The External Quality Assurance System of the WHO Global Foodborne Infections Network 2009

Public Health Agency of Canada
Agence de la santé publique du Canada

World Health Organization

CDC
Centers for Disease Control and Prevention

international network
Institut Pasteur

DTU Food
National Food Institute

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens

National Food Institute

Kemitorvet, Kgs. Lyngby, Denmark

Rene Hendriksen, Susanne Karlsmose, Valeria Bortolaia, Arne Bent Jensen, Frank Aarestrup

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

YEAR 2009

DATE OF ISSUE: DECEMBER 2010

THE EXTERNAL QUALITY ASSURANCE SYSTEM OF THE WHO GLOBAL FOODBORNE INFECTIONS NETWORK YEAR 2009

1. edition, December 2010

Photo: Mikkel Adsbøl
ISBN: 978-87-92158-88-8

The report is available at
www.food.dtu.dk

National Food Institute
Technical University of Denmark
Kemitorvet
Building 204
2800 Kgs. Lyngby
Denmark
Tel: +45 35 88 70 00
Fax +45 35 88 70 01

Contents

List of Abbreviations

AMC, Amoxicillin / Clavulanic acid

AMP, Ampicillin

AST, Antimicrobial Susceptibility Testing

CAZ, Ceftazidime

CCM, Czech Collection of Micro-organisms

CHL, Chloramphenicol

CIP, Ciprofloxacin

POD, Cefpodoxime

CRO, Ceftriaxone

CTX, Cefotaxime

DTU Food, Danish Technical University - National Food Institute

ENR, Enrofloxacin

EQAS, External Quality Assurance System

ERY, Erythromycin

EUCAST, The European Committee on Antimicrobial Susceptibility Testing

FFN, Florfenicol

FIS, Sulfisoxazole

GEN, Gentamicin

KAN, Kanamycin

MIC, Minimum Inhibitory Concentration

NAL, Nalidixic Acid

QC, Quality Control

SMX, Sulfametoxazole

STR, Streptomycin

SXT, Trimethoprim + Sulphonamides

TET, Tetracycline

TMP, Trimethoprim

WHO, World Health Organization

WHO GFN, WHO Global Foodborne Infections Network

WHO GFN CDB, WHO GFN Country Databank

WHO GSS, WHO Global Salm-Surv

XNL, Ceftiofur

1. Introduction

In January 2000, WHO launched the WHO Global Salm-Surv (WHO-GSS) which represents an international effort aiming to enhance laboratory-based surveillance of *Salmonella* infections and to promote prevention and control activities. This Program, which has been renamed "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.

An External Quality Assurance System (EQAS) program was established in 2000 to support participation of laboratories in the WHO GFN. The original goal of this program was to assess the quality of *Salmonella* serotyping and antimicrobial susceptibility testing (AST) data produced by Member States and to enhance the reliability of these data by identifying areas which could benefit from additional support. In 2003, the EQAS program was expanded to include additional foodborne pathogens, as mentioned above. The number of participants submitting data related to one or more components of the EQAS increased from 44 laboratories in 2000 to 180 laboratories in 2009. According to a goal set by WHO GFN, all national reference laboratories should 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).

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.

2. Materials and Methods

2.1 Participants

A pre-notification announcement of the EQAS 2009 was made through the WHO GFN list server on April 21, 2009 and a reminder was sent on May 4, 2009 (App. 1). The pre-notification was available in English, Spanish, Portuguese, French, Chinese and Russian, and included invitations to participate in the EQAS 2009 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 2009 from the DTU Food's strain collection. The unknown foodborne pathogen, a *Vibrio mimicus* 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* and *Shigella* strains were inoculated as agar stab cultures in nutrient agar, while the *Vibrio mimicus* strain was inoculated as agar stab cultures in Tryptic Soy 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) (5). 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* strains was determined by DTU Food and verified by CDC. The *Shigella* serotype was performed by PHAC and verified by the NCCS. DTU Food determined the antimicrobial susceptibility pattern of the *Shigella* strains, which was verified by PHAC and CDC. 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 2009 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 2009 for AST of *Campylobacter* strains: chloramphenicol, CHL; ciprofloxacin, CIP; erythromycin, ERY; gentamicin, GEN; nalidixic acid, NAL; streptomycin, STR 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-A7 (2006) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically"; Approved Standard - Seventh Edition (4), document M 100-S19 (2009) "Performance Standards for Antimicrobial Susceptibility Testing"; Nineteenth Informational Supplement (3), document M 31-A3 (2008) " Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated from Animals"; Approved Standard - Third Edition (2), and document M45-A (2006) "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria"; Approved Standard – First Edition (1). Guideline were used for interpretation of AST results with the exception of i) cefotaxime, ceftazidime and ciprofloxacin susceptibility testing for which EUCAST (www.eucast.org) epidemiological cut-off values were utilized; ii) streptomycin and ceftriaxone susceptibility testing for which DTU Food interpretative criteria were utilized; and iii) *Campylobacter* AST, for which EUCAST epidemiological cut-off values were used. 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, Aroon Bangtrakulnonth from NSSC; Thailand, Enrique Perez from Health Surveillance, Disease Prevention and Control; Brazil, Francois Xavier Weill from IP; France, Rita Tolli from Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Italy and Changwen Ke from Center for Disease Control and Prevention of Guangdong Province, China shipped to all Canadian, North American, Thai, Latin American, Francophone African, Italian and Chinese institutes, respectively. The first parcel was dispatched from DTU Food on August 25, 2009 and the last on November 12, 2009.

2.5 Procedure

Participants were instructed to download the protocol 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 2009 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 (*Vibrio mimicus*). 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 7, 2009 and closed on March 17, 2010.

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' (7). 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 $\leftrightarrow$ S or I $\leftrightarrow$R). Major deviation is the classification of a susceptible strain as resistant (*i.e.* S $\rightarrow$ R). Very major deviation is the classification of a resistant strain as susceptible (*i.e.* R $\rightarrow$ 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 192 laboratories responded to the pre-notification and were enrolled in the EQAS. When the deadline for submitting results was reached, 180 laboratories in 90 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, Gambia, Georgia, Germany, Greece, Guatemala, Honduras, Hungary, India, Iran, Ireland, Israel, Italy, Ivory Coast, Jamaica, Japan, Jordan, Kenya, Korea, Lao PDR, Lithuania, Luxembourg, Madagascar, Malaysia, Malta, Mauritius, Mexico, Moldova, Morocco, Namibia, New Zealand, Nicaragua, Nigeria, Sultanate of Oman, Panama, Paraguay, Peru, Philippines, Poland, Russia, Serbia, Singapore, Slovakia, Slovenia, South Africa, Sri Lanka, Sudan, Suriname, Taiwan,

Thailand, Trinidad and Tobago, Tunisia, Turkey, United Kingdom, Uruguay, USA, Venezuela, Vietnam, Yemen.

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

Of a total of 182 laboratories receiving *Salmonella* strains, 161 (88%) participated in the *Salmonella* serogrouping component of the EQAS, and 153 (95%) participated in the complete serotype module of the EQAS. In addition, 153 (84%) laboratories submitted AST results. Among the laboratories performing AST, 129 (84%) submitted results for the quality control (QC) strain *E. coli* ATCC 25922. The majority (n=102; 79%) of these laboratories used the disk diffusion method, while a MIC determination method was utilized by a smaller number (n=27; 21%) of laboratories.

Of 136 laboratories receiving *Shigella* strains, 118 (87%) submitted *Shigella* serogroup results (speciation) and 82 (69%) of these laboratories serogrouping the isolates further analyzed the strains to the serotype level. In addition, *Shigella* AST was performed by 111 (82%) 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 131 laboratories receiving *Campylobacter* strains, 87 (66%) reported identification results and 25 (19%) submitted AST results for both *Campylobacter* strains.

