The External Quality Assurance System of the WHO Global Foodborne Infections Network 2010

Public Health Agency of Canada
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World Health Organization

CDC
Centers for Disease Control and Prevention

international network
Institut Pasteur

DTU Food
National Food Institute

THE EXTERNAL QUALITY ASSURANCE SYSTEM OF THE WHO GLOBAL FOODBORNE INFECTIONS NETWORK YEAR 2010

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List of Abbreviations

AMP, Ampicillin

AST, Antimicrobial Susceptibility Testing

ATCC, American Type Culture Collection

CAZ, Ceftazidime

CCM, Czech Collection of Micro-organisms

CHL, Chloramphenicol

CIP, Ciprofloxacin

CDB, Country Data Bank

CDC, Centers for Disease Control and Prevention

CLSI, Clinical and Laboratory Standards Institute

CRO, Ceftriaxone

CTX, Cefotaxime

DTU Food, Technical University of Denmark - National Food Institute

ESBL, Extended Spectrum Beta-Lactamase

EQAS, External Quality Assurance System

ERY, Erythromycin

EUCAST, The European Committee on Antimicrobial Susceptibility Testing

GEN, Gentamicin

IATA, International Air Transport Association

IP. Institute Pasteur

MIC, Minimum Inhibitory Concentration

NAL, Nalidixic Acid

NSSC, National *Salmonella* and *Shigella* Center, Thailand

PHAC, Public Health Agency of Canada

QC, Quality Control

SMX, Sulfamethoxazole

STR, Streptomycin

SXT, Trimethoprim + Sulphonamides

TET, Tetracycline

TMP, Trimethoprim

TSI, Tri Sucre Iron

US, United States of America

WHO, World Health Organization

WHO GFN, WHO Global Foodborne Infections Network

1. Introduction

Since 2000, nine External Quality Assurance System (EQAS) reports have been issued with this report being the tenth. The WHO Global Foodborne Infections Network (WHO GFN)", focuses on enhancing WHO Member States' capacity to detect and respond to foodborne disease outbreaks by conducting laboratory-based surveillance of *Salmonella* and other foodborne pathogens. Since its inception, the scope of WHO GFN has expanded to include additional foodborne pathogens like *Shigella* and *Campylobacter*. *Salmonella*, *Campylobacter* and *Shigella* are among the most important foodborne pathogens worldwide and account for millions of cases of diarrheal disease and thousands of deaths per year, impacting both developing and industrialized countries. Furthermore, the increased number of *Salmonella* and *Shigella* isolates which are resistant to antimicrobials is of major concern since these isolates are associated with infections characterized by increased morbidity and mortality.

The EQAS is organized annually by the National Food Institute (DTU Food), Kgs. Lyngby, 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) in Canada; National *Salmonella* and *Shigella* Center (NSSC), National Institute of Health, Department of Medical Sciences in Thailand and Institute Pasteur (IP) in Paris, France. The technical advisory group for the WHO EQAS program consists of members of the WHO GFN Steering Committee.

Individual laboratory data are confidential and only known by the participating laboratory, the EQAS Organizer (DTU Food) and the respective WHO GFN regional centre. All summary conclusions are made public. The goal set by WHO GFN aim towards having all national reference laboratories perform *Salmonella* serotyping with a maximum of one deviation out of eight strains tested (error rate of 13%) and AST with a maximum error rate of 10% (either <5% very major / major errors and <5% minor errors, or <10% minor errors, as defined further in this report).

2. Materials and Methods

2.1 Participants

A pre-notification announcement of the EQAS 2010 was made through the WHO GFN list server on March 25, 2010 and a reminder was sent on May 10, 2010 (App. 1). The pre-notification was available in English, Spanish, Portuguese, French, Chinese and Russian, and included invitations to participate in the EQAS 2010 program for serotyping and AST of *Salmonella* and *Shigella*, identification and AST [Minimum Inhibitory Concentration (MIC) determination] of *Campylobacter*, and identification of an unknown foodborne pathogen. Participation was free of charge, but each laboratory was expected to cover expenses associated with the analyses performed.

2.2 Strains

Eight *Salmonella* strains, four *Shigella* strains, and two *Campylobacter* strains were selected for the EQAS 2010 from the DTU Food's strain collection. The unknown foodborne pathogen, a *Citrobacter spp.* strain, was selected by the Laboratory subcommittee under the WHO GFN Steering Committee, and it was provided by the US-CDC. Individual sets of *Salmonella*, *Shigella*, and *Citrobacter spp* strains were inoculated as agar stab cultures in nutrient agar. The *Campylobacter* strains were lyophilized in glass vials by Czech Collection of Micro-organisms (CCM), Czech Republic. 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 the scheme of Kaufmann-

White (2007) [1]. The *Salmonella* serotype was determined by DTU Food and verified by the CDC and IP prior to distribution. The antimicrobial susceptibility pattern of the *Salmonella* and *Shigella* strains was determined by DTU Food and verified by CDC. The *Shigella* serotype was performed by PHAC and verified by the NCCS. Finally, all results were later confirmed at DTU Food (apart from *Shigella* serotyping which is not routinely performed at DTU Food).

Furthermore, laboratories which did not formerly participate in WHO GFN EQAS AST component were provided with lyophilized international reference strains, namely *E. coli* CCM 3954 ~ ATCC 25922 and *C. jejuni* CCM 6214 ~ ATCC 33560, which were purchased at the Czech Collection of Micro-organisms (CCM); The Czech Republic.

2.3 Antimicrobials

AST of the *Salmonella, Shigella,* and *Campylobacter* strains was performed at the DTU Food, and the obtained results were used as a reference standard (App. 2). The following antimicrobials were used in the EQAS 2010 for AST of *Salmonella* and *Shigella* strains: ampicillin, AMP; cefotaxime, CTX; ceftazidime, CAZ; ceftriaxone, CRO; chloramphenicol, CHL; ciprofloxacin, CIP; gentamicin, GEN; nalidixic acid, NAL; streptomycin, STR; sulfamethoxazole, SMX; tetracycline, TET; trimethoprim, TMP and trimethoprim + sulphonamides, SXT. In addition, it was possible to confirm the presence of ESBL-producing strains by using the antimicrobials CTX and CAZ in combination with the inhibitor clavulanic acid. The following antimicrobials were used in the EQAS 2010 for AST of *Campylobacter* strains: chloramphenicol, CHL; ciprofloxacin, CIP; erythromycin, ERY; gentamicin, GEN; nalidixic acid, NAL; and tetracycline, TET.

MIC determination was performed by using Sensititre systems from Trek diagnostics Ltd, and guidelines and breakpoints by Clinical and Laboratory Standards Institute (CLSI) based on document M07-A8 (2009) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically"; Approved Standard - Eighth Edition [2], M100-S20 (2010) "Performance Standards for Antimicrobial Susceptibility Testing"; Twentieth Informational Supplement [3], document M31-A3 (2008) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated from Animals"; Approved Standard - Third Edition [4], and document M45-A2 (2010) "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria"; Approved Guideline – Second Edition [5]. Guideline were used for interpretation of AST results with the exception of i) ciprofloxacin susceptibility testing for which the EUCAST (www.eucast.org) epidemiological cut-off value was utilized; ii) streptomycin susceptibility testing for which DTU Food interpretative criteria was utilized; and iii) *Campylobacter* AST, for which EUCAST epidemiological cut-off values were used. For cefotaxime, ceftazidime and ceftriaxone values listed in CLSI M100-S20, supplemental Table 2A-S1 were utilized. All breakpoints are listed in the protocol (App. 3).

2.4 Distribution

Bacterial cultures were enclosed in double pack containers (class UN 6.2) and sent to participating laboratories according to the International Air Transport Association (IATA) regulations as "Biological Substance category B" classified UN3373. Prior to shipping, laboratories were informed about the dispatch date. 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, Chaiwat Pulsrikarn 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 Changwen Ke from Center for Disease Control and Prevention of Guangdong Province, China shipped to all Canadian, American, Thai, Latin American, Francophone African, Italian and Chinese institutes, respectively. The first parcel was dispatched from DTU Food on September 1st, 2010 and the last on October 22nd, 2010.

2.5 Procedure

Participants were instructed to download the protocol (App. 3) and additional documents (App. 4a and 4b; available only in English) from http://www.antimicrobialresistance.dk/. In addition, they were requested to subculture the strains prior to performing the method routinely used in their laboratory. The EQAS 2010 components included serotyping and AST of eight *Salmonella* and four *Shigella* strains, identification and MIC determination of two *Campylobacter* strains, AST of two quality control (QC) strains (*E. coli* CCM3954 / ATCC25922, *C. jejuni* CCM 6214 / ATCC33560), and identification of an unknown foodborne pathogen (*Citrobacter spp*). Furthermore, the laboratories were requested to save and maintain the ATCC reference strains for future proficiency tests (App. 4a and 4b).

After performing the tests, participants were requested to enter the obtained results (serotype and / or serogroup, MIC values or zone-diameter in millimeters, and antimicrobial susceptibility categories of the *Salmonella* and *Shigella* strains; identification, MIC values, and antimicrobial susceptibility categories of the *Campylobacter* strains; and identification of the unknown sample) into an electronic record sheet in the WHO GFN web-based database through a secured individual login, or alternatively, to send the record sheets from the enclosed protocol by fax to DTU Food. The database was activated on September 14, 2010 and closed on March 14, 2011.

The *Salmonella, Shigella* and *Campylobacter* strains were categorized as resistant (R), intermediate (I) or susceptible (S) to all tested antimicrobials. The interpretative criteria followed to generate the results used as reference standard were based on both clinical breakpoints and epidemiological cut-off values.

Of note, the terms 'susceptible', 'intermediate' and 'resistant' should be reserved for classifications made in relation to the therapeutic application of antimicrobial agents. When reporting data based on epidemiological cut-off values, bacteria should instead be reported as 'wild-type' or 'non-wild-type' [6]. Due to the different AST methods used by the participants and to simplify interpretation of the results, throughout this report we will maintain the terms susceptible, intermediate and resistant also when we refer to wild-type and non-wild-type strains.

Susceptibility results had to be interpreted on an individual basis for each antimicrobial tested, with the exception of cephalosporins which were interpreted according to CLSI Approved Standard – Nineteenth Edition, document M100-S19 (2009) "Performance Standards for Antimicrobial Susceptibility Testing, Table 2A". Participants were instructed to use the *Salmonella / Shigella* antisera and the antimicrobials used in the methods routinely performed. In addition, they were instructed to use their usual standard breakpoints for categorizing the results obtained by AST. All laboratories were requested to enter MIC values for the *C. jejuni* (ATCC 33560) reference strain, and either zone diameters or MIC values for the *E. coli* (ATCC 25922) reference strain. After submitting the results, participants were instructed to retrieve an instantly generated report from the secure web site. This report was created on an individual basis, and reported all deviations from the expected results and suggestions for solving or investigating the cause of error. Deviations of antimicrobial susceptibility test results from the expected results were categorized as minor, major or very major. Minor deviations are defined as classification of an intermediate strain as susceptible, resistant or vice versa (*i.e.* I ↔ S or I ↔R). Major deviation is the classification of a susceptible

strain as resistant (*i.e.* S → R). Very major deviation is the classification of a resistant strain as susceptible (*i.e.* R → S). In this report, the deviations of AST results are divided into two categories, *i.e.* critical deviations which include major and very major deviations, and total deviations which include also the minor deviations.

3. Results

A total of 188 laboratories responded to the pre-notification and were enrolled in the EQAS. When the deadline for submitting results was reached, 178 laboratories in 91 countries had uploaded data. The following countries provided data for at least one of the EQAS components (Figure 1): Albania, Algeria, Argentina, Australia, Barbados, Belarus, Belgium, Bolivia, Bosnia and Herzegovina, Brazil, Brunei Darussalam, Bulgaria, Cambodia, Cameroon, Canada, Central African Republic, Chile, China, Colombia, Democratic Republic of Congo, Costa Rica, Croatia, Cuba, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Ethiopia, Finland, France, Georgia, Germany, Ghana, Greece, Guatemala, Honduras, Hungary, India, Iran, Ireland, Israel, Italy, Ivory Coast, Jamaica, Japan, Jordan, Kenya, Korea, Lithuania, Luxembourg, Madagascar, Malawi, Malaysia, Malta, Mauritius, Mexico, Moldova, Morocco, New Zealand, Nicaragua, Nigeria, Sultanate of Oman, Panama, Paraguay, Peru, Philippines, Poland, Russia, Saudi Arabia, Serbia, Singapore, Slovakia, Slovenia, South Africa, Sri Lanka, Sudan, Suriname, Taiwan, Tanzania, Thailand, Trinidad and Tobago, Tunisia, United Kingdom, Uruguay, USA, Venezuela, Vietnam, Yemen, Zambia.

In the description of results, arbitrary thresholds of quality limits were not used. The results for AST are expressed as correct, minor, major, very major, and critical and total deviations as described above.

3.1 Methods used by EQAS participants

A total of 183 laboratories received *Salmonella* strains, and 160 (87%) participated in the *Salmonella* serogrouping component of the EQAS, whereas 148 (81%) participated in the complete serotype module of the EQAS. In addition, 152 (83%) laboratories submitted AST results. Among the laboratories performing AST, 115 (76%) submitted results for the quality control (QC) strain *E. coli* ATCC 25922. The majority (n=90; 78%) of these laboratories used the disk diffusion method, while a MIC determination method was utilized by a smaller number (n=25; 22%) of laboratories.

Of 142 laboratories receiving *Shigella* strains, 116 (82%) submitted *Shigella* serogroup results (speciation) and 78 (55%) of these laboratories serogrouping the isolates further analyzed the strains to the serotype level. In addition, *Shigella* AST was performed by 114 (80%) laboratories.

