect identifications for strains # 1 & # 2 included *Campylobacter jejuni, Campylobacter upsaliensis* and *Campylobacter coli*; these incorrect responses were reported with similar numbers for both strains.

3.5 Identification of the unknown culture

A total of 128 laboratories submitted identification results for the unknown enteric pathogen (*E. sakazakii).* Ten laboratories reported deviating results *E .coli* (1), *Salmonella* Haart (1), *Shigella spp* (1), *Enterobacter cloaccae* (4), *Hafnia alvei* (1), *Aeromonas hydrophila* (1), *Pasteurella pnemotropica* (1) (Table 13).

3.6 *Shigella* serogrouping and serotyping

In 2008, WHO GSS piloted a *Shigella* serotyping and AST trial in Latin America. Fifteen laboratories participated in this initial trial. The number of laboratories reporting serogrouping results varied by sample and ranged from 15 (WHO SH 8.2) to 10 (WHO SH 8.4). Single deviations were reported for WHO SH 8.1, WHO SH 8.3, and WHO SH 8.4. All 15 participants correctly identified WHO SH 8.2. (Table 14).
Serotype was determined by a smaller number of participants. The number of participants who reported subserotype ranged from zero to nine, no deviations were reported among this group.

3.7 Antimicrobial susceptibility testing of *Shigella*

The 15 laboratories which performed susceptibility testing of the four *Shigella* isolates had in total 95% correct with only 3% critical errors (Table 15).

The laboratories experienced some difficulties testing *Shigella* isolates against two antimicrobials. It appears that STR and TET were responsible for most of the deviations; representing 9% and 8% of total deviations, respectively (Table 16).

4. Discussion

4.1 *Salmonella* serogrouping and serotyping

In the 2008 WHO GSS EQAS trial, the selection of serovars was again based on the 15 most common serovars submitted to the WHO GSS Country Data Base (CDB) http://www.antimicrobialresistance.dk. We chose both very common and rare serovars in order to facilitate the worldwide assessment of *Salmonella* serotyping capacity. This year, we have included *Salmonella* sers. Oranienburg and Javiana which both are ranked among the top 15 most common serovars in Latin and North America. In addition, we chose *Salmonella* Thompson which happened to be one of the top 15 serovars in North America and which is also observed in many of the other regions, albeit at a lower frequency. We also included *Salmonella* Indiana which often has been reported in Europe and has been recovered from humans, animals and feed. In addition, we selected *Salmonella* Blockley a serovar more frequently reported from Europe. This year, we also decided to add two serovars which are more rarely found. *Salmonella* Meleagridis, which was observed from time to time in Europe and a much rarer serovar, *Salmonella* Hiduddify. *Salmonella* Hiduddify was first isolated in Tubingen, Germany in 1970 (Bader *et al*, 1972) and later reported in 1978 when it was isolated from the intestines of dogs in the Northern part of Nigeria (Britt *et al,* 1978). In 2006, a report on *Salmonella* Hiduddify infection in three infants in Los Angeles emphasised the zoonotic importance of this serovar (Special Studies Report, 2006). In 2009, a publication by (Raufy *et al*) found that 39 out of the 41 samples from free range chickens from Nigeria yielded *Salmonella* Hiduddify.

This year, we observed a further decrease in the number of laboratories which were able to serotype all eight strains correctly and a relatively large decrease in the total number of correctly serotyped isolates (Table 1). We believe the reason behind this result was caused primarily by the fact that the selection of the *Salmonella* strains seemed to be much harder to serotype than anticipated. In addition, it appears that this year we have more participants from developing countries compared to previous years. Based on our

experiences from the WHO GSS capacity building laboratory training course, we have learned, that countries with limited resources typically have access to limited panels of antisera and often encounter difficulties serotyping isolates that are not among the most common serovars.
The quality threshold of having at least seven strains correctly serotyped was met by only 57% - a decrease of 11% compared to 2007. In addition, the proportion of laboratories serotyping three or less isolates correct increased in 2008. This again, emphasizes that the panel of isolates in 2008 was more difficult to serotype compared to previous years.

Ninety-six percent of the laboratories correctly serotyped the internal control strain (WHO 8.3). This is the same percentage observed in 2007, which until that time, was the highest score observed to date (Table 4). In addition, the number of participants which serotyped the isolate correctly increased by four to 139. This is truly an impressive accomplishment which most likely is a result of the WHO GSS laboratory training courses, recommendations from the regional centres to participating labs on the need to utilise high quality antisera and even support to some laboratories with obtaining antisera.

While this result indicates that most laboratories worldwide have the capacity to serotype the most common serovars, (Table 3) the data also shows that some regions still lack access to reliable antisera necessary to identify other regionally prevalent serovars. The highest percentages of serotype deviations were observed in the following regions: Africa (49%), Caribbean (79%), Latin America (72%) and the Central Asia and Middle East (62%). Many countries in these regions have fewer resources available for the laboratories, and some have problems importing the needed antisera. This is an important observation as the WHO GSS would like to rely on the data uploaded to the country databank (CDB) with regards to serotype prevalence. In total, many regions appeared to obtain results from the serotyping which were less correct compared to 2007 with a few exceptions. In contrast, this year, the percent of correctly serotyped isolates dropped from 81% to only 49% in the African region, illustrating that this region needs support in retrieving a broader panel of antisera.

The problems in obtaining the correct serotype appear to have been largely due to the difficulties in characterising the flagellar antigens. One may speculate that this issue may be due to a lack of quality antisera, as laboratories often correctly identified the O antigen and one of the two flagellar antigens. In other cases the laboratories correctly indentified the O antigen and flagellar antigen complex but incorrectly identified the minor antigens within the complex. This theory is supported by findings from,

WHO 8.1 (Oranienburg / I 6,7,14:m,t:[z_{57}]), a G complex strains which accounted for a large number of deviations (33.3%) in 2008. A total of 25 laboratories reported the isolate as being *Salmonella* Othmarschen (I 6,7,14:g,m,[t]:-) and five laboratories reported this as *Salmonella* Montevideo (I 6,7,14:g,m,[p],s:[1,2,7]). For all of the three serovars one could expect not to find any second phase of the flagellar antigen but all of them differ in the G- complex with the single factors "t", "g" and "s". Isolates WHO 8.4, WHO 8.5 and WHO 8.8 all contained a L-complex which in these cases resulted in deviations of 26.8%, 24.2% and 18.3%, respectively. For WHO 8.4 (Javiana / I 1,9,12:l,z_{28}:1,5), 17 laboratories reported *Salmonella* Panama (I 1,9,12:l,v:1,5) and five laboratories *Salmonella* Itami (I 9,12:l,z_{13}:1,5) – all deviating first phase of the flagellar antigen. Similarly, with the WHO 8.8 (Hiduddify / I 6,8:l,z_{13},z_{28}:1,5) the problem lies with the first phase of the flagellar antigen as nine laboratories submit the isolate as being *Salmonella* Loanda (I 6,8:l,v:1,5). In contrast to the described two cases, the isolate WHO 8.5 (Meleagridis / I 3,10,[15],[15,34]:e,h:l,w) results in deviations due to the second phase of the flagellar antigen as 16 and three laboratories report it as being *Salmonella* Assinie (I 3,10:l,w:z_6) and *Salmonella* London (I 3,10,[15]:l,v:1,6), respectively. One could speculate that some of the 16 laboratories have guessed the isolate being *Salmonella* Assinie (I 3,10:l,w:z_6) if they initially have found the somatic and the l,w flagellar phase. One would note that only one serovar has a somatic formula of "3,10" and a first phase flagellar antigen of "l,w" if browsing through the Kaufman White scheme. One could therefore speculate if these laboratories have skipped testing the second phase of the flagellar antigen and simply guessed that the isolate was *Salmonella* Assinie (I 3,10:l,w:z_6). Unfortunately, Meleagridis / I 3,10,[15],[15,34]:e,h:l,w has the flagellar phase "l,w" listed as the second flagellar phase in the Kaufman White scheme. The isolate WHO 8.2 (Thompson / I 6,7,14:k:1,5) also resulted in many deviations. In contrast to the problems due to the G and L complexes, the deviations to Thompson was associated with one serovar in particular but to a vast panel of serotypes all differing from the antigenic formular of Thompson on the first flagellar phase. However, the nine deviations in this rapport (*Salmonella* Haart / I 8:k:1,5) to WHO 8.6 (Blockley / I 6,8:k:1,5) have not accounted as an error due to colonial form variation by minor O antigen (O:6_1).