Of the 146 laboratories receiving the unknown culture for identification, 56 (38%) submitted results.

3.2 Serogrouping and serotyping of *Salmonella* strains

In 2009, the percentage of laboratories reporting complete serotype results for all eight strains increased to 83% (n=119), thus contrasting with the decreasing trend observed in the two previous years (*e.g.* in 2008: 66%, n=100). The proportion of correctly serotyped strains increased from 83% (n=888) in 2008 to 86% (n=974) in 2009 (Table 1).

In Table 2, the number of participating laboratories is reported according to the number of correctly serotyped samples. In 2009, 76 (50%) of the 153 participating laboratories serotyped all eight strains correctly, and 29 (19%) laboratories correctly serotyped seven of the eight strains. Summarizing, in 2009, a total of 105 (69%) participating laboratories met the threshold for adequate performance of *Salmonella* serotyping, which represents a considerable increase compared to 2008 when only 86 (57%) of the participating laboratories met the performance quality threshold. In addition, 82% of the participating laboratories correctly identified half of the strains, which represents an 8% increase compared to 2008 (74%).

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 2008. The African, Latin American and Southeast Asian regions experienced the largest influx of EQAS participants with up to five new participants compared to 2008. In contrast, in the European region, participation to the EQAS 2009 decreased of three laboratories compared to 2008.
The number of tested strains increased mostly in the African, Latin American, and Southeast Asian regions and in China which tested between 18 and 55 additional strains in 2009. The accuracy of serotyping increased mostly in laboratories from Africa, Caribbean, Europe, and Latin American (between 4.7% and 26.2% increase compared to 2008). A decrease in accuracy of serotyping was observed in North America and the Asia & Middle Eastern region, with the most considerable decrease in Asia & Middle Eastern region (14.9% decrease compared with 2008).

The overall performance of laboratories performing *Salmonella* serogrouping was satisfactory, as the percentages of deviations were very low for almost all tested strains, ranging from 0.6% (WHO S9.8) to 10.9% (WHO S9.6). By excluding strain WHO S9.6, the range of deviations was from 0.6% to 3.8% (Table 4).

Of 151 laboratories performing serotyping of the internal quality control strain (WHO S9.1, used in EQAS 2000, 2001, 2004, 2006, 2007 and 2008), 141 (93.4%) reported a correct result, thus leading to a deviation rate of only 6.6% (Table 4). The ability of participating laboratories to correctly serotype the internal quality control strain was consistent through different years, and ranged from 95% in 2004 to 93% in 2009 even though the number of participating laboratories increased (Table 5).

Deviations in *Salmonella* serotyping ranged from 6.6% (WHO S9.1) to 19% (WHO S9.2) (Table 4), thus showing an improvement compared to last year when the highest percentage of deviations was 34.1%, and deviations greater than 20% were reported for four strains. In 2009, the three strains resulting in most deviations were WHO S9.2: *Salmonella* Brandenburg (I 4,12:l,v:e,n,z15) with 19% deviations, WHO S9.6: *Salmonella* Worthington (I 1,13,23:z:l,w) with 16.3 % deviations, and WHO S 9.4: *Salmonella* Bredeney (I 1,4,12:l,v:1,7) with 16% deviations (Table 4). Only WHO S9.1: *Salmonella* Enteritidis strain was serotyped satisfactorily with 6.6% deviations which is a slight decrease compared to 2008 (Table 4).

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

A total of 12,707 antimicrobial susceptibility tests was performed in 2009 by 153 participating laboratories. Of the submitted results, 94% were in agreement with the expected result, which is a 3% improvement compared to 2008 (Table 6). Minor, major and very major deviations were observed in 3%, 2% and 1% of the submitted results, respectively (Table 6).

No major difficulties in assessing antimicrobial susceptibility were encountered for any of the tested combinations of strains and antimicrobials. Mostly, difficulties were experienced in assessing susceptibility to STR, SMX and TET (Table 7).

Major deviations categorized by tested antimicrobial are reported in Table 8. Notably, a large number of critical deviations was observed for CIP (8%), STR (9%) and SMX (7%). These antimicrobials together with TET resulted also in very high numbers of total deviations (Table 8). In 2009, the average number of critical and total deviations overall observed was 3% and 6%, respectively, which represents an improvement compared to 2008.

The 2009 EQAS trial did not include any ESBL-producing *Salmonella* strain. However, participants had 1% and 2% deviating results for CAZ and CRO, and for CTX, respectively (Table 8).

In 2009, the number of laboratory participating to the AST component of EQAS decreased in Central Asia & Middle East, Caribbean, China, Europe, and North America (Table 9). In particular, compared to 2008, the Europe and the Central Asia & Middle East regions registered a decrease of 11 and 6 participants, respectively. By contrast, additional eight laboratories took part to the EQAS AST component in the Southeast Asian region. Overall, the performance of AST improved in all regions, most notably in the African and the Central Asia & Middle Eastern regions. Overall, antimicrobial

susceptibility test results were reported correctly in percentages ranging from 90.1% (Africa) to 98.7% (North America) (Table 9).

Antimicrobial susceptibility of *E. coli* ATCC 25922 was tested by 27 laboratories with the MIC determination method and by 102 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% to 21% for STR and FIS susceptibility testing, respectively (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 of CTX, CIP, and FIS susceptibility testing (Table 10).

3.4 Serogrouping and serotyping of *Shigella* strains

In 2009, the performance of *Shigella* speciation was satisfactory, as the percentages of deviations were very low for all the four test strains, ranging from 0.9% (WHO SH 9.1 - 9.3) to 4.6% (WHO SH 9.4) (Table 11). Similar results were observed among laboratories that performed full serotyping. Thus, the percentages of deviations in *Shigella* serotyping ranged from 4.2% (WHO SH 9.3) to 12.5% (WHO SH 9.4), which represents an improvement compared to the pilot study performed in 2008. The strain resulting in most deviations was WHO SH 9.4: *Shigella boydii* serotype 2, which was reported as serotype 1 by eight 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=16), Europe (n=15), China (n=13) and Southeast Asia (n=11). The accuracy of *Shigella* serotyping results ranged from 72.2% (Africa) to 100% (Oceanic, China, Central Asia & Middle East).

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

A total of 4,548 antimicrobial susceptibility tests were performed in 2009 by 111 participating laboratories. Agreement with the expected result was achieved in 96% of the reported results, which is a 1% improvement compared to 2008 (Table 13). Minor, major and very major deviations were observed in 2%, 1% and 1% of reported results, respectively (Table 13).

No major difficulties in assessing antimicrobial susceptibility were encountered for any of the tested combinations of strains and antimicrobials (Table 14). STR and TET accounted for 18.1% and 7.5% of total deviations, respectively (Table 15).
ESBL-producing *Shigella* strains were not included in the EQAS 2009 trial. However, the participating laboratories had between 0.3% and 1.1% deviating results for CAZ, CRO, and CTX (Table 15).

In 2009, laboratories in almost all regions participated in the *Shigella* AST component, except for the Oceanic region. The majority of participating laboratories was located in the European, Latin American, Southeast Asian and African regions where 22, 20, 18 and 17 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 93.3% (Africa) to 100% (North America). The African and Central Asia & Middle East regions reported results presenting the highest percentages of critical and total deviations, *i.e.* 4.3% and 4.4% critical deviations, and 6.8% and 5.2% total deviations, respectively. Also the Southeast Asian region had a considerably high number of total deviations (5.9%) (Table 16).

3.6 Identification of *Campylobacter* strains

Participation in the EQAS 2009 *Campylobacter* component was requested by 131 laboratories, but only 86 (66%) submitted results within the deadline. Of the participating laboratories, 77% and 95% performed correct species identification for strain #1 (*C. coli*) and #2 (*C. jejuni*), respectively (Table 17). This is the first time in the EQAS program that a *Campylobacter* strain has been correctly identified by such a high percentage (95%) of laboratories. Only four deviations were reported, namely three *C. upsaliensis* and one *C. lari*.