All participating laboratories were given information regarding the MIC breakpoints used for interpretation when generating the expected values, with the exception of equivalent breakpoints for disk diffusion. In addition, all participating laboratories were instructed on interpreting resistance to third generation cephalosporins and to fluoroquinolones.

Of the 130 laboratories receiving *Campylobacter* strains, 130 (100%) reported identification results and 37 (28%) submitted AST results for both *Campylobacter* strains.

Of the 134 laboratories receiving the unknown culture for identification, 115 (86%) submitted results.

3.2 Serogrouping and serotyping of *Salmonella* strains

In 2010, the percentage of laboratories reporting complete serotype results for all eight strains increased to 87% (n=129), thus continuation of the increasing trend observed in 2009 (*e.g.* 83%, n=119). The proportion of correctly serotyped strains increased from 86% (n=974) in 2009 to 89% (n=998) in 2010 (Table 1).

In Table 2, the number of participating laboratories is reported according to the number of correctly serotyped samples. In 2010, 91 (61%) of the 148 participating laboratories serotyped all eight strains correctly, and 16 (11%) laboratories correctly serotyped seven of the eight strains. Summarizing, in 2010, a total of 107 (72%) participating laboratories met the threshold for adequate performance of *Salmonella* serotyping, which represents a minor increase compared to 2009 when only 105 (69%) of the participating laboratories met the performance quality threshold. In addition, 86% of the participating laboratories correctly identified half of the strains, which represents an 4% increase compared to 2009 (82%).

In Table 3, the performance of *Salmonella* serotyping is reported on a region-based categorization of participating laboratories. Overall, the accuracy of serotyping increased in many regions compared to 2010. No regions experienced an influx of EQAS participants in 2010. In contrast, in the Chinese region, participation to the EQAS 2010 decreased of six laboratories compared to 2009.

The number of tested strains decreased in most in regions with exception in Russia and North America. The accuracy of serotyping increased mostly in laboratories from North American (7.8% increase), Southeast Asia (8.9% increase), Caribbean (9.6% increase), and Asia & Middle East (29.0% increase) compared to 2009. A decrease in accuracy of serotyping was mostly observed in Africa compared with 2009.

The overall performance of laboratories performing *Salmonella* serogrouping was less satisfactory compared to 2009 as the percentages of deviations were ranged from 2.6% (WHO S10.8) to 16.3% (WHO S10.6) (Table 4).

Of 143 laboratories performing serotyping of the internal quality control strain (WHO S10.2, used in EQAS 2000, 2001, 2004, 2006, 2007, 2008, and 2009), 138 (97%) reported a correct result, thus leading to a deviation rate of only 3% (Table 4). Thus in 2010, t he ability of participating laboratories to correctly serotype the internal quality control strain was the highest ever recorded in the seven years where it has been included (Table 5).

Deviations in *Salmonella* serotyping ranged from 3.5% (WHO S10.2) to 15.6% (WHO S10.6) (Table 4), thus showing an improvement compared to last year when the highest percentage of deviations was 19%. In 2010, four strains; WHO S10.1 *Salmonella* Muenster, WHO S10.3 *Salmonella* Bareilly, WHO S10.6 *Salmonella* Senftenberg, and WHO S10.7 *Salmonella* Kedougou resulting in most deviations with a percentage above 10% (Table 4). Only WHO S10.2 *Salmonella* Enteritidis strain was serotyped satisfactorily with 3.5% deviations which is a slight increase compared to 2009 (Table 4).

3.3 Antimicrobial susceptibility testing (AST) of *Salmonella* strains

A total of 12,580 antimicrobial susceptibility tests were performed in 2010 by 152 participating laboratories. Of the submitted results, 92% were in agreement with the expected result, which is a 2% reduction compared to 2009 (Table 6). Minor, major and very major deviations were observed in 4%, 3% and 2% of the submitted results, respectively (Table 6).

Some difficulties in assessing antimicrobial susceptibility were encountered for the tested combinations of strains and antimicrobials. The difficulties were mainly in assessing susceptibility to STR and SMX (Table 7).

Major deviations categorized by tested antimicrobial are reported in Table 8. Notably, a large number of critical deviations were observed for SMX (14%), and STR (19%). These antimicrobials together with CRO, CTX, and TET resulted also in very high numbers of total deviations (Table 8). In 2010, the average number of critical and total deviations overall observed was 5% and 9%, respectively, which represents an increased percentage of deviations compared to 2009.

In 2010, the number of laboratory participating to the AST component of EQAS decreased in China, Europe, and Southeast Asia (Table 9). In particular, compared to 2009, China registered a decrease of five participants. By contrast, additional three laboratories took part to the EQAS AST component in the Latin American region. Overall, the performance of AST differed in all regions, most notably in the African regions where the performance (percent correctly tested) decreased from 90.1% in 2009 t o 84.7% in 2010. Overall, antimicrobial susceptibility test results were reported correctly in percentages ranging from 84.7% (Africa) to 95.8% (Central Asia and Middle East) (Table 9).

Antimicrobial susceptibility to *E. coli* ATCC 25922 was tested by 25 laboratories with the MIC determination method and by 90 laboratories with the disk diffusion method. The proportion of laboratories which submitted values outside the acceptable interval for the reference strain *E. coli* ATCC 25922 is reported in Table 10. The percentages of laboratories which reported MIC values outside the intervals accepted for the QC strain ranged from 5% (NAL, STR, and TET) to 15% (TMP) (Table 10). In general, laboratories using the MIC determination method reported values within the acceptable interval in higher percentages compared to the laboratories using the disk diffusion method, with the exception to CRO and FIS testing (Table 10).

3.4 Serogrouping and serotyping of *Shigella* strains

In 2010, the performance of *Shigella* speciation was highly satisfactory, as the percentages of deviations were very low for all the four test strains, ranging from 0.0% (WHO SH 10.3) to 2.0% (WHO SH 10.4) (Table 11). In contrast, the diviations observed among laboratories performing full serotyping were quite high ranging from 13.3% (WHO SH 10.4) to 20% (WHO SH 10.3). This represents a huge decreased compared to the results of 2009. The strain resulting in most deviations was WHO SH 10.3: *Shigella flexneri* variant X, which was reported as serotype 5b, 5a, 2b, var. Y, 1b, and 2a by 13 participating laboratories.

In Table 12, the performance of *Shigella* serotyping is reported according to geographical distribution of participating laboratories. The majority of participating laboratories was located in Latin America (n=13), Southeast Asia (n=14), and Europe (n=15). The accuracy of *Shigella* serotyping results ranged from 62.5% (Africa) to 100% (Oceanic and North America).

3.5 Antimicrobial susceptibility testing (AST) of *Shigella* strains

A total of 4,517 antimicrobial susceptibility tests were performed in 2010 by 114 participating laboratories. Agreement with the expected result was achieved in 91% of the reported results, which is a 5% reduction compared to 2009 (Table 13). Minor, major and very major deviations were observed in 2%, 1% and 6% of reported results, respectively (Table 13).

Difficulties in assessing antimicrobial susceptibility to CIP was encountered (Table 14). CHL, SXT, STR, and CIP accounted for 9.2%, 13.8%, 14.6% and 77.9% of total deviations, respectively (Table 15).

ESBL-producing *Shigella* strains were not included in the EQAS 2010 trial. However, the participating laboratories had between 0.0% and 3.0% deviating results for CAZ, CRO, and CTX (Table 15).

In 2010, laboratories in all regions participated in the *Shigella* AST component. The majority of participating laboratories was located in the European, Latin American, Southeast Asian and African regions where 27, 22, 16 and 16 laboratories participated to this EQAS iteration, respectively (Table 16). By considering participating laboratories in relation to their geographical location, the percentage of correct AST results ranged from 84.8% (Africa) to 95% (North America). The African and Caribbean regions reported results presenting the highest percentages of critical and total deviations, *i.e.* 12.7% and 11.5% critical deviations, and 15.2% and 11.5% total deviations, respectively. Also the Southeast Asian, Oceanic, Central Asia & Middle East regions had a considerably high number of total deviations (9.4% to 10%) (Table 16).

3.6 Identification of *Campylobacter* strains

Participation in the EQAS 2010 *Campylobacter* component was requested by 130 laboratories, of which 99 (76%) submitted results within the deadline. Of the participating laboratories, 92% and 85% performed correct species identification for strain #1 (*C. jejuni*) and #2 (*C. coli*), respectively (Table 17). A considerable moderate number of laboratories (n = 11) reported #2 (*C. coli*) being *C. jejuni.*

In Table 18, the performance of *Campylobacter* identification is reported according to geographical location of participating laboratories. The majority (n=29; 22%) of participating laboratories were as in 2009 located in Europe (n=29), but a large number of participates were also observed Latin America (n = 19). The accuracy in *Campylobacter* identification ranged from 67% (Caribbean) to 100% (Oceanic, Russia, and Asia & Middle East). The performance has increased tremendously since 2009 in regions such as Africa and Asia & Middle East.

3.7 MIC determination of *Campylobacter* strains

A total of 404 MIC determinations were performed in 2010 by 37 participating laboratories (Table 21),. Among the reported results 91.3% were in agreement with the expected result (Table 19). Major and very major deviations were observed in 4.2% and 4.5% of reported results (Table 19).

No major difficulties in assessing antimicrobial susceptibility were encountered for any of the tested combinations of strains and antimicrobials (Table 20). However, 9.7%, 11.3%, 11.4% and 12.7%, deviations were reported for TET, Gen, STR, and ERY susceptibility testing, respectively (Table 21).

In 2010, MIC values were submitted by almost all laboratories with exception of Central Asia & Middle East, Caribbean, and Oceania (Table 22). Agreement with expected values was observed in percentages ranging from 77.2% (Southeast Asia) to 100% (Europe and China) (Table 22). The highest percentages of critical deviations were reported from laboratories in Southeast Asian regions and Russia (22.8% and 21.4%, respectively (Table 22).

MIC values of reference strain *C. jejuni* ATCC 33560 were tested by 20 (54%) laboratories. Of these, 14 laboratories used micro-dilution procedures, while six laboratories used agar-dilution procedures and tested only CIP, ERY and GEN. Overall, the percentage of laboratories which submitted values within the acceptable interval for the reference strain ranged from 75% to 90% (for CHL and CIP/ERY susceptibility testing, respectively (Table 23).

3.8 Identification of the unknown culture

Identification of the unknown enteric pathogen (*Citrobacter* spp.) was performed by 115 laboratories (Table 23). Overall, 90% of the participating laboratories identified the strain as a *Citrobacter* spp.

3.9 ESBL-producing *Salmonella*

An optional part of the EQAS was to detect and confirm Extended-Spectrum Beta-Lactamase (ESBL) production. If participating in this item of the EQAS, all strains showing reduced susceptibility to cefotaxime (CTX), ceftazidime (CAZ) and/or ceftriaxone (CRO) should be tested for ESBL production.

One of the *Salmonella* test strains was ESBL-producing (WHO S-10.3), however, it was an ampC-type ESBL-producer (harbouring an *acc*-gene) and not a so-called 'true-ESBL'. The test strain belongs to the group we usually refer to as ESC (extended spectrum cephalosporinase-producers) but the description in the protocol did not cover ampC-type ESBL-producers and therefore the results as regards ESBL-production for this test strain was disregarded in the evaluation. Uploaded results regarding ESBL-producing strains are listed in Table 25 presenting the fact that all uploaded results but one (for WHO S-10.1) was in accordance with the expected.

4. Discussion

4.1 Serogrouping and serotyping of *Salmonella* strains

As in previous years, the selection of serovars included in the 2010 WHO GFN EQAS trial was based both on the 15 most common serovars submitted to the WHO GFN Country Data Base (CDB) [7] and on various reports and scientific publications. To facilitate the global assessment of *Salmonella* serotyping capacity, we chose serovars which may be very common in certain regions and sporadically encountered in other regions. In 2010, we included *Salmonella* Kedougou and Litchfield which are common in Southeast Asia more specifically *Salmonella* Litchfield have recently caused outbreaks in Japan and *Salmonella* Kedougou seems to be endemic in regions in Thailand [8;9]. We also included *Salmonella* Kentucky which is a common serovar associated with poultry in the United States of America [10]. Furthermore, another more resistant clone has been observed in Northern Africa and European causing gastrointestinal diseases in humans [11]. Additionally, we included *Salmonella* Muenster which is reported occasionally in Europe and the United States but is very common in Africa. Likewise, *Salmonella* Amsterdam has frequently been observed in Tunisia.

The number of laboratories which serotyped all eight *Salmonella* strains increased once again from 119 (83%) in 2009 to 129 (87%) in 2010, which represents the best attempt to serotype as many serovars as possible (Table 1). Similarly, the percentage of correctly serotyped strains was higher only in 2002 compared to in 2010. However, fewer laboratories submitted results compared to 2010. Two reasons could explain the excellent results obtained in 2010. First, all the *Salmonella* strains selected for the EQAS 2010 could be fully serotyped using commonly available antisera. Second, a huge increase in performance was observed in regions such as North America, Southeast Asia, Asia & Middle East, and Caribbean. The larges effect may be originating from the performance in Southeast Asia where also a large number of participants are enrolled the EQAS in 2010. Of note, an astonishing 97% of participating laboratories correctly serotyped the internal control strain (WHO S10.2), which is again the highest percentage ever recorded since the beginning of the EQAS. The quality threshold of correctly serotyping at least seven strains was met

by 72% of participating laboratories, thus demonstrating a clear improvement compared to previous years. Once more, this result could indicate that the participants are actually improving as the panel of strains chosen in 2010 was not believed to be easier to serotype compared to the strains provided in 2008 and 2009.