4.2 Antimicrobial susceptibility testing of *Salmonella*

Over-all, the percentage of correct susceptibility testing of *Salmonella* was 91% with 5% critical deviations (Table 6). This is considered to be extremely satisfactory compared with the previous year as we in 2008 have increased the number of participants from 143 in 2007 to 168 in 2008 without jeopardising the fine results obtained the previous years.

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. Despite of the success described above, only 147 laboratories submitted results of the quality control strain (QC). We always encourage laboratories to conduct quality assurance when performing antimicrobial susceptibility testing. To facilitate internal QC, we provide each new participating laboratory with the QC strain *E.coli* ATCC 25922. Participants from previous years are asked to retain and maintain the QC strain for future use. If ≤3 out of 30 results out of range 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. For example, if utilizing a cotton swab consistently results in QC failures, we recommend dispensing different volumes of inoculum onto Müller Hinton II agar plates to determine the inoculation volume needed to have bring your results back into QC range.

Compared to previous years, we believe the issues observed in 2007, have contributed to this year's overall increase in performance. The participating laboratories were provided with MIC breakpoint guidelines. Many laboratories utilised CLSI guidelines, which instruct laboratories to extrapolate resistance to all cephalosporins, regardless of MIC, when resistance to one cephalosporin is observed. To insure that laboratories would not be penalized for reporting epidemiologic data, additional interpretive guidance for cephalosporins was also provided. The protocol also highlights that the expected results of certain antimicrobials (ceftazidime, cefotaxime, ceftriaxone and ciprofloxacin) were based on epidemiological cut off value defined by EUCAST. Thus for ciprofloxacin, a low breakpoint has been used to determine the resistance category why showing reduced susceptibility to this antimicrobial indicate the isolate being resistant to ciprofloxacin.

Susceptibility testing is particularly difficult for certain antimicrobial agents. A high percentage of deviations were observed with ciprofloxacin, streptomycin and tetracycline. Participants had obtained less than 90% correct results for two of the eight isolates tested against ciprofloxacin. Both of these isolates were expected to be resistant (reduced susceptibility) with MIC values of 1 µg/ml and 0.12 µg/ml. It is

likely to believe that for the majority of the participants the interpretation of these two isolates was based on CLSI breakpoints rather than according to EUCAST cut off values. Using epidemiological cut off values according to EUCAST for ciprofloxacin enable the user to detect both low level resistance caused by one point mutation in the *gyrase* gene and the recently discovered plasmid mediated quinolone resistance caused by the Qnr genes. In both cases, the strains exhibiting these genotypes will be interpreted as susceptible according to CLSI clinical break points and eventually results in treatment failure. Streptomycin often poses a challenge in susceptibility testing as many strains have zone diameters or MICs near the breakpoint. The results again this year illustrate the difficulties with this antimicrobial as less than 90% of the participants had errors in seven of the eight isolates. DTU Food has launched a study among 17 laboratories from Europe, China and the North America to establish a more exact breakpoint. The results ought to be ready to disseminate with the protocol of the EQAS 2009. Tetracycline again causes deviations which might be caused by the antimicrobial sensitivity to the pH of the Müller Hinton media used or that the CLSI clinical break point should be re-considered.

Regional data demonstrate important differences in antimicrobial susceptibility results. Particular focus is required for Africa and 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 last 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 continuous 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

We selected two *Campylobacter lari* isolates for inclusion in this year's EQAS. Up to 49 laboratories reported viability problems with the *Campylobacter* strains. In contrast to 2007, this year we used lyophilised vials prepared at DTU- Food. Unfortunately in 2008, we encountered shipping delays which

may have contributed to some of the viability issues reported by participants. Many laboratories had problems identifying the isolates. Compared with the *C. lari* isolate from 2007 we observed a decrease in correct identification from 72% in 2007 to 60% and 63% (for strains #1 and # 2 respectively) in 2008.

We were encouraged to select *Enterobacter sakazakii* as the unknown isolate as this agent is a rare cause of invasive infection with historically high case fatality rates (40–80%) in infants. It can cause bacteraemia, meningitis and necrotising enterocolitis and has been associated with the use of powdered infant formula even after extended period of storage for more than 2 years.
Ninety-two percent of the 128 laboratories identified the unknown sample containing *Enterobacter sakazakii* which was found to be satisfactory.

4.6 *Shigella* serogrouping and serotyping

Based on the evaluation survey conducted in 2007, we decided to launch an EQAS pilot on serotyping and susceptibility testing of *Shigella spp*. in the Latin American region. It was impressive to see that up to 15 laboratories serotyped the isolates with only a maximum of one deviation and up to nine laboratories performed serotyping on a subset of the isolates. PAHO proficiency and training programmes may help to account for the success of the *Shigella* component. *Shigella* is endemic in this region and PAHO programmes have insured that the participating laboratories are well trained and experienced in the identification and typing of *Shigella*.

4.7 Antimicrobial susceptibility testing of *Shigella*

The data of the susceptibility testing for *Shigella* reveal the exact same problems as for *Salmonella*. We observed the same high percentages of deviations to tetracycline and streptomycin. The reasons for these deviations have already been discussed in the *Salmonella* section.

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. In addition, the results show that many of the laboratories in developing countries still need supplies of antisera to facilitate serotyping of isolates with rare antigenic formulae.

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. This year's data also show that many of the laboratories were able to conduct a satisfactory confirmatory test on the ESBL producing strain. It is important to draw attention to this in the future as we have to recognise the increase of resistance to 3rd and 4th generation of cephalosporins.

Many of this year's participants had difficulties identifying *Campylobacter lari*. These results indicate a need for additional training modules on identification procedures for *Campylobacter spp*. Given the growing concerns over increasing macrolide resistance in *Campylobacter spp*., participants in the 2009 EQAS will also be offered the opportunity to perform susceptibility testing of *Campylobacter spp*. The unknown isolate; *Enterobacter sakazakii,* revealed that laboratories were able to identify the pathogen. The *Shigella* pilot program was regarded as a huge success and will be offered to all participants in the 2009 EQAS.

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Figure 1. Participating countries.

Participating countries are marked in yellow.

Table 1. The overall performance of serotyping, 2008.