In Table 18, the performance of *Campylobacter* identification is reported according to geographical location of participating laboratories. The majority (n=28; 33%) of participating laboratories were in Europe (n=28), but participation from China and Latin America [12 (14%) and 14 (16%) laboratories, respectively] was also considerable. The accuracy in *Campylobacter* identification ranged from 40% (Central Asia & Middle East) to 100% (Oceanic, Russia, and Caribbean). A high number of deviations was observed in the African region where only 53.8% of the strains were correctly identified.

3.7 MIC determination of *Campylobacter* strains

A total of 292 MIC determinations was performed in 2009 by 25 participating laboratories. Among the reported results, 91.4% were in agreement with the expected result (Table 19). Major and very major deviations were observed in 4.5% and 4.1% 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, 11.8%, 11.1%, 10.8% and 9.8%, deviations were reported for STR, TET, ERY and NAL susceptibility testing, respectively (Table 21).

In 2009, MIC values were submitted by laboratories in Africa, China, Europe, North America, Latin American, and Southeast Asia (Table 22). Agreement with expected values was observed in percentages ranging from 42.9% (Africa) to 100% (North America) (Table 22). The highest percentages of critical deviations were reported from laboratories in the African and Southeast Asian regions (50% and 28.6%, respectively; Table 22).

MIC values of reference strain *C. jejuni* ATCC 33560 were tested by 24 (96%) laboratories. Of these, 15 laboratories used micro-dilution procedures, while 9 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 72.7% to 91.7% (for ERY and NAL susceptibility testing, respectively; Table 23). Of note, only 40% of the laboratories using agar-dilution with an incubation temperature of 42°C met the quality control interval for ERY susceptibility testing (Table 23).

3.8 Identification of the unknown culture

Identification of the unknown enteric pathogen (*Vibrio mimicus)* was performed by 56 laboratories. Overall, 75% of the participating laboratories identified the strain as a *Vibrio* spp. Only 27 (48%) laboratories completely and correctly identified the strain. Thirteen (23%) laboratories reported deviating results, namely *Campylobacter* spp. (n=2), *Arizona* spp. (n=1) *Staphylococcus* spp. (n=1) *Aeromonas hydrophila* (n=1), *Salmonella enterica* Onderstepoort (n=1), *Aeromonas salmonicida salmonicida* (n=1), *Enterobacter hafnia alvei* (n=1), *Enterobacter cloacae* (n=1), *Micrococcus luteus* (n=1), *Escherichia coli* (n=1), and *Bacillus* spp. (n=1).

4. Discussion

4.1 Serogrouping and serotyping of *Salmonella* strains

As in previous years, the selection of serovars included in the 2009 WHO GFN EQAS trial was based both on the 15 most common serovars submitted to the WHO GFN Country Data Base (CDB) http://www.antimicrobialresistance.dk 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 2009, we included *Salmonella* serovar Brandenburg which is ranked among the top 15 most common serovars in the Oceanic region, more specifically in New Zealand where it has been reported as a frequently isolated serovar among humans for more than a decade (6). *S.* Brandenburg has also been often observed in Europe. In addition, we included *Salmonella* Bredeney which is frequently reported in Europe, and *Salmonella* Muenster which is reported occasionally in Europe and the United States but is very common in Africa. Then, we included *Salmonella* serovars Sandiego and Worthington which are common in the Latin American region. We also included *S.* Stanley which could be defined endemic in Southeast Asia, and it was the second most common serovar between 2002 and 2007 in Thailand. Notably, many of the *S.* Stanley observed in Europe are isolated from human patients travelling to the Southeast Asian region. Finally, we included *S.* Albany which is associated with the Southeast Asian region too.

The number of laboratories which correctly serotyped all eight *Salmonella* strains increased from 100 (66%) in 2008 to 119 (83%) in 2009, which represents the second best performance after the first EQAS in 2000 (Table 1). Similarly, the percentage of correctly serotyped strains was higher only in 2007 and 2002 when, however, fewer laboratories submitted results compared to 2009. Two reasons could explain the excellent results obtained in 2009. First, all the *Salmonella* strains selected for the EQAS 2009 could be fully serotyped using commonly available antisera. Second, the increased number of WHO GFN capacity building laboratory training courses in both Africa and Latin America may have provided a better understanding of serotyping methods and improved knowledge of the availability of good quality antisera.

Of note, only 93% of participating laboratories correctly serotyped the internal control strain (WHO S9.1), which is the lowest percentage obtained since 2001 (Table 5). This apparent contradiction with the otherwise excellent general performance support the hypothesis that the strains provided in 2009 were of easier identification compared to the test strains of previous years. The quality threshold of correctly serotyping at least seven strains was met by 69% of participating laboratories, thus demonstrating a clear

improvement compared to 2008. Once more, this result emphasizes that the panel of strains chosen in 2009 was easier to serotype compared to the strains provided in 2008.

In general, the obtained results indicate that most laboratories worldwide have the capacity to serotype the most common *Salmonella* serovars. However, the data also show that one region, *i.e.* Central Asia & Middle East, still lacks access to reliable antisera and laboratory training courses necessary to identify regionally prevalent serovars, as this region reported the highest percentages of deviations of serotyping results. Noteworthy, many regions obtained better results compared to 2008. A truly impressive accomplishment was represented by the ability of developing countries to serotype several strains correctly in 2009.

We think that 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 indentified the O antigen and the flagellar antigen complex, but incorrectly identified the minor antigens within the complex. Our proposed explanation is further supported by the results reported for strain WHO S 9.2 (Brandenburg / I 4,12: l,v:e,n,z15), which accounted for the highest number (19%) of deviations. Six laboratories reported it as *S.* Kimuenza (I 1, 4,12, 27:l,v:e,n,x) by wrongly detecting the E-complex, and three laboratories reported it as *S.* Mons (I 1, 4,12, 27:d:l,v) by incorrectly determining both the first and second flagella phase.

Similar problems were observed with strain WHO S9.4 (Bredeney / I 1,4,12:l,v:1,7); a 1-complex strain which resulted in 16% of deviations. Four laboratories reported it as *S.* Fyris (I 4,[5],12:l,v:1,2) because of incorrect detection of the second flagella phase 1,2.

The strain WHO S 9.6 *S.* Worthington (I 1,13, 23:z:l,w) was incorrectly serogrouped by 10.9 % of participating laboratories. Additionally, 16.3% of participating laboratories failed to correctly serotype it. A likely explanation is that serogrouping and serotyping this strain require a usual panel of somatic antisera; 13 and 23.

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

Overall, 94% of the *Salmonella* AST was correctly performed, and critical deviations were only 3%. This result is extremely satisfactory and represents the best achievement in the EQAS trials performed through the different years. Of note, the number of participating laboratories decreased from 168 in 2008 to 153 in 2009. Thus, the obtained results could be due to lack of participation of laboratories which performed poorly in previous years. However, the excellent results obtained could also be the consequence of better

performance by laboratories participating in training courses aiming to strengthen awareness about antimicrobial resistance.

Guidelines for MIC breakpoint interpretation were given to participating laboratories also in EQAS 2009. 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.
Participating laboratories had familiarity with the interpretation rules adopted by the EQAS organizers since they were given the interpretative guidelines and recommendations (see Materials and Methods section) in the last two years, which could have contributed to the better performance achieved in 2009.