In general, the obtained results indicate that most laboratories worldwide now have the capacity to serotype the most common *Salmonella* serovars. Noteworthy, many developing regions obtained better results compared to 2009 which are a truly impressive accomplishment.

In 2010, we have observed another kind of serotyping errors in comparison with previous years. In the previous years the main problem in identifying the correct serotype was linked to difficulties in the characterization of flagellar antigens, which could be the consequence of a lack of good quality antisera, since laboratories often correctly identified the O antigen and one of the two flagellar antigens. In other cases, participating laboratories correctly identified the O antigen and the flagellar antigen complex, but incorrectly identified the minor antigens within the complex. In 2010, the main problem seems to be related to detecting the O antigen since there are no major differences between the percentages of deviations to serogrouping and to serotyping

4.2 Antimicrobial susceptibility testing (AST) of *Salmonella* strains

Overall, 92% of the *Salmonella* AST was correctly performed, and critical deviations were 5%. This result is satisfactory but a clear decrease in performance compared to 2009. Of note, the number of participating laboratories decreased for the second year in a row to 152 in 2010. This is an unfortunate development as the level of antimicrobial resistance is increasing with a tendency of creating more multi drug resistance pathogens. We need to strengthen the awareness about antimicrobial resistance and the need for performing antimicrobial susceptibility testing accurately.

Again in 2010, guidelines for MIC breakpoint interpretation were given to participating. In addition, expert guidelines on the interpretation of cephalosporin resistance were also distributed to instruct laboratories to report resistance to all cephalosporins regardless of MIC, in case resistance to one cephalosporin was observed. Similarly, participating laboratories were asked to utilize EUCAST epidemiologic break-points for interpretation of CIP susceptibility. The EQAS organizers utilized the lower epidemiologic breakpoint for ciprofloxacin to facilitate the detection of low-level resistance which may be caused either by alteration of the drug target due to a single point mutation in the gyrase-encoding gene or by protection of the drug target due to Qnr proteins which are encoded by plasmid-mediated genes. Accurate detection of these low-level ciprofloxacin-resistant strains is essential to warrant appropriate clinical treatment. Indeed, patients infected with low-level ciprofloxacin-resistant strains may have either a higher likelihood of treatment failure or a poor clinical response if treated with fluoroquinolones. Of note, low-level ciprofloxacin-resistant strains would be interpreted as susceptible according to current CLSI clinical breakpoints. However in 2010, no such low-level ciprofloxacin-resistant strains were included the panel.

As in previous years, a high percentage of total deviations were observed for STR, SMX and TET susceptibility tests. Interestingly, CIP susceptibility tests seemed in 2010 not to creat that many deviations compared to previous years. This might be a result of the lack of low-level ciprofloxacin-resistant strains in the panel. In case of STR susceptibility test, in EQAS 2010 we observed even greater difficulties comparable to what observed in the 2009 EQAS. This might be due to lack of appropriate cut off values and zone diameters or MIC values near the breakpoint. As a consequence, DTU Food launched a study among 17 laboratories from Europe, China and North America to establish an exact breakpoint for resistance. Recently, the data of the study were published suggesting new and updated cut off values for STR [12]. In case of SMX susceptibility test, we

similarly observed more deviations in the results reported in EQAS 2010 than in the 2009 EQAS. The potency of this antimicrobial is highly dependent on the quality of the test media used for susceptibility testing, and it is well known that SMX breakpoints are difficult to interpret. Therefore, the observed deviations could have been caused by high thymidine and thymine content in the medium, which antagonize the effects of SMX and / or by difficulties in the interpretation of sulfonamide breakpoints, since it is common to observe light growth in the inhibition halo near the sulphonamide breakpoint. Of importance, sulfonamide zone diameters should be measured from the point of 80% inhibition and not from the point of complete inhibition which is typically utilized for interpretation of susceptibility tests for other classes of antimicrobials. Finally, in case of TET susceptibility test, the observed deviations could have been caused by the sensitivity of this antimicrobial to the pH of Müller Hinton media used.

In general, data from the *Salmonella* AST component of EQAS 2010 demonstrate a minor reduction in the performance compared to 2009. Of note, fewer laboratories from China, and Southeast Asian participated to this EQAS iteration compared to 2009. There is no obviously reason for the decrease in performance as the percentage of deviations seemed to decrease for all antimicrobials compared to the 2009 EQAS.

When performing AST, the inclusion of reference strains for internal QC is extremely important. If correctly used, the reference strain will provide QC for both the method and the reagents. Unfortunately, only 116 (76%) participating laboratories submitted AST results of the QC strain. We always encourage laboratories to conduct quality assurance when performing AST and, to facilitate internal QC, we provide each new participating laboratory with the reference strain *E. coli* ATCC 25922. Laboratories participating in EQAS are invited to retain and maintain the QC strain for future use. As a rule, results for the test organisms should not be reported if ≥ 3 out of 30 results for the QC strain are outside the expected interval. Unfortunately, we did not observe any improvement in AST of QC strains by using either disk diffusion or MIC determination, as a high number of laboratories reported results outside the accepted QC interval. These erroneous results typically arise from inadequate standardization of methodologies, lack of good quality culture media and improper storage of antimicrobial-containing disks. Thus, deviations in AST results can likely be corrected by improving QC practices. For example, if the use of cotton swabs for plating bacteria causes repeated failures to obtain values within the acceptable QC interval, we recommend dispensing different volumes of bacterial inoculum onto Müller Hinton II agar plates to determine the exact volume necessary to obtain acceptable results.

In conclusion, the EQAS 2010 results showed a slight decrease of performance to *Salmonella*. In addition, EQAS aims at improving the component related to AST of the QC strain which, in 2010, was less satisfactory than in previous years. It is important to emphasize that this component represents the true indicator of the quality of AST performance.

4.3 Serogrouping and serotyping of *Shigella* strains

In EQAS 2010, participating laboratories were scattered in all regions with exception of the Caribbean. Of note, a maximum of only two deviations of each isolate were observed but all of the participating laboratories serogrouped the WHO SH10.3 *Shigella flexneri* 100% correct. In addition, up to 67 participating laboratories serotyped the strains and the majority of the observed deviations were related to the same isolate as all participants had serogrouped 100% correctly. Similar problems were obverted to the other *Shigella flexneri* isolate included the panel in 2010. This might indicate some problems to either the antisera or the typing scheme. However, this needs more attention in terms of finding the real nature of the problem typing *Shigella flexneri.* Need of

improvements were identified mainly in the African region where eight laboratories performed *Shigella* serotyping with only 62% of correct results.

4.4 Antimicrobial susceptibility testing (AST) of *Shigella* strains

In EQAS 2010, AST of *Shigella* spp. was performed by 114 laboratories which is a slight increase compared to 2009. All regions submitted results with an overall regional performance similar to the one described for *Salmonella* AST. In contrast to 2009, the results reported for *Shigella* AST revealed different problems as described for *Salmonella*. Accordingly, we observed high percentages of deviations related to CIP, STR and SXT susceptibility test results. Surprisingly, participating laboratories performed SMX and TET susceptibility testing of *Shigella* more correctly then SMX susceptibility testing of *Salmonella*.

4.5 Identification of *Campylobacter* strains

In 2010, we selected both *Campylobacter jejuni* and *Campylobacter coli* strains as in 2009. We noticed a huge difference in performance per region in the different years. The regions performing less satisfactory one year performed well the following year. In 2010, the Caribbean seemed to perform worse with 67% correctly identified *Campylobacter*. Overall, the results related to *Campylobacter* identification were excellent as in 2009 with 92% of the submitted results correct for *C. jejuni*.

4.6 Antimicrobial susceptibility testing (AST) of *Campylobacter* strains

In EQAS 2010, 37 laboratories participated in the MIC determination and performed overall satisfactorily, since they obtained 91.3% correct test results. No laboratories from the Central Asia & Middle Eastern, Caribbean, and Oceanic regions participated in this EQAS component. Two participating laboratories from Africa reported more deviating results (50%) compared to laboratories from other regions in 2009. However in 2010, two laboratories again participated (not aware if they are the same) but increased the percent correct tests up to 95%.

The majority of observed deviations were linked to ERY, GEN, STR and TET susceptibility testing. Inconsistent deviations were observed for CIP and NAL susceptibility testing, which is surprising since resistance to these antimicrobials in *Campylobacter* is caused by target alteration due to the same point mutation(s) in *gyrA*, and therefore similar deviations would be expected.

Only 20 (54%) participating laboratories submitted AST results for the QC strain. The majority of deviations were observed for CHL and NAL susceptibility testing by micro-dilution at 42 °C and GEN susceptibility testing by micro-dilution at 37 °C. In general, AST of the QC strain was satisfactory. However, CHL and NAL susceptibility testing of the QC strain can be improved.

4.7 Identification of the unknown culture

In EQAS 2010, we included a *Citrobacter ssp*. strain due to its O serology to *Salmonella* O9,46+. We wanted to observe if participants would simply rely on basic biochemical reactions (eg TSI) and serology. Of 115 laboratories delivering results, 90 (78%) identified the strain completely. This indicates that most laboratories in fact are able to distinguish between *Salmonella* and *Citrobacter*.

5. Conclusions

The acceptance threshold for the *Salmonella* serotyping EQAS component was met by 72% (n=105) of the participating laboratories. In addition, 87% of the laboratories tested all eight strains

and a total of 89% of all tests were correct, thus representing an increase compared to 2009. Additionally, the ability in testing correctly the internal QC strain increased of 4% compared to 2009. Most of the regions performed satisfactorily, with a result overall similar or better than to last year. However, the *Salmonella* serotyping capacity of laboratories in African regions still needs to be improved. This year's results indicate that detection of the somatic antigen was the most critical point for obtaining satisfactory serotyping results. In addition, these results show that many laboratories in developing countries still need supplies of antisera to facilitate serotyping of strains with rare antigenic formulae.

Concerning the *Salmonella* AST component, the obtained results emphasize the importance to harmonize the methodology and to provide adequate guidelines. Indeed, analysis of the results indicate that the distribution of the latest guidelines for breakpoint interpretation and the strengthened awareness of the importance of performing an internal QC have increased the ability of most laboratories to perform correct AST. Overall, the acceptance threshold was met, and we identified 5 minor and 3 critical deviations. Notably, STR, SMX, CRO, CTX and TET caused the majority of the observed deviations as in the previous EQAS iterations. No regional underperformance was observed, and the Central Asian & Middle Eastern regions once again improved considerably compared to EQAS 2009. Unfortunately, 36 (24%) participating laboratories did not report data for AST of the QC strain despite the EQAS organizers repeatedly recommended the use of such QC strains and are willing to provide them. Once more, we want to remind the importance of the use of QC strains for optimizing the methodology in use, since many laboratories reported values out of the accepted QC range both for MIC determination and for disk diffusion.

A *Shigella* component was included also in EQAS 2010, and consisted of serogrouping, serotyping and AST. Most laboratories correctly serogrouped the four *Shigella* strains, and a maximum of 2% deviations was observed. A total of 67 laboratories performed serotyping, with a maximum of 20% deviations. Only minor regional differences were observed, and the highest number of deviations was reported from laboratories from the African region.

The results obtained in the *Shigella* AST component suggest conclusions similar to the ones reported above concerning the *Salmonella* AST.

A total of 130 laboratories requested to participate to the *Campylobacter* component of EQAS 2010, but only 88 laboratories uploaded data related to identification. The *C. jejuni* strain was correctly identified by 92% of the participating laboratories. The majority of difficulties in *Campylobacter* identification were experienced by laboratories in the African, Latin America, and the Caribbean regions.

EQAS 2010, a total of 37 laboratories participated in MIC determination. The acceptance threshold used for *Salmonella* was applied and was almost met, since we observed 8.7% critical deviations. The data revealed that ERY, GEN, STR and TET susceptibility testing were the most challenging. In addition, discrepancies between NAL and CIP susceptibility testing were observed. Of the 37 participating laboratories, 20 performed AST of the QC strain, and the majority of the results for CHL and NAL susceptibility were out of the accepted range.

The unknown strain, *Citrobacter spp*, was identified by 90% of the participating laboratories at the genus level (*Vibrio* spp.), and by 48% of the participating laboratories at the species level (*V. mimicus*).