Year	Serotyped all eight isolates		Correctly serotyped isolates	
	Number of labs		Number of correct tests	
	n	%	n	%
2000	34	92	165	76
2001	79	82	513	72
2002	80	81	668	91
2003	69	54	692	80
2004	78	61	701	81
2006	105	81	808	85
2007	109	78	920	88
2008	100	66	888	83
Overall	82	72	669	83

Table 2. The laboratories' ability to correctly serotype the test strains.

Number of correct serotypes	EQAS 2000		EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004	
	Number of laboratories		Number of laboratories		Number of laboratories		Number of laboratories		Number of laboratories	
	n	%	n	%	n	%	n	%	n	%
8	9	24	34	35	52	53	32	25	41	32
7	9	24	13	14	19	19	15	12	14	11
6	4	11	9	9	12	12	18	14	16	13
5	3	8	9	9	4	4	23	18	16	13
4	3	8	4	4	1	1	14	11	11	9
3	4	11	8	8	4	4	13	10	10	8
2	2	5	3	3	5	5	4	3	10	8
1	2	5	5	5	1	1	5	4	5	4
0	1	3	11	11	1	1	3	2	4	3
In total	N=37	100%	N=96	100%	N=99	100%	N=127	100%	N=127	100%

Number of correct serotypes	EQAS 2006		EQAS 2007		EQAS 2008		Overall EQAS 2000-2008	
	Number of laboratories		Number of laboratories		Number of laboratories		Number of laboratories	
	n	%	n	%	n	%	N	%
8	42	32	66	47	50	33	41	36
7	35	27	29	21	36	24	21	18
6	19	15	13	9	11	7	13	11
5	12	9	11	8	14	9	12	11
4	7	5	7	5	12	8	7	6
3	5	4	6	4	9	6	7	6
2	3	2	2	1	8	6	5	4
1	4	3	6	4	9	6	5	4
0	3	2	0	0	2	1	3	2
In total	N=130	100%	N=140	100%	N=151	100%	N=114	100%

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

Region:	Year:	Number of laboratories (n)	Number of strains serotyped (n)	Percent strains correctly serotyped (%)	Participating countries in the 2008 Iteration
Africa	2001	6	37	73.0	Cameroon, Central African Republic, Democratic Republic of Congo, Ivory Coast, Madagascar, Mauritius, Morocco, South Africa, Tunisia
	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	
	2008	10	71	49.3	
Asia & Middle East	2001	10	60	50.0	Egypt, Israel, Jordan, Oman, Saudi Arabia
	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	
	2008	5	34	61.8	
Caribbean	2001	0	0	0	Barbados, Suriname, Trinidad and Tobago.
	2002	0	0	0	
	2003	3	18	61.1	
	2004	2	8	87.5	
	2006	3	14	78.6	
	2007	2	9	77.8	
	2008	3	14	78.6	
China	2001	4	32	96.9	China
	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	
	2008	15	108	94.4	
Europe	2001	43	323	80.5	Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Italy, Lithuania, Luxembourg, Malta, Republic of Moldova, Netherlands, Poland, Serbia, Slovak Republic, Slovenia, Turkey, United Kingdom.
	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	
	2008	50	379	82.3	
North America	2001	4	32	87.5	Canada, United States of America.
	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	
	2008	11	84	95.2	
Oceanic	2001	4	30	100.0	Australia,New Zealand
	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	
	2008	4	30	93.3	
Russia	2001	1	8	12.5	Belarus, Georgia, Russia
	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	
	2008	6	40	90.0	
Latin America	2001	11	78	57.7	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Honduras, Mexico, Paraguay, Peru, Venezuela.
	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	
	2008	17	120	71.7	
Southeast Asia	2001	15	113	54.0	Cambodia, Japan, Malaysia, Philippines, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam.
	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	
	2008	18	125	81.6	

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

Year	Labs serotyped Enteritidis correctly	
	Number of labs	
	n	%
2000	34	92%
2001	64	84%
2004	113	95%
2006	116	94%
2007	135	96%
2008	139	96%

Table 5. List of *Salmonella* serogroups, serotypes and deviations, 2008

Strain	Correct serotype		No. of labs: serogrouping	Deviations (%)	Deviating results	No. of labs: serotyping	Deviations (%)	Deviating results
WHO8.1	Oranienburg	6,7:m,t:-	171	0.6	O:6, 14 (1)	129	34.1	Othmarschen (25), Montevideo (5), Winston (4), Salmonella II (3), Oakey (2), Riggil (1), II 6,7:m,t:- (1), Concord (1), Edinburg (1), Colindale (1)
WHO8.2	Thompson	6,7:k:1,5	172	2.3	O:6, 14(2) O:8 (1) O:11 (1)	132	22.7	Irumu (3), Bareilly (3), Alamo (2), Poitiers (2), Tennessee (2), Infantis (2), Afula (1), Isangi (1), Somone (1), Salmonella II (1), Montevideo II (1), II 6,7:z6:1,7 (1), Leopoldville (1), Choleraesuis (1), Livingstone (1), Oritamerin (1), Montevideo (1), Nessziona (1), Colindale (1), Bulovka (1), Sanjuan (1), Concord (1)
WHO8.3	Enteritidis	9,12:g,m:-	175	1.7	O:4 (1) O:6, 14 (1) O:9, 46 (1)	145	4.1	Berta (1), Blegdam (1), Jamaica (1), Mendoza (1), Naestved (1), Gallinarum (1)
WHO8.4	Javiana	9,12:l,z28:1,5	174	1.7	O:9, 46 (3)	138	26.8	Panama (17), Itami (5), Victoria (4), Salmonella II (1), Enteritidis (1), Eastbourne (1),Lawndale (1), Powell (1), Houston (1), Mendoza (1), Kapemba (1), Ndolo (1), Berta (1), Lome (1).
WHO8.5	Meleagridis	3,10:e,h:l,w	169	5.9	O:4 (2) O:8 (2) O:9 (2) O:1, 3, 19 (4)	128	25.0	Assinie (16), London (3), Give (2), Fulda (2), 3,10:l,w:- (2), Ruzizi (1), Newrochelle (1), Langensalza (1), Birmingham (1), Lexington (1), Drumfries (1), Sinsfort (1).
WHO8.6	Blockley	6,8:k:1,5	172	6.4	O:2 (1) O:4 (2) O:6, 14 (1) O:7 (6) O:9 (1)	138	10.1	*Haardt (9), Kallo (2), Thompson (2), Schwerin (2), Kisii (1), Tshiongwe (1), Charlottenburg (1), Bonariensis (1), Tennessee (1), Loanda (1), Muenchen (1), Goldcoast (1).
WHO8.7	Indiana	4,5:z:1,7	173	1.2	O:6, 14 (1) O:8 (1)	136	11.8	Bredeney (4), Shubra (2), Salmonella II (2), Typhimurium (1), Thayngen (1), Kaapstad (1), Stanley (1), Remo (1), Coeln (1), Kiambu (1), Kubacha (1).
WHO8.8	Hiduddify	6,8:l,z13,z28:1,5	168	3.6	O:6, 14 (2) O:7 (4)	131	19.1	Loanda (9), Edmonton (2), Breukelen (2), Kivu (1), Goldcoast (1), Lindenburg (1), Tananarive (1), Bovismorbificans (1), Litchfield (1), Nessziona (1), Kunduchi (1), Fayed II (1), Fayed (1), Hidalgo (1), Loanda (1).

*: The serovar "*S.* Haardt" have not been accounted as an error due to colonial form variation by minor O antigen ($O:6_1$).

Table 6. The number of susceptibility tests performed from 2000 to 2008.