As in previous years, a high percentage of total deviations was observed for CIP, STR, SMX and TET susceptibility tests. In case of CIP susceptibility test, the participating laboratories obtained less than 90% correct results for strain WHO S-9.7. This strain had MIC values of 0.12 µg/ml, and therefore should have been considered resistant (since it had reduced susceptibility) to ciprofloxacin. Likely, participating laboratories interpreted the results according to CLSI breakpoints instead of the recommended EUCAST cut-off values. In case of STR susceptibility test, in EQAS 2009 we observed difficulties comparable to what observed in previous EQAS iterations, since many strains had zone diameters or MIC values near the breakpoint. In 2009, less than 90% of the participants had correct interpretation in six of the eight strains (Table 7). As a consequence, DTU Food launched a study among 17 laboratories from Europe, China and North America to establish an exact breakpoint for resistance, and the obtained results have now been submitted to a scientific journal for editorial revision. In case of SMX susceptibility test, we observed more deviations in the results reported in EQAS 2009 than in previous EQAS iterations. 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. Although four (50%) of the strains included in EQAS 2009 were susceptible to SMX, less than 90% of the laboratories obtained correct results, and they instead classified these strains as resistant. 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 or they might indicate that the CLSI clinical breakpoint should be reconsidered.

In general, data from the *Salmonella* AST component of EQAS 2009 demonstrate an overall improvement which could be the consequence of i) participation of a decreased number of laboratories from Central Asia & Middle East compared to 2008; ii) test strains typeable more easily than strains provided in previous EQAS iterations; and iii) improved awareness concerning antimicrobial resistance also due to WHO GFN laboratory training courses. Of note, fewer laboratories from China, Europe and North America and more laboratories from the Southeast Asian and African regions participated to this EQAS iteration compared to 2008.

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 129 (84%) 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 2009 results showed an improvement in *Salmonella* AST which, however, still need harmonization. In addition, EQAS aims at improving the component related to AST of the QC strain which, in 2009, 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 2009, the component related to *Shigella* serotyping was available for all regions. Participating laboratories were scattered in all regions excluding the Caribbean. Of note, between 103 (95%) and 114 (99%) participating laboratories serogrouped the test strains with maximum one deviation for three of the strains and five deviations for the *Shigella boydii* strain. In addition, up to 70 participating laboratories serotyped the strains and, as for the serogrouping, the majority of the observed deviations was related to *Shigella boydii* typing. Surprisingly, eight laboratories reported the same erroneous serotype (serotype 1). Need of improvements were identified mainly in the African region where eight laboratories performed *Shigella* serotyping with only 72% of correct results. The considerable number of laboratories participating to this EQAS component indicates that *Shigella* is an important human pathogen and that the inclusion of this microorganism in EQAS was a wise decision.

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

In EQAS 2009, AST of *Shigella* spp. was available for all regions, and was performed by 111 laboratories. All regions submitted results with the exception of the Oceanic region, and the overall regional performance was similar to the one described for *Salmonella* AST. The results reported for *Shigella* AST revealed similar problems as described for *Salmonella*. Accordingly, we observed high percentages of deviations related to TET and STR susceptibility test results. Possible reasons for these deviations have already been discussed in section 4.2. SMX and CIP susceptibility test results were not as deviating as described for *Salmonella*. A likely explanation is represented by the fact that all *Shigella* strains were susceptible to CIP, and the participants could then avoid misinterpretation of strains having reduced susceptibility to CIP. Surprisingly, participating laboratories performed SMX susceptibility testing of *Shigella* more correctly then SMX susceptibility testing of *Salmonella*.

4.5 Identification of *Campylobacter* strains

In 2009, we selected both *Campylobacter jejuni* (not included in EQAS trials since 2006) and *Campylobacter coli* strains. To avoid viability problems, the stability of lyophilized cultures was tested by DTU Food prior to appoint the producer, and was confirmed by viability testing of the lyophilized cultures in January 2010. A large number (34%) of laboratories requesting the strains did not submit results, as it was observed in previous years. Probably, this represents a lack of capacity to grow *Campylobacter* strains which require incubation in microaerophilic atmosphere. We did not examine if this lack of capacity was regional-based, but analysis of the results strongly indicate that participating laboratories in the African and Central Asian & Middle Eastern region had the worst performance. As a consequence, the WHO GFN planned to conduct a specific laboratory training course on isolation of *Campylobacter* for English-speaking African laboratories in 2010. Overall, the results related to *Campylobacter* identification were excellent, and 95% of the submitted results for *C. jejuni* were correct. This is the first EQAS iteration in which the percentage of correctly identified *Campylobacter* strains is higher than 90%.

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

In EQAS 2009, a *Campylobacter* AST component was added. However, only data obtained through the MIC determination method were accepted, since international recognized disk diffusion interpretation guidelines do not exist. In addition, epidemiological cut-off values recommended by EUCAST were used for AST interpretation, which allows to categorize strains in susceptible (wild-type) or resistant (non-wild-type). The 25 participating laboratories performed satisfactorily, since they obtained 91.4% correct test results, which is very close to the threshold criteria set for *Salmonella*. No laboratories from the Central Asia & Middle Eastern, Caribbean, Oceanic and Russian regions participated in this EQAS component. The two participating laboratories from Africa reported more deviating results compared to laboratories from other regions.

The majority of observed deviations was linked to ERY, NAL, 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. A total of 24 (96%) participating laboratories submitted AST results for the QC strain. In this case, it was possible to upload data for four different MIC determination methods, *i.e.* micro- and agar-dilution performed at two different incubation conditions (37 °C and 42 °C). The majority of deviations was observed for CIP susceptibility testing by micro-dilution at 42 °C and ERY susceptibility testing by agar-dilution at 42 °C. Surprisingly, the

participating laboratories performed better when testing NAL susceptibility for the QC strain than for the test strains, while in the case of CIP susceptibility testing, they obtained better result for the tests strains than for the QC strain. In general, AST of the QC strain was satisfactory. However, ERY and GEN susceptibility testing of the QC strain can be improved.

4.7 Identification of the unknown culture

In EQAS 2009, we included a *Vibrio mimicus* strain as we aim to strengthen the ability of diagnostic laboratories to differentiate different *Vibrio* species. Of 56 laboratories delivering results, only 27 (48%) identified the strain completely. In EQAS 2007, we included a *Vibrio parahaemolyticus* strain which was tested by 86 laboratories, thus showing that in EQAS 2009 the performance of this component was poor probably due to low viability of the strain.

5. Conclusions

The acceptance threshold for the *Salmonella* serotyping EQAS component was met by 69% (n=105) of the participating laboratories. In addition, 83% of the laboratories tested all eight strains and a total of 86% of all tests were correct, thus representing an increase compared to 2008. However, the ability in testing correctly the internal QC strain decreased of 3% compared to 2008. Many of the regions performed satisfactorily, with a result overall similar to last year. However, the *Salmonella* serotyping capacity of laboratories in African and Central Asian & Middle Eastern regions still needs to be improved. Future training efforts should aim at enhancing the capability to detect the flagella phases, and at distributing protocols for preparing high quality swarm agar plates. The obtained results indicate that detection of the phase two flagellar antigen is 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 3 minor and 3 critical deviations. Notably, STR, SMX, CIP 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 improved considerably compared to EQAS 2008. Unfortunately, 24 (16%) 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 2009, and consisted of serogrouping, serotyping and AST. Most laboratories (n=103; 87%) correctly serogrouped the four *Shigella* strains, and a maximum of 4.6% deviations was observed. A total of 70 laboratories performed serotyping, with a maximum of 12.5% 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 131 laboratories requested to participate to the *Campylobacter* component of EQAS 2009, but only 86 (66%) uploaded data related to identification. The *C. jejuni* strain was correctly identified by 95% of the participating laboratories. The majority of difficulties in *Campylobacter* identification were experienced by laboratories in the African and Central Asian & Middle Eastern regions. Therefore, a laboratory training course on *Campylobacter* identification has been scheduled in Africa in 2010.
EQAS 2009 included an AST component for *Campylobacter*, where only MIC determinations were considered acceptable. A total of 25 laboratories participated to this component. The acceptance threshold used for *Salmonella* was applied and was almost met, since we observed 0.7% minor and 8.6% critical deviations. The data revealed that ERY, NAL, STR and TET susceptibility testing were the most challenging. In addition, discrepancies between NAL and CIP susceptibility testing were observed. Of the 25 participating laboratories, 24 performed AST of the QC strain, and the majority of the results for ERY and GEN susceptibility was out of the accepted range.