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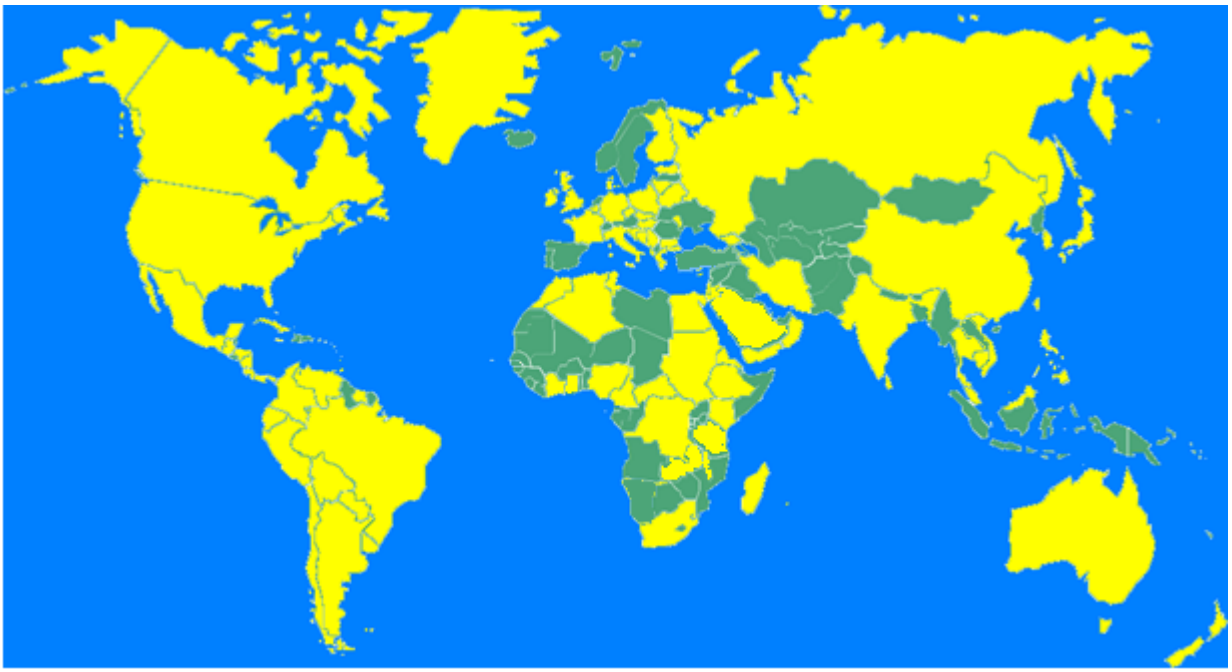
Figure and Tables

Figure 1. Countries participating* in the WHO EQAS 2010



*marked in yellow

Table 1. EQAS participating laboratories' performance of *Salmonella* serotyping

EQAS iteration	Labs serotyping all provided strains		Correct test results	
	No.	%	No.	%
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
2009	119	83	974	86
2010	129	87	998	89
Average	90	77	733	84

Table 2. Ability of EQAS participating laboratories to serotype the test *Salmonella* strains

Number of strains correctly serotyped	Participating laboratories											
	EQAS 2000		EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004		EQAS 2006	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
8	9	24	34	35	52	53	32	25	41	32	42	32
7	9	24	13	14	19	19	15	12	14	11	35	27
6	4	11	9	9	12	12	18	14	16	13	19	15
5	3	8	9	9	4	4	23	18	16	13	12	9
4	3	8	4	4	1	1	14	11	11	9	7	5
3	4	11	8	8	4	4	13	10	10	8	5	4
2	2	5	3	3	5	5	4	3	10	8	3	2
1	2	5	5	5	1	1	5	4	5	4	4	3
0	1	3	11	11	1	1	3	2	4	3	3	2
In total	37	100	96	100	99	100	127	100	127	100	130	100

Number of strains correctly serotyped	Participating laboratories									
	EQAS 2007		EQAS 2008		EQAS 2009		EQAS 2010		AVERAGE EQAS 2000 - 2010	
	No.	%	No.	%	No.	%	No.	%	No.	%
8	66	47	50	33	76	50	91	61	493	41
7	29	21	36	24	29	19	16	11	215	18
6	13	9	11	7	7	5	12	8	121	10
5	11	8	14	9	13	8	9	6	114	9
4	7	5	12	8	5	3	6	5	70	6
3	6	4	9	6	7	5	2	1	68	6
2	2	1	8	6	5	3	2	1	44	4
1	6	4	9	6	6	4	7	5	50	4
0	0	0	2	1	5	3	3	2	33	3
In total	140	100	151	100	153	100	148	100	1208	100

Table 3. Region-based categorization of EQAS participants' performance of *Salmonella* serotyping

Region	EQAS iteration	No. of labs	No. of strains serotyped	% strains correctly serotyped	Countries participating in EQAS 2010
Africa	2001	6	37	73.0	Algeria, Cameroon, Central African Republic, Democratic Republic of Congo, Ghana, Ivory Coast, Kenya, Madagascar, Malawi, 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	
	2009	15	94	75.5	
	2010	13	83	67.5	
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	
	2009	5	32	46.9	
	2010	5	22	75.9	
Caribbean	2001	0	0	0	Barbados, 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	
	2009	3	12	83.3	
	2010	2	13	92.9	
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	
	2009	16	126	95.2	
	2010	10	74	92.5	
Europe	2001	43	323	80.5	Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Luxembourg, Malta, Republic of Moldova, Poland, Serbia, Slovak Republic, Slovenia, 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	
	2009	47	362	93.1	
	2010	45	332	94.1	

Table 3 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* serotyping

Region	EQAS iteration	No. of labs	No. of strains serotyped	% strains correctly serotyped	Countries participating in EQAS 2010
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	
	2009	12	90	92.2	
	2010	13	103	100.0	
Oceania	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	
	2009	4	32	96.9	
	2010	4	32	100.0	
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	
	2009	7	49	91.8	
	2010	8	54	87.1	
Latin America	2001	11	78	57.7	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemale, Mexico, Nicaragua, Panama, Paraguay, Peru, Uruguay,
	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	
	2009	21	150	77.3	
	2010	22	132	80.0	
Southeast Asia	2001	15	113	54.0	Brunei, Cambodia, Japan, Malaysia, Philippines, Singapore, 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	
	2009	23	180	81.1	
	2010	24	172	90.5	

Table 4. *Salmonella* serogroups (SG), serotypes (ST) and deviations (D), WHO EQAS 2010

Strain ID	Correct serotype		No. of labs reporting SG	% D_{SG}	No. of labs reporting ST	% D_{ST}	Deviating results (*)
WHO S-10.1	Muenster	I 3,10:e,h:1,5	160	8,8	144	13,2	Lamberhurst (3), Nyborg(2), Sekondi (2), Vejle (2), Vilvoorde (2), Aminatu (1), Anatum var. 15+,34+ (1), Enterica (1), Lexington (1), Newlands (1), S. Vejle (1), Salmonella spp (1), Typhi (1).
WHO S-10.2	Enteritidis	I 9,12:g,m:-	159	3,9	143	3,5	Dublin (1), Gueuelatapee (1), Hillingdon (1), Wernigerode (1), Salmonella spp (1)
WHO S-10.3	Bareilly	I 6,7:y:1,5	159	3,9	138	14,5	Richmond (8), Colorado (1), Gatow (1), Hartford (1), Nessziona (1), Oyonnax (1), Rissen (1), S. Montevideo II (1), Sanjuan (1), Trachau (1),Thompson (1), V (1), Salmonella spp. (1),
WHO S-10.4	Amsterdam	I 3,10:g,m,s:-	156	6,9	143	9,1	Suberu (3), II 3,.15.:g,m,s,t:- (2), Hato II 3,10:-:- (1), Kingston (1), Mokola (1), S. Suberu (1), Westhampton (1), Subsp Salamae (1), Salmonella spp. (1).
WHO S-10.5	Litchfield (or Pakistan)	I 6,8:l,v:1,2	158	6,0	139	10,1	Fayed (3), Manchester (2), Bonn (1), Concord (1), IIIb (1), Lindenburg (1), Loanda (1), Nagoya (1),Phaliron (1), S.Bsilla (1), Typhimurium (1)
WHO S-10.6	Senftenberg	I 1,3,19:g,s,t:-	157	16,3	141	15,6	Westhampton (7), Dessau (4), Salmonella spp (2),Agona (1), Budapest (1), Cannstatt (1), Juba (1), Kiel (1), Kouka (1), Petahtikve (1), Rideau (1),S.Kouka (1)
WHO S-10.7	Kedougou	I 13,23:i:l,w	149	11,2	132	12,1	Zuilen (2), V (2), Chagoua (1), Hoboken (1), Idikan (1), Jukestown (1), Koessen (1), Ordonez (1), S. Sanktjohann (1), Wichita (1), Bongori (1), Salmonella spp(1)
WHO S-10.8	Kentucky	I 8,20:i:z6	157	2,6	140	9,3	Magherafelt (4), Lindenburg (3), Altona (1), Bargny (1), Malmoe (1), S. Banalia (1), Warnow (1), Salmonella spp (1)

*number of participants reporting the specified deviating result

Table 5. EQAS participating laboratories' performance of internal quality control strain (WHO S-10.2, *Salmonella* Enteritidis) serotyping

EQAS iteration	Labs serotyping *S.* Enteritidis correctly	
	No.	%
2000	34	92
2001	64	84
2004	113	95
2006	116	94
2007	135	96
2008	139	96
2009	141	93
2010	138	97
Average	110	94

Table 6. EQAS participating laboratories' performance of antimicrobial susceptibility testing of *Salmonella* strains

EQAS iteration	No. of EQAS participating laboratories	% correct test results	% minor deviations (S ↔ I or I ↔ R)†	% major deviations (S → R)†	% very major deviations (R→ S)†	% critical deviations (R→ S & S → R)†	% total deviations (S → R & R → S & S ↔ I or I ↔ R)†
2000	44	92	4	4	0	4	8
2001	108	91	6	2	1	3	9
2002	119	92	6	2	1	3	9
2003*	147	93	4	3	0	3	7
2004	152	93	4	2	1	3	7
2006	143	88	8	3	1	4	12
2007	143	93	4	2	1	3	7
2008	168	91	4	2	3	5	9
2009	153	94	3	2	1	3	6
2010	152	92	4	3	2	5	8
Average*	133	92	5	2	1	3	8

*Data do not include one strain which may have lost resistance due to transport or storage stress

†S, susceptible; I, intermediate; R, resistant

Table 7. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2010 *Salmonella* strains*

Strain	Antimicrobial†												
	AMP	**CTX**	**CAZ**	**CRO**	**CHL**	**CIP**	**GEN**	**NAL**	**STR**	**SMX**	**SXT**	**TET**	**TMP**
WHO S-10.1	5/1/**140**	4/3/**121**	3/2/**112**	3/0/**95**	1/2/**137**	3/0/**147**	3/1/**138**	2/4/**131**	**10**/12/78	7/3/**60**	6/0/**121**	6/9/**128**	1/0/**74**
WHO S-10.2	9/8/**131**	4/2/**124**	3/ 2/**113**	0/1/**98**	2/2/**137**	3/1/**146**	**137**/1/4	2/2/**134**	**90**/3/7	**64**/0/6	6/0/**121**	9/9/**123**	1/0/**75**
WHO S-10.3	**143**/2/2	**80**/35/14	**115**/2/2	**55**/31/14	1/2/**135**	4/0/**146**	5/1/**136**	0/3/**133**	4/**17**/79	12/5/**52**	5/0/**122**	5/4/**132**	2/0/**75**
WHO S-10.4	6/1/**140**	3/0/**125**	1/2/**114**	3/0/**94**	0/1/**139**	4/0/**145**	4/0/**138**	0/2/**135**	3/8/**89**	16/2/**51**	4/0/**123**	4/6/**132**	0/0/**76**
WHO S-10.5	5/0/**142**	3/0/**125**	1/0/**116**	2/0/**96**	3/0/**137**	3/0/**146**	5/2/**135**	0/1/**135**	**11**/34/55	10/6/**54**	3/0/**124**	2/3/**138**	0/0/**75**
WHO S-10.6	5/0/**141**	3/1/**124**	2/ 1/**114**	3/0/**95**	2/1/**137**	6/0/**143**	4/0/**138**	2/0/**136**	7/**29**/64	7/1/**61**	3/0/**123**	4/4/**134**	1/0/**74**
WHO S-10.7	7/0/**139**	3/0/**124**	1/0/**114**	3/1/**94**	2/3/**134**	9/0/**139**	5/2/**133**	1/2/**134**	7/**31**/62	9/2/**58**	3/0/**122**	4/9/**128**	0/0/**74**
WHO S-10.8	7/1/**138**	5/1/**122**	4/0/**113**	4/0/**95**	1/1/**138**	**142**/2/5	**110**/7/24	**134**/1/2	**83**/5/12	**58**/0/12	8/3/**115**	**120**/2/19	2/1/**73**

*In bold: expected interpretation. Grey cell: <90% of laboratories did correct interpretation. R, resistant; I, intermediate; S, susceptible.

†For antimicrobial abbreviations: see List of Abbreviations page 1

Table 8. EQAS participants' performance of *Salmonella* strains antimicrobial susceptibility testing categorized by antimicrobial

EQAS iteration	No. of labs	Performance	Antimicrobial[∞]																
			AMC	AMP	CAZ	CHL	CIP	POD	CRO	CTX	GEN	KAN	NAL	SMX	STR	SXT	TET	TMP	XNL	OVERALL
2000	44	No. of tests	-	343	-	343	334	-			343	312	328	248	312	-	335	295	-	3193
		% critical deviations*	-	6	-	4	1	-			4	4	1	3	4	-	6	1	-	3
		% total deviations^	-	8	-	7	6	-			5	16	4	5	12	-	13	1	-	8
2001	108	No. of tests	-	822	-	814	813	-			821	623	726	431	679	757	804	416	-	7706
		% critical deviations*	-	4	-	2	1	-			2	2	2	6	7	2	7	1	-	3
		% total deviations^	-	7	-	3	4	-			4	7	8	9	27	5	18	2	-	9
2002	119	No. of tests	-	918	-	903	911	-			905	680	885	495	718	724	861	499	-	8499
		% critical deviations*	-	2	-	2	0	-			2	2	2	4	4	7	3	3	-	3
		% total deviations^	-	3	-	3	2	-			16	10	4	4	34	10	7	3	-	9
2003•	147	No. of tests	-	1019	-	996	995	-			993	738	947	615	768	929	995	582	-	9577
		% critical deviations*	-	2	-	1	0	-			2	2	1	4	9	2	4	1	-	3
		% total deviations^	-	4	-	2	1	-			2	6	4	5	39	2	11	1	-	7
2004	152	No. of tests	973	1178	-	1159	1162	-	-	995	1201	-	1130	734	947	1051	1122	729	-	12381
		% critical deviations*	6	3	-	2	0	-	-	0	2	-	1	5	1	3	5	2	-	3
		% total deviations^	12	5	-	2	1	-	-	14	3	-	4	8	21	4	11	2	-	7
2006	143	No. of tests	950	1092	769	1060	1110	305	-	956	1078	-	1035	649	896	996	1054	607	225	12782
		% critical deviations*	9	2	7	3	2	1	-	7	3	-	2	6	5	3	9	1	2	4
		% total deviations^	22	3	11	15	6	26	-	15	7	-	6	7	22	5	20	2	9	12
2007	143	No. of tests	908	1114	830	1105	1101	389	-	914	1111	-	1092	678	875	971	1047	583	258	12976
		% critical deviations*	6	5	1	0	1	4	-	1	3	-	2	5	4	3	4	1	0	3
		% total deviations^	17	7	1	6	1	16	-	2	4	-	3	6	26	3	11	2	6	7
2008	168	No. of tests	-	1331	961	1226	1307	-	791	1104	1265	-	1168	718	867	1155	1249	696	-	13858
		% critical deviations*	-	3	3	1	19	-	3	3	4	-	2	4	7	3	6	2	-	5
		% total deviations^	-	8	6	11	21	-	6	6	6	-	4	5	25	4	13	2	-	9
2009	153	No. of tests	-	1206	921	1108	1190	-	775	1009	1143	-	1095	624	864	1042	1114	616	-	12707
		% critical deviations*	-	3	1	1	8	-	0	1	2	-	1	7	9	3	4	1	-	3
		% total deviations^	-	6	1	2	10	-	1	2	3	-	3	9	30	4	10	1	-	6
2010	152	No. of tests	-	1173	937	1118	1194	-	787	1026	1133	-	1096	566	800	1012	1134	604	-	12580
		% critical deviations*	-	4	2	1	3	-	4	4	5	-	1	14	19	4	5	1	-	5
		% total deviations^	-	5	3	2	3	-	8	8	6	-	2	17	55	4	9	1	-	9
Average•	133	No. of tests	944	1020	884	983	1012	347	784	1001	999	588	950	576	773	960	972	563	242	10626
		% critical deviations*	7	3	3	2	4	3	2	3	3	3	2	6	7	3	5	1	1	4
		% total deviations^	17	6	4	5	6	21	5	8	6	10	4	8	29	5	12	2	8	8