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 I 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	92	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
2008	168	10.3	91	4	2	3	5	9
Overall*	130	9.6	92	5	2	1	3	8

*: Data is exclusive one strain which may have lost resistance due to transport or storage stress in 2003.

Table 7. Susceptibility test results (no. R/I/S) of the *Salmonella* strains tested in 2008

Strain	AMP	CTX	CAZ	CRO	CHL	CIP	GEN	NAL	STR	SMX	SXT	TET	TMP
WHO S-8.1	1/6/**158**	4/0/**134**	1/0/**117**	1/0/**99**	1/2/**148**	1/0/**161**	3/1/**153**	3/4/**140**	4/23/**81**	5/0/**84**	4/1/**139**	9/18/**127**	0/0/**86**
WHO S-8.2	12/20/**135**	3/2/**133**	4/1/**115**	3/0/**95**	7/**44**/103	**43**/23/97	4/0/**155**	**144**/0/6	6/22/**81**	4/1/**85**	4/0/**141**	**145**/1/11	4/1/**82**
WHO S-8.3	9/11/**147**	5/0/**133**	4/2/**115**	3/1/**95**	0/4/**150**	2/1/**161**	**151**/1/7	3/1/**145**	**99**/3/8	**87**/1/2	1/1/**143**	14/16/**125**	0/2/**85**
WHO S-8.4	2/8/**157**	3/0/**135**	2/0/**119**	1/0/**99**	0/1/**153**	2/0/**163**	7/2/**149**	2/8/**138**	13/33/**62**	6/1/**83**	5/0/**140**	6/12/**138**	0/0/**87**
WHO S-8.5	6/5/**155**	3/2/**132**	2/2/**117**	2/1/**97**	1/3/**149**	1/1/**161**	3/1/**154**	4/4/**140**	10/12/**85**	5/0/**84**	2/2/**139**	9/12/**135**	0/0/**88**
WHO S-8.6	**158**/2/7	**106**/27/5	**86**/24/11	**68**/23/9	2/4/**148**	5/0/**159**	5/1/**153**	7/7/**134**	9/31/**68**	6/1/**83**	3/0/**142**	7/10/**139**	2/1/**84**
WHO S-8.7	**160**/2/4	3/3/**132**	2/3/**114**	2/1/**93**	**148**/2/3	**158**/1/4	**130**/16/13	**147**/1/1	**105**/2/3	**90**/0/0	**132**/2/9	**146**/2/11	**82**/0/5
WHO S-8.8	4/2/**160**	2/1/**136**	2/1/**117**	2/0/**96**	2/1/**150**	**26**/2/135	5/1/**150**	**147**/1/1	9/29/**69**	3/4/**83**	6/1/**138**	8/12/**136**	1/1/**85**

Numbers in bold: Number with expected interpretation. Grey cell: < 90% of laboratories determined correct interpretation.

Table 8. Number of tests performed and percentage of major deviations for each antimicrobial 2000 – 2008.

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)			EQAS 2008 (N=168)		
	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	1331	3	8
Amoxicillin / Clavulanic acid	973	6	12	950	9	22	908	6	17	-	-	-
Ceftazidime	-	-	-	769	7	11	830	1	1	961	3	6
Ceftiofur	-	-	-	225	2	9	258	0	6	-	-	-
Ceftriaxone	-	-	-	-	-	-	-	-	-	791	3	6
Chloramphenicol	1159	2	2	1060	3	15	1105	0	6	1226	1	11
Ciprofloxacin	1162	0	1	1110	2	6	1101	1	1	1307	19	21
Cefotaxime	995	0	14	956	7	15	914	1	2	1104	3	6
Gentamicin	1201	2	3	1078	3	7	1111	3	4	1265	4	6
Kanamycin	-	-	-	-	-	-	-	-	-	-	-	-
Nalidixic acid	1130	1	4	1035	2	6	1092	2	3	1168	2	4
Cefpodoxime	-	-	-	305	1	26	389	4	16	-	-	-
Sulfamethoxazole	734	5	8	649	6	7	678	5	6	718	4	5
Streptomycin	947	1	21	896	5	22	875	4	26	867	7	25
Sulphonamides + Trimethoprim	1051	3	4	996	3	5	971	3	3	1155	3	4
Tetracycline	1122	5	11	1054	9	20	1047	4	11	1249	6	13
Trimethoprim	729	2	2	607	1	2	583	1	2	696	2	2
OVERALL	12381	3	7	12782	4	12	12976	3	7	13858	5	9

Table 8a. Number of tests performed and percentage of major deviations for each antimicrobial 2000 – 2008 cont.

Antimicrobial	Overall EQAS 2000 -2008* (N=1024)		
	Total no of determinations	% critical deviations	% total deviations
Ampicillin	7817	3	5
Cefotaxime	3969	3	9
Ceftazidime	2560	4	6
Ceftriaxone	791	3	6
Chloramphenicol	7606	2	6
Ciprofloxacin	7733	4	6
Gentamicin	7717	3	6
Nalidixic acid	7311	1	4
Sulfamethoxazole	4568	5	6
Streptomycin	6062	5	26
Sulphonamides + Trimethoprim	6583	3	5
Tetracycline	7467	5	13
Trimethoprim	4407	1	2
OVERALL	74591	3	8

Table 9. Proportion of laboratories that obtained the expected result. Number and percentages of laboratories which correctly detected and confirmed the ESBL and non ESBL producing *Salmonella* strains.

Isolate no.	Expected interpretation	Confirmatory tests	
		CAZ/CL:CAZ	CTX/CL:CTX
WHO S-8.1	non ESBL	15/16 (94%)	14/14 (100%)
WHO S-8.2	non ESBL	14/14 (100%)	14/14 (100%)
WHO S-8.3	non ESBL	16/16 (100%)	16/16 (100%)
WHO S-8.4	non ESBL	15/15 (100%)	14/14 (100%)
WHO S-8.5	non ESBL	15/15 (100%)	13/13 (100%)
WHO S-8.6	ESBL	63/65 (97%)	62/68 (91%)
WHO S-8.7	non ESBL	19/20 (95%)	17/18 (94%)
WHO S-8.8	non ESBL	15/15 (100%)	13/14 (93%)

Table 10. The number of laboratories having deviating results in per year and region.