The unknown strain, *Vibrio mimicus*, was identified by 75% of the participating laboratories at the genus level (*Vibrio* spp.), and by 48% of the participating laboratories at the species level (*V. mimicus*).

Reference List

1. Clinical and Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. M45-A. 2006. Wayne, PA, USA.

2. Clinical and Laboratory Standards Institute. 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. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. M100-S19. Nineteenth Informational Supplement. 2009. Wayne, PA, USA.

4. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. M07-A7. 7th Edition[Approved Standard]. 2006. Wayne, PA, USA.

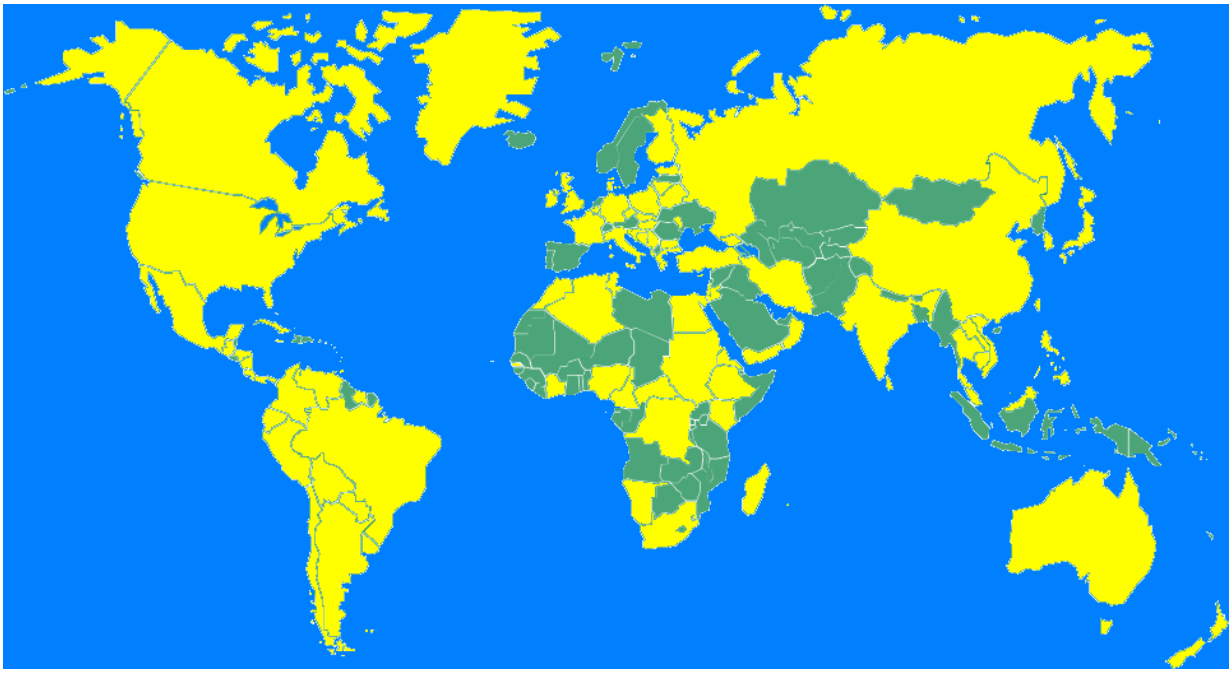
5. Grimont, P. A. D. Antigenic formulae of the *Salmonella* serovars. F.X.Weil. [9th ed.]. 2007. Paris, France., WHO Collaborating Center for Reference and Research on *Salmonella*, Institut Pasteur.

6. Herikstad, H., Y. Motarjemi, and R. V. Tauxe. 2002. *Salmonella* surveillance: a global survey of public health serotyping. Epidemiol.Infect. 129(1):1-8.

7. Schwarz, S., P. Silley, S. Simjee, N. Woodford, D. E. van, A. P. Johnson, and W. Gaastra. 2010. Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J.Antimicrob.Chemother. 65(4):601-604.

Figure and Tables

Figure 1. Countries participating* in the WHO EQAS 2009



*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
Average	86	75	703	82

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	
	No.	%	No.	%	No.	%	No.	%	No.	%
8	9	24	34	35	52	53	32	25	41	32
7	9	24	13	14	19	19	15	12	14	11
6	4	11	9	9	12	12	18	14	16	13
5	3	8	9	9	4	4	23	18	16	13
4	3	8	4	4	1	1	14	11	11	9
3	4	11	8	8	4	4	13	10	10	8
2	2	5	3	3	5	5	4	3	10	8
1	2	5	5	5	1	1	5	4	5	4
0	1	3	11	11	1	1	3	2	4	3
In total	37	100	96	100	99	100	127	100	127	100
Number of strains correctly serotyped	**Participating laboratories**									
	EQAS 2006		EQAS 2007		EQAS 2008		EQAS 2009		AVERAGE EQAS 2000 - 2009	
	No.	%	No.	%	No.	%	No.	%	No.	%
8	42	32	66	47	50	33	76	50	44	37
7	35	27	29	21	36	24	29	19	20	18
6	19	15	13	9	11	7	7	5	12	11
5	12	9	11	8	14	9	13	8	11	10
4	7	5	7	5	12	8	5	3	7	6
3	5	4	6	4	9	6	7	5	7	7
2	3	2	2	1	8	6	5	3	4	4
1	4	3	6	4	9	6	6	4	4	4
0	3	2	0	0	2	1	5	3	3	3
In total	130	100	140	100	151	100	153	100	114	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 2009
Africa	2001	6	37	73.0	Algeria, Cameroon, Central African Republic, Democratic Republic of Congo, Gambia, Ivory Coast, Kenya, Madagascar, Mauritius, Morocco, South Africa, Tunisia
	2002	9	62	87.1	
	2003	11	70	71.4	
	2004	9	51	62.7	
	2006	16	95	71.6	
	2007	11	73	80.8	
	2008	10	71	49.3	
	2009	15	94	75.5	
Asia & Middle East	2001	10	60	50.0	Egypt, Israel, Jordan, Oman, Yemen
	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	
Caribbean	2001	0	0	0	Barbados, Suriname, Trinidad and Tobago
	2002	0	0	0	
	2003	3	18	61.1	
	2004	2	8	87.5	
	2006	3	14	78.6	
	2007	2	9	77.8	
	2008	3	14	78.6	
	2009	3	12	83.3	
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	
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, Turkey, United Kingdom
	2002	50	384	90.0	
	2003	60	401	84.8	
	2004	57	392	84.7	
	2006	52	403	86.4	
	2007	54	415	89.4	
	2008	50	379	82.3	
	2009	47	362	93.1	

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 2009
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	
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	
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	
Latin America	2001	11	78	57.7	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemale, Honduras, Mexico, Nicaragua, Paraguay, Peru, Venezuela
	2002	11	82	87.8	
	2003	13	83	75.9	
	2004	15	88	79.5	
	2006	13	84	84.5	
	2007	15	107	88.8	
	2008	17	120	71.7	
	2009	21	150	77.3	
Southeast Asia	2001	15	113	54.0	Brunei, Cambodia, Japan, Lao, 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	

Table 4. *Salmonella* serogroups (SG), serotypes (ST) and deviations (D), WHO EQAS 2009