[∞]For antimicrobial abbreviations: see List of Abbreviations page 1

*R→ S & S → R (R, resistant; S, susceptible)

^S→R & R→S & S↔I or I↔R (I, intermediate)

• Data do not include one strain which may have lost resistance due to transport or storage stress

-, not determined

Table 9. Region-based categorization of EQAS participants' performance of *Salmonella* antimicrobial susceptibility testing

Region	EQAS iteration	No.of labs	% correct test result	% minor deviations (S ↔ I or I ↔ R)	% major Deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2010 iteration
Africa	2001	7	80.1	9.6	7.7	2.5	10.2	19.8	Algeria, Cameroon, Central African Republic, Congo, Côte d´Ivoire, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mauritius, Morocco, Nigeria, South Africa, Sudan, Tanzania, 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	
	2009	22	90.1	4.5	3.6	1.8	5.4	9.9	
	2010	22	84.7	6.0	6.5	2.8	9.3	15.3	
Central Asia & Middle East	2001	10	87.7	6.3	5.2	0.8	6.0	12.3	Egypt, Iran, 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	
	2009	6	93.7	4.3	0.9	1.1	2.0	6.3	
	2010	7	95.8	2.6	0.2	1.4	1.6	4.2	
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	
	2009	4	93.2	1.8	3.2	1.8	5.0	6.8	
	2010	4	90.9	5.4	2.7	0.7	3.4	8.8	
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	
	2009	14	94.8	2.2	2.1	0.8	2.9	5.1	
	2010	9	92.1	4.5	1.6	1.8	3.4	7.9	
Europe	2001	47	91.3	5.7	2.7	0.3	3.0	8.7	Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Italy, Lithuania, Luxembourg, Malta, Poland, Serbia, Slovakia, Slovenia, United Kingdom, Moldova
	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	
	2009	40	95.1	2.6	1.3	0.9	2.2	4.8	
	2010	39	92.4	4.1	1.2	2.3	3.5	7.6	

Table 9 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* antimicrobial susceptibility testing

Region	EQAS iteration	No.of labs	% correct test result	% minor deviations (S ↔ I or I ↔ R)	% major Deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2010 iteration
North 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	
	2009	10	98.7	0.0	0.4	0.9	1.3	1.3	
	2010	11	94.8	2.6	0.2	2.4	2.6	5.2	
Oceania	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	
	2009	4	95.9	3.2	0.3	0.6	0.9	4.1	
	2010	4	92.5	4.6	0.6	2.3	2.9	7.5	
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	
	2009	6	93.8	2.1	3.3	0.8	4.1	6.2	
	2010	8	94.3	3.3	1.3	1.1	2.4	5.7	
Latin America	2001	11	90.8	6.9	1.4	1.0	2.4	9.2	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, 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	
	2009	20	95.6	2.1	1.1	1.2	2.3	4.4	
	2010	23	90.8	2.1	5.6	1.4	7.1	9.2	
Southeast Asia	2001	16	88.1	7.7	2.3	1.9	4.2	11.9	Brunei Darussalam, Cambodia, India, Japan, Korea, Malaysia, Philippines, Sri Lanka, Thailand, Viet Nam
	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	
	2009	27	91.8	4.1	3.0	1.2	4.2	8.3	
	2010	25	92.8	3.8	1.5	1.9	3.4	7.2	

^S, susceptible; I, intermediate; R, resistant

Table 10. EQAS participants' performance of antimicrobial susceptibility testing of quality control strain *Escherichia coli* ATCC 25922

		Method	Labs' perfor-mance[4,5]	Antimicrobial[0]																	
				AMC	AMP	CAZ	CHL	CIP	POD	CRO	CTX	ENR	FFN[2]	FIS	GEN	NAL	SMX	STR	SXT	TET	TMP	XNL
Accepted interval[1]		**MIC (µg/ml)**		2-8	2-8	0.06-0.5	2-8	0.004-0.016	0.25-1	0.03-0.12	0.03-0.12	0.008-0.03	2-8	0.004-0.015	0.25-1	1-4	8-32	4-16[3]	≤0.5/9.5	0.5-2	0.5-2	0.25-1
		Disks (mm)		8-24	16-22	25-32	21-27	30-40	23-28	29-35	29-35	32-40	22-28	15-23	19-26	22-28	15-23	12-20	23-29	18-25	21-28	26-31
EQAS iteration (total no. of participants)	**2000** (44)	MIC & Disk	No.[4]	-	37	-	38	35	-	-	-	-	-	-	39	37	19	36	-	42	31	-
			%[5]	-	27	-	37	20	-	-	-	-	-	-	23	35	53	22	-	42	30	-
	2001 (107)	MIC & Disk	No.[4]	-	97	-	97	97	-	-	-	-	-	-	99	74	53	81	90	96	50	-
			%[5]	-	19	-	20	14	-	-	-	-	-	-	12	14	34	12	14	22	22	-
	2002 (114)	MIC & Disk	No.[4]	-	109	-	107	108	-	-	-	-	-	-	108	102	57	82	102	102	66	-
			%[5]	-	16	-	15	14	-	-	-	-	-	-	12	14	26	11	12	13	11	-
	2003 (144)	MIC & Disk	No.[4]	-	140	-	137	138	-	-	-	-	-	-	138	132	82	105	129	137	79	-
			%[5]	-	14	-	22	9	-	-	-	-	-	-	9	16	17	9	14	19	14	-
	2004 (140)	MIC & Disk	No.[4]	117	132	-	128	132	-	-	111	-	-	-	134	126	84	110	120	129	87	-
			%[5]	13	10	-	13	8	-	-	18	-	-	-	10	9	16	6	11	13	9	-
	2006 (137)	MIC & Disk	No.[4]	116	133	96	126	127	39	-	115	19	-	-	131	122	74	106	122	125	74	32
			%[5]	9	14	15	18	8	12	-	21	63	-	-	14	20	29	11	19	12	17	22
	2007 (126)	MIC & Disk	No.[4]	102	124	92	123	121	47	-	104	-	13	-	124	120	64	97	107	117	67	35
			%[5]	8	11	9	14	12	9	-	16	-	0	-	6	7	22	6	13	7	10	11
	2008 (147)	MIC & Disk	No.[4]	-	147	111	135	144	-	-	124	-	-	71	145	136	-	101	129	139	79	-
			%[5]	-	12	9	10	8	-	-	14	-	-	14	8	8	-	12	13	7	13	-
		MIC	No.[4]	-	33	23	24	33	-	-	23	-	-	18	31	23	-	19	22	28	16	-
			%[5]	-	0	5	0	6	-	-	9	-	-	11	0	0	-	11	9	0	13	-
		Disk	No.[4]	-	114	89	112	111	-	-	101	-	-	53	114	113	-	82	107	111	63	-
			%[5]	-	16	10	12	8	-	-	15	-	-	15	11	10	-	12	14	9	13	-
	2009 (129)	MIC & Disk	No.[4]	-	128	100	121	124	-	88	107	-	-	63	123	117	-	98	113	122	70	-
			%[5]	-	16	13	15	7	-	16	10	-	-	11	18	13	-	10	14	14	11	-
		MIC	No.[4]	-	27	19	24	26	-	20	20	-	-	14	25	24	-	19	21	27	25	-
			%[5]	-	11	11	8	8	-	15	15	-	-	21	12	8	-	5	19	11	13	-
		Disk	No.[4]	-	101	81	97	98	-	68	87	-	-	49	98	93	-	79	92	95	55	-
			%[5]	-	16	14	16	6	-	16	9	-	-	10	18	14	-	11	12	15	11	-
	2010 (116)	MIC & Disk	No.[4]	-	114	97	108	115	-	79	100	-	-	51	112	104	-	84	101	110	63	-
			%[5]	-	11	9	9	6	-	10	14	-	-	11	11	5	-	5	12	5	15	-
		MIC	No.[4]	-	25	15	21	25	-	15	17	-	-	12	24	19	-	17	17	24	11	-
			%[5]	-	12	20	10	8	-	7	18	-	-	8	13	16	-	18	18	17	36	-
		Disk	No.[4]	-	89	82	87	90	-	64	83	-	-	39	88	85	-	67	84	86	52	-
			%[5]	-	9	6	8	4	-	9	11	-	-	10	9	2	-	1	10	1	8	-

[0]For antimicrobial abbreviations: see List of Abbreviations page 1

[1]CLSI standard, Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 19th Informational supplement. CLSI document M100-S19, Wayne, Pennsylvania

[2]CLSI standars, Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for bacteria Isolated from Animals. M31-A3. 3rd Edition[Approved Standard]. 2008. Wayne, PA, USA

[3]Quality control range developed by the manufacturer of Sensititre®

[4]No., number of labs performing the analysis

[5]%, percentage of labs reporting erroneous results

-, not determined

Table 11. *Shigella* serotypes (ST) and deviations (D), WHO EQAS 2010

Strain	Correct serotype	No. of labs reporting correct identification	D (%)	Deviating results (*)	No. of labs reporting correct ST	D (%)	Deviating results (*)
WHO SH-10.1	*S. flexneri* var Y	114	0.9	1	67	13.4	2a (3), 1b, 3a, 3b, 4a, 4c, 5a
WHO SH-10.2	*S. sonnei*	114	1.7	2	N/A	N/A	N/A
WHO SH-10.3	*S. flexneri* var X	114	0.0	0	66	20.0	5b (4), 5a (3), 2b (2), var Y (2), 1b, 2a
WHO SH-10.4	*S. dysenteriae* serotype 3	100	2.0	2	60	13.3	1 (7), 2

*number of participants reporting deviating result

Table 12. Region-based categorization of laboratories performing *Shigella* serotyping in 2010

Region	Year	No. of laboratories	No. of strains serotyped	Strains serotyped correctly (%)	Countries participating in the 2010 iteration
Africa	2009	8	18	72.2	Cameroon, Côte d´Ivoire, Kenya, Algeria, Mauritius, South Africa, Tunisia
	2010	7	16	62.5	
Asia & Middle East	2009	3	5	100.0	Israel, Saudi Arabia, Yemen
	2010	3	6	83.3	
Caribbean	2009	-	-	-	-
	2010	-	-	-	
China	2009	13	35	100.0	China
	2010	9	23	91.3	
Europe	2009	15	40	92.5	Italy, Luxembourg, Malta, Albania, Denmark, Ireland, Belgium, Bulgaria, Czech Republic, Finland, Lithuania, Moldova, Serbia, Slovenia
	2010	15	35	85.7	
North America	2009	7	18	100.0	Canada, United States of America
	2010	7	20	100.0	
Oceanic	2009	3	8	100.0	Australia, New Zealand
	2010	3	8	100.0	
Russia	2009	6	18	83.3	Belarus, Georgia, Russia
	2010	7	20	75.0	
Latin America	2009	16	40	97.5	Ecuador, Mexico, Colombia, Panama, Argentina, Brazil, Chile, Costa Rica, Honduras, Nicaragua, Paraguay, Peru, Venezuela
	2010	13	33	78.8	
Southeast Asia	2009	11	30	90.0	Cambodia, India, Philippines, Korea, Sri Lanka, Taiwan, United Kingdom, Japan, Malaysia, Thailand
	2010	14	32	87.5	

Table 13. EQAS participating laboratories' performance of *Shigella* strains antimicrobial susceptibility testing

EQAS iteration	No. of participating laboratories	% correct test results	% minor deviations (S ↔ I or I ↔ R)^	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S → R & R → S & S ↔ I or I ↔ R)^
2008	15	95	2	2	1	3	5
2009	111	96	2	1	1	2	4
2010	114	91	2	1	6	7	9

^S, susceptible; I, intermediate; R, resistant

Table 14. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2010 *Shigella* strains*

Strain	Antimicrobial∞												
	AMP	**CTX**	**CAZ**	**CRO**	**CHL**	**CIP**	**GEN**	**NAL**	**STR**	**SMX**	**SXT**	**TET**	**TMP**
WHO SH-9.1	**108**/0/1	2/2/**93**	0/1/**87**	0/0/**74**	**92**/6/4	**47**/5/58	2/2/**99**	**97**/1/0	**67**/0/5	3/1/**45**	9/7/**76**	**100**/0/5	**53**/0/2
WHO SH-9.2	**108**/0/1	1/0/**96**	0/1/**87**	0/0/**74**	2/0/**102**	**47**/4/61	**96**/3/4	**94**/0/3	**66**/0/5	**47**/0/3	**90**/1/3	**99**/2/4	**54**/0/1
WHO SH-9.3	**105**/0/2	3/0/**92**	1/0/**87**	0/0/**73**	**90**/10/3	**50**/4/56	3/2/**97**	**93**/2/2	**66**/1/4	3/1/**45**	14/6/**72**	**100**/2/3	**55**/0/1
WHO SH-9.4	**95**/1/3	3/0/**85**	1/0/**79**	0/0/**70**	**84**/4/5	2/0/**100**	5/0/**90**	3/0/**87**	**41**/12/8	0/0/**46**	4/0/**81**	**88**/1/6	4/0/**48**

*****In bold: expected interpretation. Grey cell: <90% of laboratories did correct interpretation. R, resistant; I, intermediate; S, susceptible.