Region:	Year:	Number of laboratories (n)	Percent correct test result	Percent minor deviations (S to I or I to R) switch	Percent major deviations (S to R) switch	Percent very major deviations (R to S) switch	Percent critical deviations	Percent total deviations	Participating countries in the 2008 Iteration
Africa	2001	7	80.1	9.6	7.7	2.5	10.2	19.8	Cameroon, Central African Republic, Democratic Republic of Congo, Ethiopia, Ghana, Ivory Coast, Madagascar, Mauritius, Morocco, Namibia, Senegal, South Africa, Sudan, Tunisia, Zambia.
	2002	10	94.3	4.1	1.0	0.6	1.6	5.7	
	2003	13	86.9	6.6	2.8	3.7	6.5	13.1	
	2004	11	85.7	7.2	5.2	1.9	7.1	14.3	
	2006	20	85.8	7.5	4.1	2.7	6.8	14.3	
	2007	16	90,7	4.4	4.0	0.9	4.9	9.3	
	2008	19	83.8	6.5	5.5	4.2	9.7	16.2	
Central Asia & Middle East	2001	10	87.7	6.3	5.2	0.8	6.0	12.3	Egypt, India, Israel, Jordan, Oman, Saudi Arabia, Yemen.
	2002	6	83.4	9.8	6.6	0.2	6.8	16.6	
	2003	8	89.9	4.5	4.0	1.6	5.6	10.1	
	2004	10	87.5	6.7	5.5	0.3	5.8	12.5	
	2006	7	79.2	10.5	9.8	0.5	10.3	20.8	
	2007	8	87,8	5.0	6.2	1.1	7.3	12.2	
	2008	12	86.1	6.5	4.0	3.4	7.4	13.9	
Caribbean	2001	2	83.5	9.5	7.0	0.0	7.0	16.5	Barbados, Jamaica, Suriname, Trinidad and Tobago.
	2002	1	95.8	4.2	0.0	0.0	0.0	4.2	
	2003	8	91.7	6.4	1.5	0.5	2.0	8.4	
	2004	8	94.1	3.1	1.9	0.9	2.8	5.9	
	2006	5	92.1	5.4	1.6	1.0	2.6	8.0	
	2007	4	95.0	3.1	0.9	0.9	1.8	5.0	
	2008	5	90.7	5.5	0.9	2.9	3.8	9.3	
China	2001	4	98.9	0.8	0.0	0.3	0.3	1.1	China
	2002	3	96.0	4.0	0.0	0.0	0.0	4.0	
	2003	8	90.1	3.6	2.8	3.6	6.4	10.0	
	2004	8	96.0	3.2	0.7	0.1	0.8	4.0	
	2006	6	89.6	7.0	2.9	0.5	3.4	10.4	
	2007	10	98.3	1.1	0.3	0.2	0.5	1.6	
	2008	18	92.8	3.7	0.8	2.7	3.5	7.2	
Europe	2001	47	91.3	5.7	2.7	0.3	3.0	8.7	Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Italy, Lithuania, Luxembourg, Malta, Republic of Moldova, Poland, Serbia, Slovak Republic, Slovenia, Turkey, United Kingdom
	2002	57	92.7	5.2	1.2	0.9	2.1	7.3	
	2003	64	92.9	3.8	1.0	2.3	3.3	7.1	
	2004	58	93.5	4.3	1.4	0.8	2.2	6.5	
	2006	54	88.7	7.0	3.8	0.6	4.4	11.3	
	2007	49	94.2	3.7	1.6	0.4	2.0	5.7	
	2008	51	91.2	4.4	2.5	1.9	4.4	8.8	
Nouth America	2001	4	95.8	3.8	0.0	0.4	0.4	4.2	Canada, United States of America.
	2002	3	90.5	6.9	0.6	2.0	2.6	9.5	
	2003	7	93.4	5.2	0.0	1.4	1.4	6.6	
	2004	9	94.2	4,2	1.8	0.0	1.8	6.0	
	2006	8	94.8	2.9	1.0	1.3	2.3	5.2	
	2007	10	95.4	2.9	0.8	0.8	1.6	4.6	
	2008	14	96.4	0.6	0.4	2.6	3.0	3.6	
Oceanic	2001	6	91.8	4.7	2.7	0.9	3.6	8.2	Australia, New Zealand
	2002	7	91.7	6.2	0.0	2.0	2.0	8.3	
	2003	9	94.3	2.5	1.2	2.0	3.2	5.7	
	2004	11	97.1	2.5	0.3	0.1	0.4	2.9	
	2006	7	93.4	4.6	0.9	1.1	2.0	6.6	
	2007	1	98.9	1.1	0.0	0.0	0.0	1.1	
	2008	4	93.9	3.8	0.0	2.3	2.3	6.1	
Russia	2001	1	81,9	15,3	2,8	0.0	2.8	18.1	Belarus, Georgia, Russia
	2002	1	84,5	9,9	5,6	0.0	5.6	15.5	
	2003	1	100.0	0.0	0.0	0.0	0.0	0.0	
	2004	4	91.2	6.6	1.5	0.7	2.2	8.8	
	2006	5	87.4	8.2	2.7	1.7	4.4	12.6	
	2007	8	88.9	5.8	4.8	0.4	5.2	11.0	
	2008	6	92.2	4.7	1.4	1.7	3.1	7.8	
South America	2001	11	90.8	6.9	1.4	1.0	2.4	9.2	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Honduras, Mexico, Nicarragua, Panama, Paraguay, Peru, Uruguay, Venezuela.
	2002	13	93.7	4.6	0.7	1.0	1.7	6.3	
	2003	12	90.8	4.2	2.0	3.0	5.0	9.2	
	2004	17	94.4	4.7	0.8	0.1	0.9	5.6	
	2006	16	88.7	6.3	4.5	0.6	5.1	11.3	
	2007	17	94.9	1.8	1.9	1.4	3.3	5.0	
	2008	20	93.0	3.4	1.5	2.1	3.6	7.0	
Southeast Asia	2001	16	88.1	7.7	2.3	1.9	4.2	11.9	Cambodia, Japan, Malaysia, Nepal, Philippines, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam.
	2002	18	89.0	8.1	1.4	1.6	3.0	11.0	
	2003	17	87.4	5.2	4.7	2.7	7.4	12.6	
	2004	16	92.8	4.4	2.3	0.5	2.8	7.2	
	2006	15	90.0	8.1	1.2	0.8	2.0	10.0	
	2007	20	93.9	4.0	1.4	0.7	2.1	6.1	
	2008	19	90.5	4.7	2.2	2.6	4.8	9.5	

Table 11a. Range of obtained values for *E. coli* ATCC 25922.

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)		EQAS 2006 (N=137)		EQAS 2007 (N=126)	
	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]	% of labs	N[3]	% of labs	N[3]
Ampicillin	2-8	16-22	27	37	19	97	16	109	14	140	10	132	14	133	11	124
Amoxicillin / Clavulanic acid	2-8	8-24	-	-	-	-	-	-	-	-	13	117	9	116	8	102
Ceftazidime	0.06-0.5	25-32	-	-	-	-	-	-	-	-	-	-	15	96	9	92
Chloramphenicol	2-8	21-27	37	38	20	97	15	107	22	137	13	128	18	126	14	123
Ciprofloxacin	0.004-0.016	30-40	20	35	14	97	14	108	9	138	8	132	8	127	12	121
Cefotaxime	0.03-0.12	29-35	-	-	-	-	-	-	-	-	18	111	21	115	16	104
Ceftiofur	0.25-1	26-31	-	-	-	-	-	-	-	-	-	-	22	32	11	35
Enrofloxacin	0.008-0.03	32-40	-	-	-	-	-	-	-	-	-	-	63	19	-	-
Florfenicol													-	-	0	13
Gentamicin	0.25-1	19-26	23	39	12	99	12	108	9	138	10	134	14	131	6	124
Nalidixic acid	1-4	22-28	35	37	14	74	14	102	16	132	9	126	20	122	7	120
Cefpodoxime	0.25-1	23-28	-	-	-	-	-	-	-	-	-	-	12	39	9	47
Sulfamethoxazole	8-32	15-23	53	19	34	53	26	57	17	82	16	84	29	74	22	64
Streptomycin	4-16[2]	12-20	22	36	12	81	11	82	9	105	6	110	11	106	6	97
Sulphonamides / Trimethoprim	≤0.5/9.5	23-29	-	-	14	90	12	102	14	129	11	120	19	122	13	107
Tetracyclin	0.5-2	18-25	42	42	22	96	13	102	19	137	13	129	12	125	7	117
Trimethoprim	0.5-2	21-28	30	31	22	50	11	66	14	79	9	87	17	74	10	67

[1] CLSI standard, Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 18th Informational suppl.CLSI document M100-S18, Wayne, Pennsylvania.[2] QC range developed by the manufacturer of Sensititre®[3] The total number of laboratories performing the test.