Strain ID	Correct serotype		No. of labs reporting SG	% D_{SG}	No. of labs reporting ST	% D_{ST}	Deviating results (*)
WHO S-9.1	Enteritidis	9,12:g,m:-	159	3.1	151	6.6	Dublin (3), Nitra (1), Moscow (1), Blegdam (1), Stanley (1), Rostock (1), Onarimon (1), Paratyphi A (1)
WHO S-9.2	Brandenburg	4,12:l,v:e,n,z15	157	1.3	142	19.0	Kimuenza (6), Mons (3), Typhimurium (2), Sandiego (2), Essen(1), Fyris (1), Texas (1), Azteca (1), Kisangani (1), Reinickendorf (1), Wilhelmsburg (1), Tsevie (1), Brezany (1), Koessen (1), Wagenia (1), Chartres (1), Duisburg (1), II (1)
WHO S-9.3	Muenster	3,10:e,h:1,5	152	3.3	142	12.7	Vejle (2), Newlands (2), Lamberhurst (2), Lamin (1), Ratchaburi (1), Aminatu (1), Vilvoorde (1), Lorochelle (1), Sekondi(1), II (1), Anatum (1),
WHO S-9.4	Bredeney	1,4,12:l,v:1,7	157	3.8	144	16.0	Fyris (4), Brandenburg (3), Saintpaul (2), Give (1), Hato (1), Togo (1), Schwarzzengrund (1), Typhimurium (1), Azteca (1), Svedvi (1), Concord (1), Kubacha (1), Reading (1), Stanley (1), Kaapstad (1), Parkroyal (1), II 1,9,12:l,w:e,n,z
WHO S-9.5	Sandiego	4,5,12:e,h:e,n,z15	157	0.6	142	14.8	Chester (8), Saintpaul (4), Reading (2), Chartres (2), Typhimurium (2), Duisburg (1), Brandenburg (1), Arechavaleta (1)
WHO S-9.6	Worthington	1,13,23:z:l,w	147	10.9	129	16.3	Carno (3), Nanga (3), Tanzania (2), Gabon (1), Poona (1), Vridi (1), Paratyphi A (1), Enteritidis (1), Wortington (1), Washington (1), Remiremont (1), Ajiobo (1), Alkmaar (1), Koessen (1), Marburg (1), II 1,13,23:z:1,5
WHO S-9.7	Albany	8,20:z4,z24:-	155	2.6	134	10.4	Corvallis (3), Altona (2), Tallahassee (2), Dabou (1), Cocody (1), Kalamu (1), Sindelfingen (1), Manhattan (1), Blockley (1),Yovokome (1)
WHO S-9.8	Stanley	4,5,12:d:1,2	156	0.6	143	13.3	Typhimurium (5), Duisburg (3), Schwarzengrund (3), Fyris (1), Typhi (1), Clackamas (1), Saintpaul (1), Paratyphi B var. Java (1), Paratyphi B (1), Eppendorf (1), Ayinde (1)

*number of participants reporting the specified deviating result

Table 5. EQAS participating laboratories' performance of internal quality control strain (WHO S-9.1, *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
Average	106	93

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

EQAS iteration	No. of EQAS participating laboratories	Average no. of antimicrobial agents tested	% 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	9.1	92	4	4	0	4	8
2001	108	8.9	91	6	2	1	3	9
2002	119	8.9	92	6	2	1	3	9
2003*	147	8.1	93	4	3	0	3	7
2004	152	10.2	93	4	2	1	3	7
2006	143	11.2	88	8	3	1	4	12
2007	143	10.8	93	4	2	1	3	7
2008	168	10.3	91	4	2	3	5	9
2009	153	10.4	94	3	2	1	3	6
Average*	131	9.8	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 2009 *Salmonella* strains*

Strain	Antimicrobial^												
	AMP	**CTX**	**CAZ**	**CRO**	**CHL**	**CIP**	**GEN**	**NAL**	**STR**	**SMX**	**SXT**	**TET**	**TMP**
WHO S-9.1	10/10/**132**	3/2/**121**	1/2/**113**	0/0/**98**	2/1/**137**	0/1/**149**	**142**/0/2	1/0/**138**	**104**/1/4	**76**/1/3	5/0/**126**	14/19/**105**	1/1/**75**
WHO S-9.2	6/3/**142**	1/1/**124**	0/0/**115**	0/2/**96**	1/0/**138**	0/2/**147**	4/2/**137**	1/3/**134**	9/33/**65**	10/3/**65**	4/2/**125**	1/9/**130**	2/0/**75**
WHO S-9.3	5/4/**142**	1/1/**124**	0/0/**115**	0/0/**98**	2/1/**135**	0/1/**148**	1/1/**141**	2/4/**131**	9/16/**83**	12/1/**65**	6/1/**122**	6/14/**120**	1/0/**77**
WHO S-9.4	3/2/**146**	1/0/**125**	0/1/**114**	0/1/**97**	1/0/**138**	1/1/**147**	2/3/**138**	1/3/**133**	11/21/**76**	7/1/**70**	4/1/**127**	4/7/**129**	1/0/**75**
WHO S-9.5	4/2/**144**	1/0/**124**	0/0/**115**	0/0/**97**	1/0/**137**	1/2/**145**	1/3/**138**	1/3/**133**	6/31/**71**	11/3/**63**	4/1/**125**	5/8/**125**	1/0/**76**
WHO S-9.6	5/2/**143**	1/1/**124**	0/1/**114**	1/1/**94**	3/0/**135**	0/2/**146**	1/5/**136**	3/3/**130**	**104**/0/4	**75**/1/2	**122**/1/6	**135**/0/3	**77**/0/0
WHO S-9.7	**147**/0/3	1/2/**124**	0/1/**114**	0/0/**94**	**136**/1/1	**45**/7/97	5/1/**137**	**130**/1/4	23/49/**36**	**77**/0/1	**129**/0/1	**132**/5/3	**76**/0/1
WHO S-9.8	5/3/**143**	1/1/**125**	0/0/**115**	0/0/**96**	**134**/1/3	0/1/**147**	3/2/**138**	3/3/**131**	11/34/**63**	**77**/0/0	**126**/1/3	**124**/12/4	**77**/0/0

^For antimicrobial abbreviations: see List of Abbreviations page 1

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

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
Average•	1531	No. of tests	-	9023	3481	8714	8923	-	1566	4978	8860	-	8406	5192	6926	7625	8581	5023	-	87298
		% critical deviations*	-	3	3	2	5	-	2	3	3	-	1	5	5	3	5	1	-	3
		% total deviations^	-	5	5	5	6	-	3	8	6	-	4	6	27	5	13	2	-	8

Legend Figure 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 2009 iteration
Africa	2001	7	80.1	9.6	7.7	2.5	10.2	19.8	Algeria, Cameroon, Central African Republic, Democratic Republic of Congo, Ethiopia, Gambia, Ivory Coast, Kenya, Madagascar, Mauritius, Morocco, Namibia, Nigeria, South Africa, Sudan, Tunisia
	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	
Central Asia & Middle East	2001	10	87.7	6.3	5.2	0.8	6.0	12.3	Egypt, Iran, Israel, Jordan, Oman, 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	
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	
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	
Europe	2001	47	91.3	5.7	2.7	0.3	3.0	8.7	Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Denmark, Finland, France, Greece, Hungary, Ireland, Italy, Lithuania, Luxembourg, Malta, Republic of Moldova, Poland, Serbia, Slovak Republic, Slovenia, Turkey, United Kingdom
	2002	57	92.7	5.2	1.2	0.9	2.1	7.3	
	2003	64	92.9	3.8	1.0	2.3	3.3	7.1	
	2004	58	93.5	4.3	1.4	0.8	2.2	6.5	
	2006	54	88.7	7.0	3.8	0.6	4.4	11.3	
	2007	49	94.2	3.7	1.6	0.4	2.0	5.7	
	2008	51	91.2	4.4	2.5	1.9	4.4	8.8	
	2009	40	95.1	2.6	1.3	0.9	2.2	4.8	