∞For antimicrobial abbreviations: see List of Abbreviations page 1

Table 15. EQAS laboratories' performance of *Shigella* strains antimicrobial susceptibility testing categorized by antimicrobial

EQAS iteration	No. of labs	Lab performance	Antimicrobial													
			AMP	CAZ	CHL	CIP	CTX	GEN	NAL	SMX	STR	SXT	TET	TMP	CRO	OVERALL
2008	15	No. of tests	52	44	51	48	48	50	52	7	27	52	52	4	42	529
		% critical deviations*****	1	2	1	-	2	1	-	-	4	2	4	-	2	1.5
		% total deviations^	1	2	1	-	2	1	-	-	9	2	8	-	2	2.2
2009	111	No. of tests	423	358	388	426	372	396	388	211	293	388	386	218	301	4548
		% critical deviations*****	2.4	0.3	2.1	0.2	1.1	2.5	0.5	3.8	5.8	2.3	2.8	1.8	0.3	1.9
		% total deviations^	3.8	0.3	4.6	0.9	1.1	3.5	1.5	3.8	18.1	3.6	7.5	1.8	0.6	3.8
2010	114	No. of tests	424	344	402	434	377	403	382	194	275	363	410	218	291	4517
		% critical deviations*	1.7	0.6	3.5	40.8	2.4	3.5	2.1	4.6	8.0	8.3	4.4	3.7	0.0	6.4
		% total deviations^	1.9	1.2	9.2	77.9	3.0	5.5	3.0	6.0	14.6	13.8	5.9	3.8	0.0	11.2

[∞]For antimicrobial abbreviations: see List of Abbreviations page 1

*****R→ S & S → R (R, resistant; S, susceptible)

^S→R & R→S & S↔I or I↔R (I, intermediate)

-, not determined

Table 16. Region-based categorization of EQAS participating laboratories' performance of antimicrobial susceptibility tests for *Shigella* strains in 2010

Region	Year	No. of labs	% correct test result	% minor deviations (S↔I or I↔R)^	% major deviations (S→R)^	% very major deviations (R→ S)^	% critical deviations (R→ S & S → R)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2010 iteration
Africa	2009	17	93.3	2.4	3.5	0.8	4.3	6.8	Algeria, Côte d´Ivoire, Tanzania, Ghana, Congo, Ethiopia, Cameroon, Kenya, Nigeria, Sudan, South Africa, Tunisia, Central African Republic, Mauritius
	2010	16	84.8	2.5	2.7	10.0	12.7	15.2	
Central Asia & Middle East	2009	5	94.8	0.9	3.0	1.3	4.4	5.2	Iran, Israel, Jordan, Oman, Saudi Arabia, Yemen
	2010	6	90.6	1.2	1.6	6.7	8.3	9.4	
Caribbean	2009	4	95.6	1.5	0.7	2.2	2.9	4.4	Barbados, Jamaica, Suriname, Trinidad and Tobago
	2010	4	88.5	1.5	3.8	6.2	10.0	11.5	
China	2009	12	96.3	2.2	1.0	0.5	1.5	3.7	China
	2010	8	92.7	1.2	0.6	5.5	6.1	7.3	
Europe	2009	22	98.1	1.1	0.7	0.1	0.8	1.9	Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Denmark, Finland, Greece, Ireland, Italy, Lithuania, Luxembourg, Malta, Moldova, Poland, Serbia, Slovakia, Slovenia, United Kingdom
	2010	27	93.6	1.5	0.9	3.9	4.8	6.4	
North America	2009	6	100.0	0.0	0.0	0.0	0.0	0.0	Canada, United States of America
	2010	7	95.0	0.0	0.0	5.0	5.0	5.0	
Oceanic	2009	-	-	-	-	-	-	-	Australia
	2010	1	90.0	10.0	0.0	0.0	0.0	10.0	
Russia	2009	6	95.5	1.6	1.6	1.3	2.9	4.6	Belarus, Georgia, Russia
	2010	7	92.1	2.9	1.5	3.5	5.0	7.9	
Latin America	2009	20	98.3	1.1	0.4	0.3	0.7	1.7	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Uruguay, Venezuela
	2010	22	92.1	1.3	2.1	4.5	6.6	7.9	
Southeast Asia	2009	18	94.1	3.9	0.3	1.7	2.0	5.9	Cambodia, India, Japan, Korea, Malaysia, Philippines, Sri Lanka, Thailand
	2010	16	90.5	2.4	0.7	6.4	7.1	9.5	

^S, susceptible; I, intermediate; R, resistant.

Table 17. EQAS participating laboratories' performance of *Campylobacter* strains identification

EQAS iteration	No. of labs	Correct species	Strain no.	No. of results submitted	% correct identification	Deviating results (*)
2003	97	*C. jejuni*	# 1	92	87%	*C. coli* (9) *C. lari* (3)
	97	*C. coli*	# 2	92	83%	*C. jejuni* (7) *C. lari* (4) *C. upsaliensis* (4)
2004	109	*C. lari*	# 1	95	80%	*C. coli* (11) *C. jejuni* (8)
	109	*C. jejuni*	# 2	107	87%	*C. coli* (8) *C. lari* (4) *C. upsaliensis* (2)
2006	99	*C. jejuni*	# 1	86	90%	*C. lari* (3) *C. coli* (3) *C. upsaliensis* (3)
	99	*C. coli*	# 2	94	66%	*C. lari* (19) *C. jejuni* (11) *C. upsaliensis* (2)
2007	142	*C. lari*	# 1	95	72%	*C. jejuni* (10) *C. coli* (9) *C. upsaliensis* (7)
	142	*C. coli*	# 2	99	74%	*C. lari* (3) *C. jejuni* (20) *C. upsaliensis* (2)
2008	154	*C. lari*	# 1	105	63%	*C. coli* (14) *C. jejuni* (18) *C. upsaliensis* (7)
	154	*C. lari*	# 2	105	60%	*C. coli* (10) *C. jejuni* (19) *C. upsaliensis* (13)
2009	131	*C. coli*	# 1	87	77%	*C. upsaliensis* (10) *C. jejuni* (9) *C. lari* (1)
	131	*C. jejuni*	# 2	87	95%	*C. upsaliensis* (3) *C. lari* (1)
2010	130	*C. jejuni*	# 1	88	92%	*C. coli* (4) *C. lari* (3) *C. upsaliensis* (1)
	130	*C. coli*	# 2	84	85%	*C. jejuni* (11) *C. lari* (2) *C. upsaliensis* (2)

*number of participants reporting the specified deviating result

Table 18. Region-based categorization of EQAS 2010 participating laboratories' performance of *Campylobacter* strains identification

Region	Year	No. of labs	No. of strains identified	% strains correctly identified	Countries participating in the 2010 iteration
Africa	2009	8	13	54	Algeria, Central African Republic, Ethiopia, Kenya, Mauritius, South Africa, Tunisia
	2010	7	13	77	
Asia & Middle East	2009	3	5	40	Israel, Oman, Saudi Arabia
	2010	3	6	100	
Caribbean	2009	2	4	100	Barbados, Jamaica, Trinidad and Tobago
	2010	3	6	67	
China	2009	12	24	92	China
	2010	10	20	85	
Europe	2009	28	53	89	Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Germany, Greece, Hungary, Italy, Lithuania, Luxembourg, Malta, Moldova, Poland, Serbia, Slovakia, Slovenia
	2010	29	57	96	
North America	2009	10	19	90	Canada, United States of America
	2010	11	22	86	
Oceania	2009	2	4	100	Australia, New Zealand
	2010	2	3	100	
Russia	2009	2	4	100	Belarus, Georgia
	2010	2	4	100	
Latin America	2009	14	26	89	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Paraguay, Peru, Uruguay, Venezuela
	2010	19	37	78	
Southeast Asia	2009	10	20	90	Brunei Darussalam, Cambodia, Japan, Korea, Malaysia, Philippines, Sri Lanka, Taiwan, Thailand, Viet Nam
	2010	14	27	93	

Table 19. EQAS participants' performance of *Campylobacter* strains antimicrobial susceptibility testing

EQAS iteration	No. of labs	% correct test results	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (R → S & S → R)^
2009	25	91.4	4.5	4.1	8.6
2010	37	91.3	4,2	4,5	8,7

^S, susceptible; R, resistant

Table 20. Antimicrobial susceptibility test results (number of R/S) for the EQAS 2010 *Campylobacter* strains*

Strain	Antimicrobial^						
	CHL	**CIP**	**ERY**	**GEN**	**NAL**	**STR**	**TET**
WHO C-10.1	1/**20**	**32**/2	**32**/3	**26**/3	**23**/3	**17**/2	**30**/3
WHO C-10.2	1/**22**	3/**33**	5/**31**	3/**27**	1/**26**	**18**/2	**3**/32

^For antimicrobial abbreviations, see List of Abbreviations page 1

*In bold: expected interpretation. R, resistant; S, susceptible

Table 21. EQAS participants' performance of *Campylobacter* antimicrobial susceptibility testing categorized by antimicrobial

EQAS iteration	No. of labs	Lab performance	Antimicrobial						
			CHL	**CIP**	**ERY**	**GEN**	**NAL**	**STR**	**TET**
2009	25	No. of tests	37	46	46	43	41	34	45
		% critical deviations*	8.1	6.5	10.8	2.3	9.8	11.8	11.1
2010	37	No. of tests	44	70	71	59	53	39	68
		% critical deviations*	4,8	7,7	12,7	11,3	8,2	11,4	9,7

^For antimicrobial abbreviations, see List of Abbreviations page 1

*R→ S & S → R (R, resistant; S, susceptible)

Table 22. Region-based categorization of EQAS 2010 participants' performance of antimicrobial susceptibility testing of *Campylobacter* strains

Region		No. of labs	% correct test result	% major deviations (S → R)^	% very major deviations (S → R)^	% critical deviations (R→S & S→R)^	Countries participating in the 2010 iteration
Africa	2009	2	50.0	21.4	28.6	50.0	Algeria, Tunisia
	2010	2	95.2	0.0	4.8	4.8	
Central Asia & Middle East	2009	0	-	-	-	-	-
	2010	0	-	-	-	-	
Caribbean	2009	0	-	-	-	-	-
	2010	0	-	-	-	-	
China	2009	2	95.2	4.8	0.0	4.8	China
	2010	1	100.0	0.0	0.0	0.0	
Europe	2009	9	98.3	1.7	0.0	1.7	Bosnia and Herzegovina, Bulgaria, Denmark, Greece, Italy, Luxembourg, Malta, Poland, Slovenia
	2010	13	100.0	0.0	0.0	0.0	
North America	2009	2	100.0	0.0	0.0	0.0	Canada, United States of America.
	2010	5	93.8	6.3	0.0	6.3	
Oceania	2009	0	-	-	-	-	-
	2010	0	-	-	-	-	
Russia	2009	0	-	-	-	-	Georgia
	2010	1	78.6	7.1	14.3	21.4	
Latin America	2009	5	93.2	6.8	0.0	6.8	Argentina, Brazil, Chile, Costa Rica, Cuba, Paraguay, Uruguay
	2010	8	89.6	6.0	4.5	10.4	
Southeast Asia	2009	4	71.4	0.0	28.6	28.6	Thailand, Philippines, Sri Lanka, Korea, Japan
	2010	7	77.2	9.8	13.0	22.8	

^S, susceptible; R, resistant

Table 23. EQAS 2010 participants' performance of antimicrobial susceptibility testing of *Campylobacter jejuni* ATCC 33560

	Method used	Incubation conditions	Labs' performance	Antimicrobial[3]					
				CHL	CIP	ERY	GEN	NAL	TET
EQAS 2009 (N=24)	Microdilution	42°C / 24h	No.[1]	6	9	10	9	7	9
			%[2]	83.3	66.7	80	88.9	100	88.9
	Microdilution	36-37°C / 48h	No.[1]	5	5	5	5	5	5
			%[2]	80	80	80	80	80	80
	Agardilution	42°C / 24h	No.[1]	0	5	5	6	0	0
			%[2]	-	100	40	66.7	-	-
	Agardilution	36-37°C / 48h	No.[1]	0	2	2	2	0	0
			%[2]	-	100	100	100	-	-
	Overall	Overall	No.[1]	11	21	22	22	12	14
			%[2]	81.8	81	72.7	75	91.7	85.7
EQAS 2010 (N=20)	Microdilution	42°C / 24h	No.[1]	3	6	6	6	4	6
			%[2]	67	83	100	83	75	83
	Microdilution	36-37°C / 48h	No.[1]	5	8	8	8	7	8
			%[2]	80	88	88	75	86	88
	Agardilution	42°C / 24h	No.[1]	0	6	6	6	0	0
			%[2]	0	100	83	83	0	0
	Agardilution	36-37°C / 48h	No.[1]	0	0	0	0	0	0
			%[2]	0	0	0	0	0	0
	Overall	Overall	No.[1]	8	20	20	20	11	14
			%[2]	75	90	90	80	82	86

[1]No., number of labs performing the analysis
[2]%, percentage of labs reporting correct results
[3]For antimicrobial abbreviations: see List of Abbreviations page 1
-, not determined

Table 24. EQAS participating laboratories' performance of unknown strain identification

EQAS iteration	Strain ID	No. of participating labs	Pecentage (%) of labs performing correct identification
2003	*E. coli* O157	115	99
2004	*Shigella flexneri*	121	94 (*Shigella)* 74 (*S. flexneri)*
2006	*Yersinia enterocolitica* O3	134	93 (*Yersinia)* 89 (*Y. enterocolitica)* 66 (*Y. enterocolitica* O3)
2007	*Vibrio parahaemolyticus*	86	83
2008	*Enterobacter sakazakii*	128	92
2009	*Vibrio mimicus*	56	48
2010	*Citrobacter spp.*	115	90

Table 25. Proportion of laboratories that obtained the expected result. Number (n/N) and percentages of laboratories which correctly detected and confirmed the ESBL and non ESBL producing *Salmonella* and *Shigella* strains.