Table 11b. Range of obtained values for *E. coli* ATCC 25922 by disk diffusion and MIC determinations.

Antimicrobial	EQAS 2008 (N=147)					
	All		MIC (N=33)		Disk (N=114)	
	% of labs	N [3]	% of labs	N [3]	% of labs	N [3]
Ampicillin	12	147	0	33	16	114
Ceftazidime	9	111	5	23	10	89
Chloramphenicol	10	135	0	24	12	112
Ciprofloxacin	8	144	6	33	8	111
Cefotaxime	14	124	9	23	15	101
Gentamicin	8	145	0	31	11	114
Nalidixic acid	8	136	0	23	10	113
Sulfisoxazole	14	71	11	18	15	53
Streptomycin	12	101	11	19	12	82
Sulphonamides / Trimethoprim	13	129	9	22	14	107
Tetracyclin	7	139	0	28	9	111
Trimethoprim	13	79	13	16	13	63

[1] CLSI standard, Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 18th Informational suppl.CLSI document M100-S18, Wayne, Pennsylvania. [2] QC range developed by the manufacturer of Sensititre® [3] The total number of laboratories performing the test.

Table 12. Laboratories which successfully species identified *Campylobacter.*

Year	Number. of participating 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)* *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. jejuni*	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)*
2007	142	*C. lari*	Strain # 1	95	72%	*C. jejuni (n:10)* *C. coli (n:9)* *C. upsaliensis (n:7)*
2007	142	*C. coli*	Strain # 2	99	74%	*C. lari (n:3)* *C. jejuni (n:20)* *C. upsaliensis (n:2)*
2008	154	*C. lari*	Strain # 1	105	63%	*C. coli (n:14)* *C. jejuni (n:18)* *C. upsaliensis (n:7)*
2008	154	*C. lari*	Strain # 2	105	60%	*C. coli (n:10)* *C. jejuni (n:19)* *C. upsaliensis (n:13)*

Table 13. Laboratories which successfully identified *E.sakazakii*.

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**
2007	**86**	**83% *Vibrio parahaemolyticus***
2008	**128**	**92% *E. sakazakii***

Table 14. List of *Shigella* serotypes, variants and deviations, 2008

Strain	Correct serotype Include full serotype (e.g. flex 2a)	No. of labs: serogrouping	Deviations (%)	Deviating results	No. of labs: serotype	Deviations (%)	Deviating results
WHO SH 8.1	*S. boydii*	13	7.1	*S. flexneri* (1)	6	0.0	-
WHO SH 8.2	*S. sonnei*	15	0.0	-	0	0.0	-
WHO SH 8.3	*S. flexneri*	14	7.1	*S. dysenteriae* (1)	9	0.0	-
WHO SH 8.4	*S. dysenteriae*	10	10.0	*S. boydii* (1)	5	0.0	-

Table 15. The number of susceptibility test performed in 2008 of *Shigella*.

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 I 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
2008	15	8.8	95	2	2	1	3	5

Table 16. Number of tests performed and percentage of major deviations for each antimicrobial in 2008.

Antimicrobial	Overall EQAS 2008* (N=1024)		
	Total no of determinations	% critical deviations	% total deviations
Ampicillin	52	1	1
Ceftazidime	44	2	2
Chloramphenicol	51	1	1
Ciprofloxacin	48	-	-
Cefotaxime	48	2	2
Gentamicin	50	1	1
Nalidixic acid	52	-	-
Sulfamethoxazole	7	-	-
Streptomycin	27	4	9
Sulphonamides + Trimethoprim	52	2	2
Tetracycline	52	4	8
Trimethoprim	4	-	-
Ceftriaxone	42	2	2
OVERALL	529	19	28

WHO Global Salm-Surv Electronic Discussion Group
English Version
Subject: Signing up for EQAS 2008
Greetings WHO Global Salm-Surv Members!

WHO Global Salm-Surv strive to increase the quality of laboratory-based surveillance of *Salmonella* and other foodborne pathogens and have just closed the year 2007 WHO Global Salm-Surv External Quality Assurance System (EQAS).

We are now pleased to announce the launch of EQAS 2008.

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?
This year's WHO EQAS offers serogrouping, serotyping and antimicrobial susceptibility testing of eight *Salmonella* isolates, species identification of two *Campylobacter* isolates and identification of one unknown bacterial sample.

WHO SHOULD PARTICIPATE IN EQAS 2008?
All national or regional reference laboratories performing work on *Salmonella* and/or *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 WHO GSS Regional Centres in cooperation with the EQAS Coordinator will evaluate the list of participants. Laboratories which signed up and received strains in year 2007 but **did not submit** any data should explain the reason for this in order to participate in 2008.

COST FOR PARTICIPATING IN EQAS
Participation is free of charge. Nevertheless, 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 2008
This link will take you to a sign up webpage: 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 2008
- Level of reference function in your country

If you experience any problems when you sign up electronically, please try again a few days

later and contact the EQAS Coordinator Rene Hendriksen by e-mail (rsh@food.dtu.dk) or fax (+45 7234 6001).

SHIPPING AND TIMELINE TO RECEIVE ISOLATES AND PROTOCOLS
Shipping of the bacterial isolates will be taken care of by a number of institutes because of the increasing number of participants. **Please remember to provide the coordinator with a valid import permission in order to minimize delay in shipping the isolates to your laboratory.** As means of avoiding passing the deadline we ask you to apply for an import permit already at this stage. **Please apply for a permit to receive the following "Biological Substance Category B": eight *Salmonella* strains, two *Campylobacter*, one *Campylobacter* reference strain (new participants), one *E .coli* reference strain (new participants) and an unknown sample (enterobacteriaceae) between August and September 2008.**

The isolates will be shipped in August - September 2008. The protocol as well as additional information needed for the EQAS will be made available for download from the website www.antimicrobialresistance.dk/who.

TIMELINE FOR RESULTS TO BE TURNED INTO THE NATIONAL FOOD INSTITUTE
Results must be returned to the National Food Institute (DTU Food) by 31st of December 2008. 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 DTU Food 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 this EQAS: April the 1st, 2008
****

Posted by Rene Hendriksen rsh@food.dtu.dk WHO GSS EQAS Coordinator, DTU Food, The National Food Institute, Denmark.