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 2009 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	
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	
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	
South America	2001	11	90.8	6.9	1.4	1.0	2.4	9.2	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemale, 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	
Southeast Asia	2001	16	88.1	7.7	2.3	1.9	4.2	11.9	Brunei, Cambodia, India, Japan, Lao, Malaysia, Nepal, Philippines, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam
	2002	18	89.0	8.1	1.4	1.6	3.0	11.0	
	2003	17	87.4	5.2	4.7	2.7	7.4	12.6	
	2004	16	92.8	4.4	2.3	0.5	2.8	7.2	
	2006	15	90.0	8.1	1.2	0.8	2.0	10.0	
	2007	20	93.9	4.0	1.4	0.7	2.1	6.1	
	2008	19	90.5	4.7	2.2	2.6	4.8	9.5	
	2009	27	91.8	4.1	3.0	1.2	4.2	8.3	

^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	-

Legend table 10

[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 2009

Strain	Correct serotype	No. of labs reporting correct identification	D (%)	Deviating results (*)	No. of labs reporting correct ST	D (%)	Deviating results (*)
WHO SH-9.1	*S. sonnei*	114	0.9	(1)	N/A	N/A	N/A
WHO SH-9.2	*S. flexneri* serotype 6	112	0.9	(1)	70	5.4	2a (1), 2b (1), 4a (1)
WHO SH-9.3	*S. flexneri* serotype 2a	111	0.9	(1)	68	4.2	6 (1), 4a (1), var Y (1)
WHO SH-9.4	*S. boydii* serotype 2	103	4.6	(5)	56	12.5	1 (8)

*number of participants reporting deviating result

Table 12. Region-based categorization of laboratories performing *Shigella* serotyping in 2009

Region	No. of laboratories	No. of strains serotyped	Strains serotyped correctly (%)	Countries participating in the 2009 iteration
Africa	8	18	72.2	Cameroon, Central African Republic, Ethiopia, Gambia, Kenya, Mauritius, South Africa, Tunisia
Asia & Middle East	3	5	100.0	Israel, Oman, Yemen
Caribbean	0	0	0	-
China	13	35	100.0	China
Europe	15	40	92.5	Albania, Belgium, Czech Republic, Denmark, Finland, Ireland, Italy, Lithuania, Luxembourg, Republic of Moldova, Serbia, Slovenia, Turkey, United Kingdom
North America	7	18	100.0	Canada, United States of America
Oceanic	3	8	100.0	Australia, New Zealand
Russia	6	18	83.3	Belarus, Georgia, Russia
Latin America	16	40	97.5	Argentina, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Honduras, Mexico, Nicaragua, Paraguay, Peru, Uruguay, Venezuela
Southeast Asia	11	30	90.0	Japan, Lao, Philippines, South Korea, Sri Lanka, Taiwan, Thailand

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

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

Table 14. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2009 *Shigella* strains*

Strain	Antimicrobial[∞]												
	AMP	**CTX**	**CAZ**	**CRO**	**CHL**	**CIP**	**GEN**	**NAL**	**STR**	**SMX**	**SXT**	**TET**	**TMP**
WHO SH-9.1	**105**/0/1	1/0/**93**	0/0/**90**	0/1/**76**	1/0/**96**	0/0/**107**	2/1/**96**	0/1/**96**	**70**/0/3	**53**/0/0	**96**/1/1	**78**/14/5	**54**/0/0
WHO SH-9.2	4/4/**98**	1/0/**91**	0/0/**90**	0/0/**75**	1/0/**96**	0/2/**105**	2/1/**97**	1/2/**94**	11/33/**29**	3/0/**48**	2/2/**94**	1/1/**95**	2/0/**51**
WHO SH-9.3	**100**/1/4	2/0/**90**	1/0/**87**	1/0/**72**	**82**/10/4	0/1/**105**	4/2/**93**	0/1/**95**	**71**/0/1	**51**/0/1	**93**/1/2	**90**/2/3	**54**/0/0
WHO SH-9.4	**100**/1/1	0/0/**90**	0/0/**86**	0/0/**72**	2/0/**92**	1/0/**101**	2/0/**92**	1/0/**93**	**67**/2/2	**47**/0/4	4/1/**87**	**90**/1/2	2/0/**51**

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

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

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	19
		% total deviations^	1	2	1	-	2	1	-	-	9	2	8	-	2	28
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

[∞]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 2009

Region	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 2009 iteration
Africa	17	93.3	2.4	3.5	0.8	4.3	6.8	Algeria, Cameroon, Central African Republic, Democratic Republic of Congo, Ethiopia, Gambia, Ivory Coast, Kenya, Madagascar, Mauritius, Nigeria, South Africa, Sudan, Tunisia
Central Asia & Middle East	5	94.8	0.9	3.0	1.3	4.4	5.2	Iran, Israel, Jordan, Oman, Yemen
Caribbean	4	95.6	1.5	0.7	2.2	2.9	4.4	Barbados, Jamaica, Suriname, Trinidad and Tobago
China	12	96.3	2.2	1.0	0.5	1.5	3.7	China
Europe	22	98.1	1.1	0.7	0.1	0.8	1.9	Albania, Belgium, Bosnia and Herzegovina, Denmark, Finland, Greece, Ireland, Italy, Lithuania, Luxembourg, Republic of Moldova, Poland, Serbia, Slovak Republic, Slovenia, United Kingdom
North America	6	100.0	0.0	0.0	0.0	0.0	0.0	United States of America
Oceanic	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
Russia	6	95.5	1.6	1.6	1.3	2.9	4.6	Belarus, Georgia, Russia
South America	20	98.3	1.1	0.4	0.3	0.7	1.7	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemale, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Uruguay, Venezuela
Southeast Asia	18	94.1	3.9	0.3	1.7	2.0	5.9	Cambodia, India, Japan, Lao, Malaysia, Philippines, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam

^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)

*number of participants reporting the specified deviating result

Table 18. Region-based categorization of EQAS 2009 participating laboratories' performance of *Campylobacter* strains identification

Region	No. of labs	No. of strains identified	% strains correctly identified	Countries participating in the 2009 iteration
Africa	8	13	53.8	Algeria, Cameron, Democratic Republic of Congo, Ethiopia, Tunisia
Asia & Middle East	3	5	40	Egypt, Israel, Oman
Caribbean	2	4	100	Barbados, Trinidad and Tobago
China	12	24	91.7	China
Europe	28	53	88.7	Cyprus, Czech Republic, Denmark, Finland, Germany, Greece, Hungary, Italy, Lithuania, Luxembourg, Republic of Moldova, Poland, Serbia, Slovak Republic, Slovenia, Turkey
North America	10	19	89.5	Canada, United States of America
Oceania	2	4	100	Australia, New Zealand
Russia	2	4	100	Belarus, Georgia
South America	14	26	88.5	Argentina, Brazil, Chile, Colombia, Costa Rica, Cuba, Guatemale, Paraguay, Perù, Uruguay, Venezuela
Southeast Asia	10	20	90	Cambodia, Japan, Philippines, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam

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

^S, susceptible; R, resistant

Table 20. Antimicrobial susceptibility test results (number of R/S) for the EQAS 2009 *Campylobacter* strains*

Strain	Antimicrobial^						
	CHL	CIP	ERY	GEN	NAL	STR	TET
WHO C-9.1	1/**17**	0/**23**	**21**/2	0/**21**	0/**20**	3/**13**	2/**20**
WHO C-9.2	2/**15**	**19**/2	2/**19**	0/**20**	**16**/3	1/**15**	**19**/2