Isolate no.	Expected interpretation	Confirmatory tests	
		CAZ/CL:CAZ	CTX/CL:CTX
WHO S-10.1	non ESBL	18/18 (100%)	19/20 (95%)
WHO S-10.2	non ESBL	18/18 (100%)	19/19 (100%)
WHO S-10.3	Disregarded*	-	-
WHO S-10.4	non ESBL	18/18 (100%)	19/19 (100%)
WHO S-10.5	non ESBL	17/17 (100%)	18/18 (100%)
WHO S-10.6	non ESBL	16/16 (100%)	18/18 (100%)
WHO S-10.7	non ESBL	18/18 (100%)	19/19 (100%)
WHO S-10.8	non ESBL	17/17 (100%)	19/19 (100%)
WHO SH-10.1	non ESBL	12/12 (100%)	12/12 (100%)
WHO SH-10.2	non ESBL	10/10 (100%)	11/11 (100%)
WHO SH-10.3	non ESBL	9/9 (100%)	11/11 (100%)
WHO SH-10.4	non ESBL	9/9 (100%)	10/10 (100%)

*Strain WHO S-10.3 was an ESBL-producing strain, however, not a so-called 'true-ESBL'. Due to this fact it was disregarded in the evaluation of the results regarding ESBL-production (see also description in the report).

Appendixes (1, 2, 3, 4a, 4b)

Appendix 1	Prenotification
Appendix 2	Expected results
Appendix 3	Protocol
Appendix 4a	Subculture and Maintenance of QC strains
Appendix 4b	Instructions for opening and reviving lyophlised cultures

WHO Global Foodborne Infections Network Electronic Discussion Group
Subject: Signing up for EQAS 2010

Greetings WHO Global Foodborne Infections Network (WHO GFN) Members:
WHO GFN strives to increase the quality of laboratory-based surveillance of *Salmonella* and other foodborne pathogens by encouraging national or regional reference laboratories that have attended WHO GFN training courses to participate in the External Quality Assurance System (EQAS). The 2009 EQAS cycle has closed, and we are pleased to announce the launch of the 2010 EQAS cycle.

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;
- serotyping and antimicrobial susceptibility testing of four *Shigella* isolates;
- species identification and antimicrobial susceptibility testing of two *Campylobacter* isolates;
- identification of one unknown bacterial sample.

WHO SHOULD PARTICIPATE IN EQAS 2010?
All national or regional reference laboratories that are performing work on *Salmonella*, *Shigella* and/or *Campylobacter* and are interested in participating in a quality assurance program are invited to participate.

We expect that all national or regional reference laboratories that have attended WHO GFN Training Courses will participate in EQAS.

The WHO GFN Regional Centers, in cooperation with the EQAS Coordinator, will evaluate the list of participants that wish to enroll in EQAS 2010. Laboratories which signed up and received strains in year 2009, but did not submit any data, should explain the reason for this in order to participate in 2010.

COST FOR PARTICIPATING IN EQAS
There is no charge for participating in EQAS 2010; however, laboratories which are capable of paying for shipping the parcel should intend to do so. If your country has an agreement with FedEx, regarding importing Biological Substance Category B (UN3373) please forward your FedEx import account number in the sign-up form, or alternatively to the EQAS Coordinator (contact information below),. Having this information before sending out the isolates saves time and resources. Participating laboratories are responsible for paying any expenses related to getting the parcel through customs, additional taxes or customs fees.

SIGNING UP FOR THE EQAS 2010
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 (no post-office box number)
- Telephone, fax, e-mail
- FedEx import account number (if such one is available)
- Approximate number of *Salmonella* isolates annually serogrouped/serotyped
- Approximate number of *Salmonella* isolates annually tested for antimicrobial susceptibility
- Level of participation in EQAS 2010
- Level of reference function in your country

If you experience any problems enrolling electronically, please try again a few days later. If you are still unsuccessful after attempting to enroll, please contact the EQAS Coordinator, Susanne Karlsmose, by e-mail (suska@food.dtu.dk) or fax (+45 3588 6341).

SHIPPING AND TIMELINE TO RECEIVE ISOLATES AND PROTOCOLS
Due to the increased number of participants in EQAS, a number of different institutions will ship the bacterial isolates. You will be informed of the institution which will ship your parcel. In order to minimize the delay in

shipping the isolates to your laboratory, please **provide the coordinator with a valid import permission.** Please apply for a permit to receive the following (according to your level of participation): “Biological Substance Category B”: eight *Salmonella* strains, four *Shigella* strains, two *Campylobacter*, one *Campylobacter* reference strain (for participants performing antimicrobial susceptibility testing on *Campylobacter*), one *E .coli* reference strain (for new participants performing antimicrobial susceptibility testing on *Salmonella* and/or *Shigella*) and an unknown sample (enteric bacteria) between August and September 2010.

The isolates will be shipped between August and September 2010. The protocol as well as additional information needed for EQAS will be made available for download from the website. http://www.antimicrobialresistance.dk/233-169-215-eqas.htm.

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 2010** via the password protected website. Immediately upon receiving the results, an evaluation report will be generated. Full anonymity is ensured; only DTU Food and the WHO GFN Regional Centre in your region will be given access to your results.

Deadline for signing up to participate in this EQAS: May 21st, 2010
****

Posted by Susanne Karlsmose, suska@food.dtu.dk, WHO GFN EQAS Coordinator, DTU Food, National Food Institute, Denmark.

			Ampicillin		cefotaxime		Ceftazidime		Ceftriaxone		Chloramphenicol		Ciprofloxacin		Gentamicin		Nalidixic acid		Streptomycin		Sulfonamides		Tetracycline		Trimethoprim		Trim/Sulfa	
			AMP		CTX		CAZ		CRO		CHL		CIP		GEN		NAL		STR		SMX		TETRA		TMP		SXT	
WHO 2010 S-10.1	*Salmonella* Muenster	I 3,10:e,h:1,5	<= 1	SUSC	<= 0.12	SUSC	= 0.50	SUSC	= 0.125	SUSC	= 8	SUSC	= 0.03	SUSC	<= 0.5	SUSC	= 4	SUSC	= 32	RESIST	= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.064	SUSC
WHO 2010 S-10.2	*Salmonella* Enteritidis	I 9,12:g,m:-	= 2	SUSC	= 0.25	SUSC	= 1.0	SUSC	= 0.50	SUSC	= 8	SUSC	= 0.06	SUSC	> 16	RESIST	= 4	SUSC	> 128	RESIST	> 1024	RESIST	= 4	SUSC	<= 1	SUSC	= 0.064	SUSC
WHO 2010 S-10.3	*Salmonella* Bareilly	I 6,7:y:1,5	= 32	RESIST	> 4	RESIST	= 64	RESIST	= 16	RESIST	= 4	SUSC	= 0.03	SUSC	<= 0.5	SUSC	= 4	SUSC	= 16	INTER	= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.064	SUSC
WHO 2010 S-10.4	*Salmonella* Amsterdam	I 3,10:g,m,s:-	<= 1	SUSC	<= 0.12	SUSC	= 0.50	SUSC	= 0.125	SUSC	= 4	SUSC	= 0.03	SUSC	<= 0.5	SUSC	= 2	SUSC	<= 8	SUSC	= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.064	SUSC
WHO 2010 S-10.5	*Salmonella* Litchfield	I 6,8:l,v:1,2	<= 1	SUSC	<= 0.12	SUSC	= 0.50	SUSC	= 0.064	SUSC	= 4	SUSC	= 0.03	SUSC	<= 0.25	SUSC	= 2	SUSC	= 32	RESIST	= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.064	SUSC
WHO 2010 S-10.6	*Salmonella* Senftenberg	I 1,3,19:g,s,t:-	<= 1	SUSC	<= 0.12	SUSC	= 0.50	SUSC	= 0.125	SUSC	= 8	SUSC	<= 0.015	SUSC	<= 0.25	SUSC	= 4	SUSC	= 16	INTER	= 32	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.064	SUSC
WHO 2010 S-10.7	*Salmonella* Kedougou	I 13,23:i:l,w	<= 1	SUSC	<= 0.12	SUSC	= 0.25	SUSC	= 0.125	SUSC	= 4	SUSC	= 0.03	SUSC	<= 0.25	SUSC	= 4	SUSC	= 16	INTER	= 32	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.064	SUSC
WHO 2010 S-10.8	*Salmonella* Kentucky	I 8,20:i:z6	<= 1	SUSC	<= 0.12	SUSC	= 0.50	SUSC	= 0.125	SUSC	= 4	SUSC	> 4	RESIST	> 16	RESIST	> 64	RESIST	> 128	RESIST	> 1024	RESIST	> 32	RESIST	<= 1	SUSC	= 0.064	SUSC
WHO 2010 SH-10.1	*Shigella* flexneri var Y		> 32	RESIST	<= 0.12	SUSC	= 0.25	SUSC	= 0.064	SUSC	= 64	RESIST	= 1	RESIST	= 1	SUSC	> 64	RESIST	= 128	RESIST	<= 16	SUSC	> 32	RESIST	> 32	RESIST	= 0.25	SUSC
WHO 2010 SH-10.2	*Shigella* sonnei		> 32	RESIST	<= 0.12	SUSC	= 0.064	SUSC	= 0.016	SUSC	= 4	SUSC	= 0.25	RESIST	> 16	RESIST	> 64	RESIST	> 128	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST	> 32	RESIST
WHO 2010 SH-10.3	*Shigella* flexneri var X		> 32	RESIST	<= 0.12	SUSC	= 0.25	SUSC	= 0.064	SUSC	= 64	RESIST	= 1	RESIST	= 1	SUSC	> 64	RESIST	= 128	RESIST	<= 16	SUSC	> 32	RESIST	> 32	RESIST	= 0.25	SUSC
WHO 2010 SH-10.4	*Shigella* dysenteriae 3		> 32	RESIST	<= 0.12	SUSC	= 0.064	SUSC	= 0.032	SUSC	> 64	RESIST	= 0.03	SUSC	= 1	SUSC	= 2	SUSC	= 64	RESIST	<= 16	SUSC	> 32	RESIST	<= 1	SUSC	= 0.064	SUSC

			Chloramphenicol		Ciprofloxacin		Erythromycin		Gentamicin		Nalidixic acid		Streptomycin		Tetracycline	
WHO 2010 C-10.1	*C. jejuni*		= 8	SUSC	= 32	RESIST	> 64	RESIST	> 32	RESIST	> 64	RESIST	> 16	RESIST	> 64	RESIST
WHO 2010 C-10.2	*C. coli*		= 4	SUSC	<= 0.06	SUSC	= 2	SUSC	= 0.25	SUSC	= 8	SUSC	> 16	RESIST	= 0.25	SUSC

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PROTOCOL for

- serotyping and susceptibility testing of *Salmonella*
- serotyping and susceptibility testing of *Shigella*
- identification and susceptibility testing of *Campylobacter*
- identification of an unknown enteric pathogen

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1 INTRODUCTION

In 2000, the Global Foodborne Infections Network (formely known as WHO Global Salm-Surv) 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 GFN.

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

The WHO EQAS 2010 includes

- serotyping and susceptibility testing of eight *Salmonella* strains,
- serotyping and susceptibility testing of four *Shigella* strains,
- susceptibility testing of the *E. coli* reference strain for quality control (ATCC 25922 (CCM 3954)),
- identification and susceptibility testing of two thermophilic *Campylobacter* isolates
- susceptibility testing of the *C. jejuni* reference strain for quality control (ATCC 33560 (CCM 6214)),
- and identification of one 'unknown' bacterial isolate.

All participants will receive the strains relevant to their laboratory according to the sign-up information.

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 and should be used for future internal quality control for susceptibility testing in your laboratory. Please take proper care of the strains. Handle and maintain them as suggested in the manual 'Subculture and Maintenance of QC Strains' available on the WHO CC website (see www.antimicrobialresistance.dk).

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. The laboratory work for this EQAS should be done by the methods routinely used in your laboratory.