Appendix 2

			AMPICl AMP	cefotaxime CTX	CTX/CL : CTX	ceftazidim CAZ	CAZ/CL : CAZ	Ceftriax CRO	CHLORA CHL	CIPROF CIP	GENTAM GEN	NALAC NAL	STREPT STR	SULFIZ SMX	TETRA TET	Trim TMP	TRISUL SXT
WHO S-8.1	Oranienburg	I 6,7:m,t:-	<= 1	= 0.25	<0,25/0,125	0,5	<0,5/0,125	<= 0.25	= 8	= 0.03	<= 0.5	<= 4	<= 8	<= 64	<= 2	<= 1	= 0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-8.2	Thompson	I6,7:k:1,5	= 4	= 0.25	<0,25/0,125	0,5	<0,50/0,125	<= 0.25	= 16	= 1	<= 0.5	> 64	<= 8	<= 64	> 32	<= 1	= 0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	INTER	RESIST	SUSC	RESIST	SUSC	SUSC	RESIST	SUSC	SUSC
WHO S-8.3	Enteritidis	I 9,12:g,m:-	= 4	= 0.25	0,25/0,25	1	1,0/0,50	<= 0.25	= 8	= 0.03	> 16	<= 4	= 64	> 1024	= 4	<= 1	= 0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	RESIST	SUSC	RESIST	RESIST	SUSC	SUSC	SUSC
WHO S-8.4	Javiana	I 9,12:l,z28:1,5	<= 1	0,125	<0,25/0,064	0,25	<0,50/0,064	<= 0.25	= 4	<= 0.015	<= 0.5	<= 4	<= 8	<= 64	<= 2	<= 1	= 0.032
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-8.5	Meleagridis	I 3,10:e,h:l,w	= 2	<= 0.12	<0,25/0,125	1	0,50/0,50	<= 0.25	= 4	<= 0.015	<= 0.5	<= 4	<= 8	<= 64	<= 2	<= 1	= 0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-8.6	Blockley	I 6,8:k:1,5	> 32	16	>16/0,032	32	>32/0,125	= 4	= 4	= 0.03	<= 0.5	<= 4	<= 8	<= 64	<= 2	<= 1	= 0.032
			RESIST	RESIST	ESBL	RESIST	ESBL	RESIST*	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-8.7	Indiana	I 4,12:z:1,7	> 32	<= 0.12	<0,25/0,064	0,5	<0,50/0,125	<= 0.25	> 64	> 4	= 16	> 64	> 128	> 1024	> 32	> 32	> 32
			RESIST	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST
WHO S-8.8	Hiduddify	I 6,8:l,z13,z28:1,5	<= 1	<= 0.12	<0,25/0,032	0,25	<0,5/0,125	<= 0.25	= 4	= 0.12	<= 0.5	> 64	<= 8	<= 64	<= 2	<= 1	= 0.032
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	RESIST	SUSC	RESIST	SUSC	SUSC	SUSC	SUSC	SUSC

*regarded as resistant to CRO due to the isolate being resistant to CAZ and CTX (see description in the protocol)

WHO C-8.1 *Campylobacter lari*
WHO C-8.2 *Campylobacter lari*

WHO B-8.1 *Enterobacter sakazakii*

DTU Food
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 (DTU Food), in collaboration with partners and Regional Sites in WHO GSS.

As previous years the WHO EQAS 2008 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 strains, these are included in the parcel. The reference strains will not be included in the years to come. The reference strains are original CERTIFIED cultures and are free of charge. Please take proper care of the strains. Handle and maintain them as suggested in the enclosed manual 'Subculture and Maintenance of QC Strains'. Please use them for future internal quality control for susceptibility testing in your laboratory.

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of 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 2008

3.1 Shipping, receipt and storage of strains

In September 2008 around 190 laboratories from all parts of the world will receive a parcel containing eight *Salmonella* strains, two *Campylobacter* strains and one 'unknown' bacterial isolate (according to information when signing up). An *E. coli* reference strain is included for participants who have not previously received these. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains among the selected material.

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).

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 DTU Food (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).

Antimicrobials	**Reference value, MIC (µg/mL)**		
	Sensitive	**Intermediate**	**Resistant**
Ampicillin, AMP*	≤8	16	≥32
Cefotaxime, CTX**	≤0.5	-	>0.5
Ceftazidime, CAZ**	≤2	-	>2
Ceftriaxone, CRO**	≤0.125	-	>0.125
Chloramphenicol, CHL*	≤8	16	≥32
Ciprofloxacin, CIP**	<0.125	-	≥0.125
Gentamicin, GEN*	≤4	8	≥16
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 (epidemiological cut off values) ***DTU Food

For ceftriaxone, please note that the breakpoint used for this antimicrobial is the epidemiological cut off value defined by EUCAST for *E. coli*.

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 susceptible against cefotaxime (CTX), ceftazidime (CAZ) or ceftriaxone (CRO) 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 a β-lactamase inhibitor (clavulanic acid). Synergy is defined as a 3 dilution steps difference between the two compounds in at least one of the two cases (MIC ratio ≥ 8, E-test 3 dilution steps) or an increase in zone diameter ≥ 5 mm. (CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

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

3.4 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 Handling the *Campylobacter* ampoules

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.

a. Check the number of the culture written on the label.
b. Make a file cut on the ampoule just above the shoulder of the ampoule.
c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool.
d. Crack the glass using sterile gauze or cotton to protect your fingers.
e. Add to the dried suspension about 0.5 ml appropriate broth or a sterile 0.9% NaCl solution using a pipette. Mix carefully to avoid creating aerosols.
f. Inoculate the suspension on a suitable agar plate with a 10µl loop or a cotton swab.
g. Transfer the rest of the content in the ampoule to a test tube containing 5-6 ml of a suitable liquid media.
h. Incubate the agar plate and liquid media at a temperature of 42°C at microaerobic conditions for 24-48 hours.
i. Inoculate a second agar plate from the liquid media with a 10µl loop or a cotton swab if the initial plate had inadequate growth.
j. Select a pure culture with vigorous growth from the agar plate for further work.

Please note that:

- Cultures may need at least one sub-culturing before they can be optimally used
- Unopened ampoules should be kept in a dark and cool place!

For reconstitution of the *E. coli* reference strain: Please see the document 'Instructions for opening and reviving lyophilised cultures' on the WHO CC website (see www.antimicrobialresistance.dk).

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 December 31st, 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 do not hesitate to contact the EQAS Coordinator:

Mr. Rene Hendriksen

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27 Bülowsvej, DK-1790 Copenhagen V - DENMARK

Tel: +45 7234 6288, Fax: +45 7234 6001

E-mail: rshe@food.dtu.dk

It is possible to communicate with the EQAS organisers in other languages than English. However, this is not a direct contact with the EQAS organisers since translation of the message is required. The following languages may be used: Russian, Chinese, French, Spanish or Portuguese.

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 'Key activities'
    b. Click on the link 'http://www.who.int/salmsurv/activities/GSS_EQAS/en'
    c. Click on 'Data entry for the year 2008'
    d. Write your username and password in low 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 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 than 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. Browse through the input pages and 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.

8) When you have seen all pages in the report, you will find a new menu. You can choose 'EQAS 2008 start page', 'Review evaluated results' (a printer friendly version of the evaluation report is also available) or 'Go to Global Salm-Surv homepage'.

   End of entering your data – thank you very much!

SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1.1 Purpose

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

1.2 References

M100-S18, January 2008 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A7, January 2006 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard)

1.3 Definition of Terms

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

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

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

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

1.4 Important Considerations

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

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

1.5 Storage of Reference Strains

Preparation of stock cultures

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

Working cultures

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

1.6 Frequency of Testing

Weekly vs. daily testing

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

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

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

Corrective Actions

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

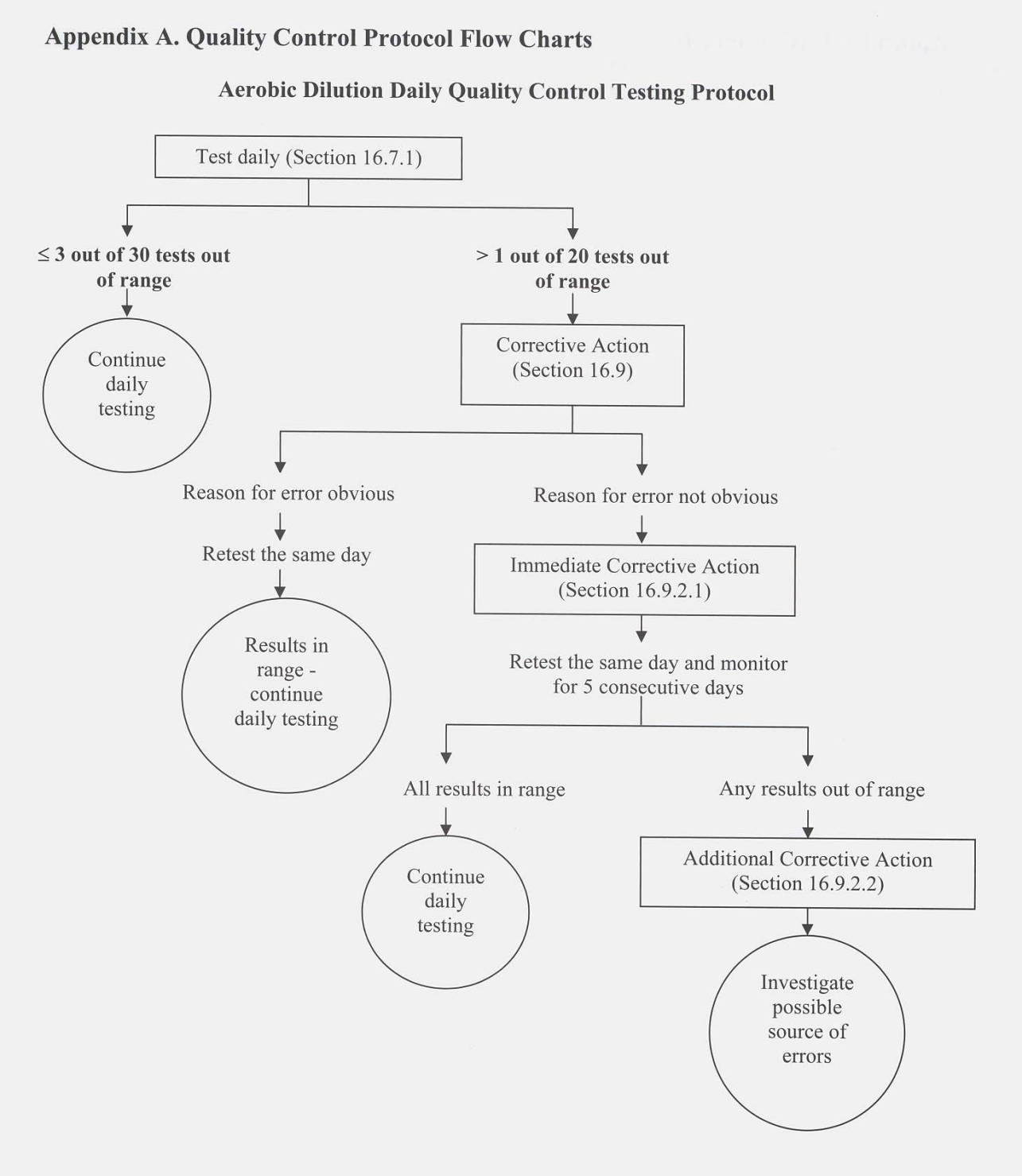
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

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

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

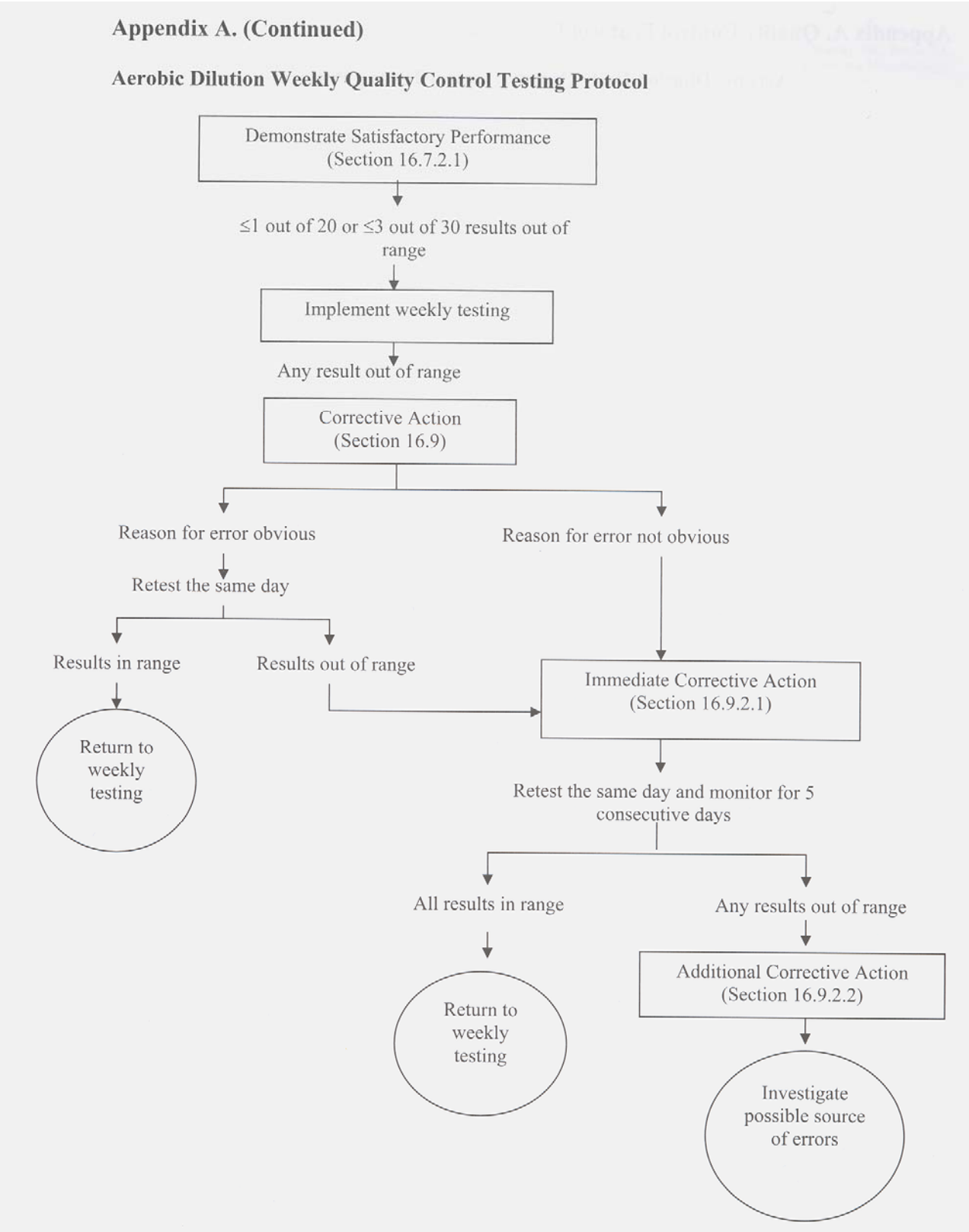
Repeat the 30 days validation before resuming weekly testing.

DAILY MIC QC CHART



Reference: CLSI M7-A7, page 39

WEEKLY MIC QC CHART



Reference: CLSI M7-A7, page 40

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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