3 OUTLINE OF THE EQAS 2010

3.1 Shipping, receipt and storage of strains

In August/September 2010 more than 180 laboratories from all parts of the world will receive a parcel containing eight *Salmonella* strains, four *Shigella*, two *Campylobacter* strains and one 'unknown' bacterial isolate (according to information when signing up). An *E. coli* reference strain and a *C. jejuni* reference strain will be included for participants who have signed up to perform antimicrobial susceptibility testing (AST) and 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 confirmation form enclosed in the shipment

The reference strains and the *Campylobacter* strains are shipped lyophilised, whereas the *Salmonella* and *Shigella* strains, as well as the 'unknown' isolate are agar stab cultures. On arrival, the agar stab cultures must be subcultured and prepared for storage in the strain collection (e.g. in a -80 °C freezer). This set of cultures serves as reference if discrepancies are detected when testing the strains (errors such as mis-labelling or contamination can be verified). 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-Le Minor (Grimont and Weill, 2007. 9th ed. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

When uploading the data, please fill in the information on the brand of antisera used in the typing. Also, we ask you to comment on what antisera you think is required to complete your serotyping.

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

The *Salmonella* and *Shigella* strains as well as 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.

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).

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* and *Shigella* should not be reported as susceptible.

In this EQAS the breakpoints used as a key to interpreting MIC results are CLSI values, supplemented with values from EUCAST (www.eucast.org) 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).

For ciprofloxacin, please note that a low breakpoint has been used to determine resistance category (the lower breakpoint is epidemiological breakpoint based on mechanism of resistance e.g. *qnr*-genes and one point-mutation in the gyrase gene (from EUCAST, see Table 1)). Considering the expected results of this EQAS, microorganisms are considered resistant to ciprofloxacin when showing reduced susceptibility to this antimicrobial.

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Antimicrobials	Reference value, MIC (µg/mL)			Reference value, Disk diffusion (mm)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ampicillin, AMP	≤8	16	≥32	≥17	14-16	≤13
Cefotaxime, CTX	<1	-	≥1	>27	-	≤27
Ceftazidime, CAZ	<1	-	≥1	>22	-	≤22
Ceftriaxone, CRO	<1	-	≥1	>25	-	≤25
Chloramphenicol, CHL	≤8	16	≥32	≥18	13-17	≤12
Ciprofloxacin, CIP	<0.125*	-	≥0.125*	≥23mm (1µg)*** or ≥30mm (5µg)***	-	<23mm (1µg)*** or <30mm (5µg)***
Gentamicin, GEN	≤4	8	≥16	≥15	13-14	≤12
Nalidixic acid, NAL	≤16	-	≥32	≥19	14-18	≤13
Streptomycin, STR	≤8**	16**	≥32**	≥15	12-14	≤11
Sulfonamides, SMX	≤256	-	≥512	≥17	13-16	≤12
Tetracycline, TET	≤4	8	≥16	≥15	12-14	≤11
Trimethoprim, TMP	≤8	-	≥16	≥16	11-15	≤10
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT	≤2/38	-	≥4/76	≥16	11-15	≤10

Table 1: Reference values used are according to CLSI, apart from:
* EUCAST (epidemiological cut off values)
** DTU Food
*** The EUCAST cut-off value for microbroth is considerably lower than the CLSI breakpoint for zone diameters. As EUCAST does not suggest a cut-off value when susceptibility testing *Salmonella* by disk diffusion, the article by Cavaco LM and Aarestrup FM (J Clin Microbiol. 2009 Sep;47(9):2751-8) gives the background for the interpretative criteria for susceptibility testing *Salmonella* by disk diffusion in the WHO GFN EQAS. In the article, *Shigella* has not been included; however, the same interpretative criteria will be used in this context.

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.

Concerning cefotaxime (CTX), ceftazidime (CAZ) and/or ceftriaxone (CRO) used when detecting ESBL-producing strains in the EQAS: Obtained values and interpretations for these antimicrobials should be reported as found (according to EUCAST expert rules).

3.4 Handling the *Campylobacter* strains

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 *C. jejuni* reference strain: Please see the document 'Instructions for opening and reviving lyophilised cultures' on the WHO CC website (see www.antimicrobialresistance.dk).

3.5 Identification of *Campylobacter*

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

3.6 Susceptibility testing of *Campylobacter* and *C. jejuni* ATCC 33560

The *Campylobacter* test strains as well as the *C. jejuni* reference strain should be susceptibility tested towards as many as possible of the antimicrobials mentioned in the test form. It should be noted that for AST of *Campylobacter* only MIC methods are recommendable, i.e. broth or agar dilution methods. Neither the use of disk diffusion nor E-test is recommendable for AST of *Campylobacter*.

In this EQAS the breakpoints used as a key to interpreting MIC results for *Campylobacter* are epidemiological cut off values. The reference values used are from EUCAST (www.eucast.org; see list below). This allows only two categories of characterisation – resistant or sensitive. Interpretations in concordance with the expected value will be categorised as 'correct', whereas deviations from the expected interpretation are categorizes as 'incorrect'.

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).

Note that the interpretation requires knowledge about the species. If you do no identify *Campylobacter* but perform AST on *Campylobacter*, you may contact the EQAS Coordinator to obtain information regarding the identity of the *Campylobacter* test strains.

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

Table 2: Reference values for interpretation of *Campylobacter* results are according to EUCAST

The sub-cultured *Campylobacter* should be used for the MIC-testing after incubation at 36-37ºC for 48 hours or 42ºC for 24 hours; possibly two subcultures are needed to ensure good growth before testing.

3.7 Identification and of the unknown enteric pathogen

The 'unknown' isolate should be identified to species level and further typed if relevant.

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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, 2010**. 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 GFN 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:

Susanne Karlsmose

National Food Institute, Technical University of Denmark

Kemitorvet, Building 204 ground floor, DK-2800 Lyngby - DENMARK

Tel: +45 3588 6601, Fax: +45 3588 6341

E-mail: suska@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 CC website (from http://www.antimicrobialresistance.dk), then
    a. Click on 'EQAS'
    b. Click on the link for the interactive database
    c. Write your username and password in low letters and click on 'Login'.

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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. In the comments-field, fill in what antisera you think is required to complete your serotyping
   f. 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':

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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 2010 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!

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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.

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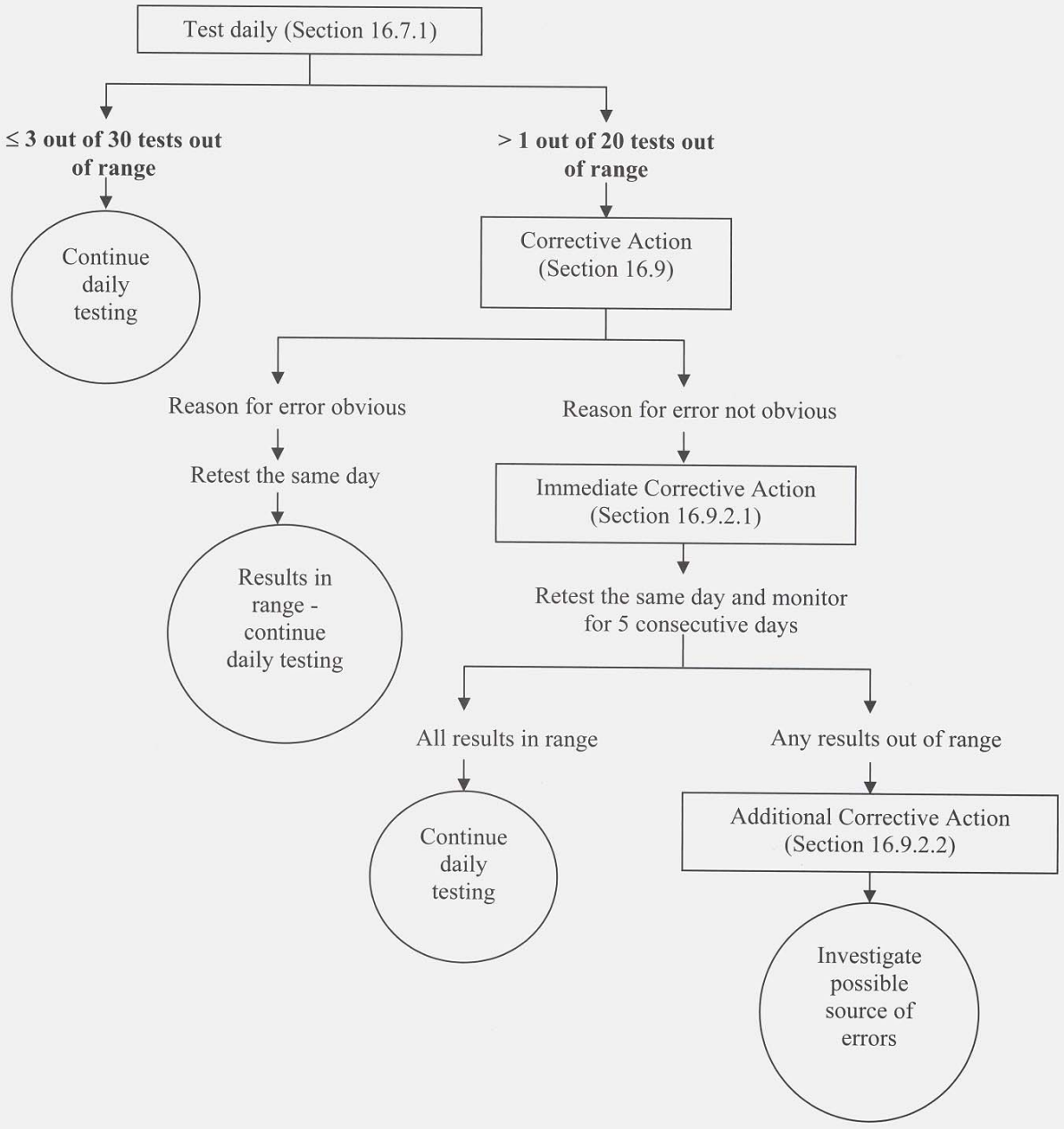
DAILY MIC QC CHART

Appendix A. Quality Control Protocol Flow Charts

Aerobic Dilution Daily Quality Control Testing Protocol



Reference: CLSI M7-A7, page 39

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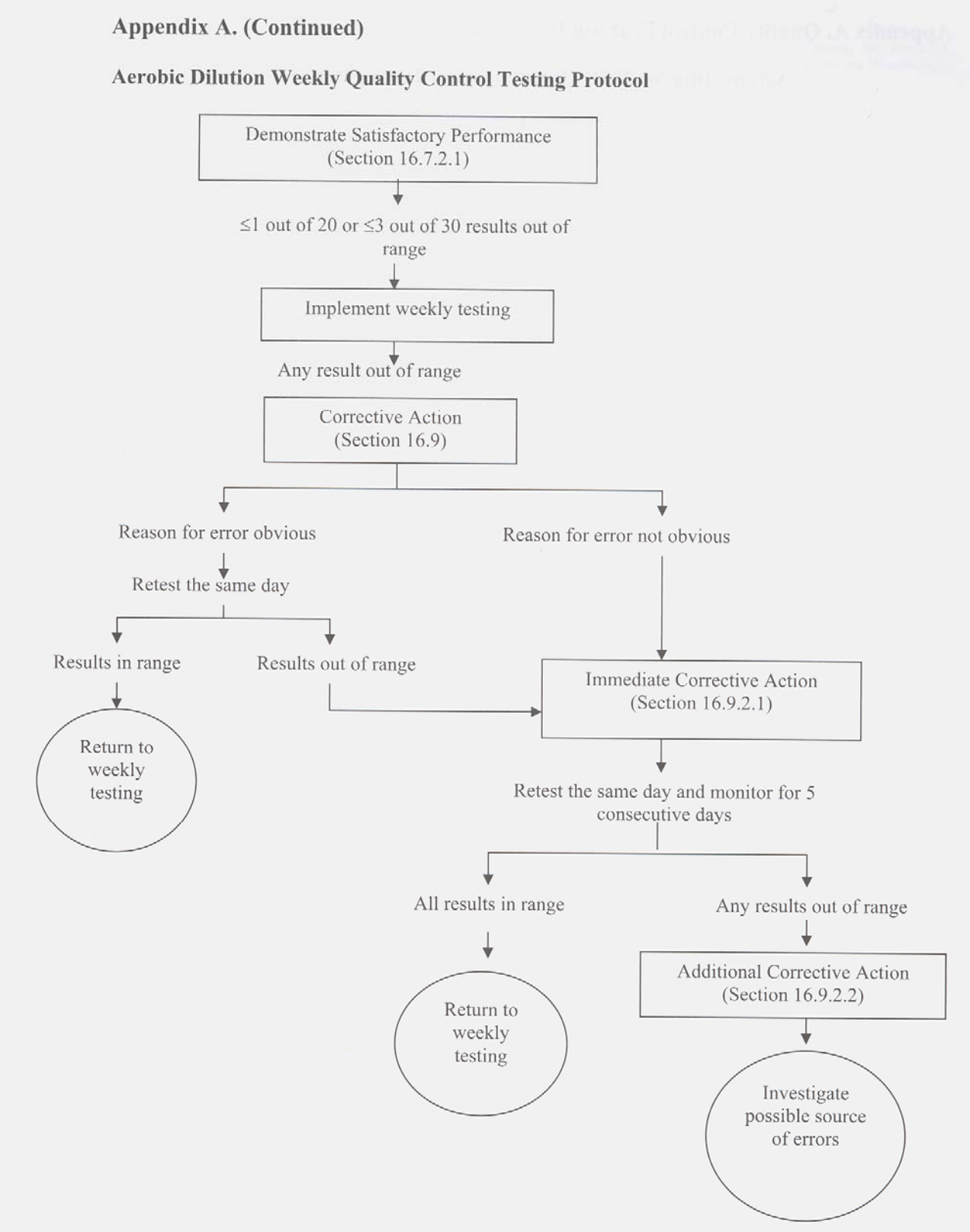
WEEKLY MIC QC CHART



Reference: CLSI M7-A7, page 40

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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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