^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

^For antimicrobial abbreviations, see List of Abbreviations page 1

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

Table 22. Region-based categorization of EQAS 2009 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 2009 iteration
Africa	2	50.0	21.4	28.6	50.0	Algeria, Tunisia
Central Asia & Middle East	0	-	-	-	-	-
Caribbean	0	-	-	-	-	-
China	2	95.2	4.8	0.0	4.8	China
Europe	9	98.3	1.7	0.0	1.7	Denmark, Greece, Italy, Luxembourg, Poland, Slovenia
North America	2	100.0	0.0	0.0	0.0	United States of America.
Oceania	0	-	-	-	-	-
Russia	0	-	-	-	-	-
South America	5	93.2	6.8	0.0	6.8	Argentina, Brazil, Costa Rica, Paraguay
Southeast Asia	4	71.4	0.0	28.6	28.6	Thailand, Phippines, Sri Lanka, Korea

^S, susceptible; R, resistant

Table 23. EQAS 2009 participants' performance of antimicrobial susceptibility testing of *Campylobacter jejuni* ATCC 33560

	Method used	Incubation conditions	Labs' performance[1, 2]	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

[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 sakasakii*	128	92
2009	*Vibrio mimicus*	56	48

Appendixes (1-4b)

WHO Global Salm-Surv Electronic Discussion Group
Subject: Signing up for EQAS 2009

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 2008 EQAS cycle has closed, and we are pleased to announce the launch of the 2009 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 2009?
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 2009. Laboratories which signed up and received strains in year 2008, but did not submit any data, should explain the reason for this in order to participate in 2009.

COST FOR PARTICIPATING IN EQAS
There is no charge for participating in EQAS 2009; 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 2009
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 2009
- 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 7234 6001).

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 2009.

The isolates will be shipped between August and September 2009. 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 2009** 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 Global Salm-Surv Regional Centre in your region will be given access to your results.

Deadline for signing up to participate in this EQAS: April 30th, 2009
****

Posted by Susanne Karlsmose, suska@food.dtu.dk, WHO Global Foodborne Infections Network EQAS Coordinator, DTU Food, The National Food Institute, Denmark.

Salmonella

			Ampicillin **AMP**	Cefotaxime **CTX**	**CTX/CL : CTX**	Ceftazidime **CAZ**	**CAZ/CL : CAZ**	Ceftriaxone **CRO**	Chloramphenicol **CHL**	Ciprofloxacin **CIP**	Gentamicin **GEN**	Nalidixic acid **NAL**	Streptomycin **STR**	Sulfiz. **SMX**	Tetracycline **TET**	Trimethoprim **TMP**	TriSul **SXT**
WHO S-9.1	*S.* Enteritidis	9,12:g,m:-	4	0.25	<0,25/0,125	1.0	<0,25/0,125	0.25	8	0.030	>16	4	64	>1024	4	<=1	0.125
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	RESIST	SUSC	RESIST	RESIST	SUSC	SUSC	SUSC
WHO S-9.2	*S.* Brandenburg	4,12:l,v:e,nz15	<=1	<=0.12	<0,25/0,125	0.25	<0,25/0,125	0.064	4	<=0.015	<=0.5	2	<=8	32	<=2	<=1	0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-9.3	*S.* Muenster	3,10:e,h,1,5	<=1	<=0.12	<0,25/0,125	0.5	<0,25/0,125	0.125	8	<=0.015	1	4	<=8	<=16	<=2	<=1	0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-9.4	*S.* Bredeney	1,4,12:l,v,1,7	<=1	<=0.12	<0,25/0,125	0.25	<0,25/0,125	0.064	4	0.030	<=0.5	2	<=8	<=16	<=2	<=1	0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-9.5	*S.* Sandiego	4,5,12:e,h:e,n,z15	<=1	<=0.12	<0,25/0,125	0.25	<0,25/0,125	0.064	8	0.030	0.5	2	<=8	32	<=2	<=1	0.064
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO S-9.6	*S.* Worthington	1,13,23:z:l,w	<=1	<=0.12	<0,25/0,125	0.25	<0,25/0,125	0.064	8	<=0.015	0.5	2	128	>1024	>32	>32	>32
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST
WHO S-9.7	*S.* Albany	8,20:z4,z24:-	>32	<=0.12	<0,25/0,125	0.5	<0,25/0,125	0.125	>64	0.25	<=0.5	>64	<=8	>1024	>32	>32	>32
			RESIST	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	RESIST	RESIST	SUSC	RESIST	SUSC	RESIST	RESIST	RESIST	RESIST
WHO S-9.8	*S.* Stanley	4,5,12:d:1,2	<=1	<=0.12	<0,25/0,125	0.25	<0,25/0,125	0.032	>64	<=0.015	<=0.5	4	<=8	>1024	>32	>32	>32
			SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	RESIST	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST	RESIST	RESIST

Shigella

		Ampicillin **AMP**	Cefotaxime **CTX**	**CTX/CL : CTX**	Ceftazidime **CAZ**	**CAZ/CL : CAZ**	Ceftriaxone **CRO**	Chloramphenicol **CHL**	Ciprofloxacin **CIP**	Gentamicin **GEN**	Nalidixic acid **NAL**	Streptomycin **STR**	Sulfiz. **SMX**	Tetracycline **TET**	Trimethoprim **TMP**	TriSul **SXT**
WHO SH-9.1	*S. sonnei*	>32	<=0.12	<0,25/0,125	0.125	<0,25/0,125	0.016	<=2	<=0.015	<=0.5	1	>128	>1024	>32	>32	>32
		RESIST	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST
WHO SH-9.2	*S. flexneri* type 6	4	<=0.12	<0,25/0,125	0.125	<0,25/0,125	0.032	<=2	<=0.015	<=0.5	1	<=8	<=16	<=2	<=1	0.064
		SUSC	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
WHO SH-9.3	*S. flexneri* type 2a	>32	<=0.12	<0,25/0,125	0.125	<0,25/0,125	0.064	64	<=0.015	<=0.5	1	>128	>1024	>32	>32	>32
		RESIST	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	RESIST	SUSC	SUSC	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST
WHO SH-9.4	*S. boydii* type 2	>32	<=0.12	<0,25/0,125	0.064	<0,25/0,125	0.032	<=2	<=0.015	<=0.5	1	64	>1024	32	<=1	0.064
		RESIST	SUSC	non-ESBL	SUSC	non-ESBL	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST	RESIST	SUSC	SUSC

Campylobacter

		Chloramph. **CHL**	Ciproflox. **CIP**	Erythromycin **ERY**	Gentamicin **GEN**	Nalidixic acid **NAL**	Streptom. **STR**	Tetracycline **TETRA**
WHO C-9.1	*C. coli*	= 4	0.25	>32	0.5	= 8	<=1	= 2
		SUSC	SUSC	RESIST	SUSC	SUSC	SUSC	SUSC
WHO C-9.2	*C. jejuni*	= 4	>4	= 2	0.5	>64	<=1	>16
		SUSC	RESIST	SUSC	SUSC	RESIST	SUSC	RESIST

WHO B-9.1 *Vibrio mimicus*

DTU Food
National Food Institute

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk

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

---

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 2009 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 2009

3.1 Shipping, receipt and storage of strains

In August/September 2009 around 190 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 stab cultures. On arrival, the stab cultures must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of lyophilized strains is presented below.

3.2 Serotyping of *Salmonella*

The eight *Salmonella* strains should be serotyped by the method routinely used in the laboratory. If you do not have all the antisera please go as far as you can, and please report the serogroup, since also serogrouping results will be evaluated. When reporting serogroups, please use terms according to Kaufman-White (Popoff and Le Minor, 2001. 8th ed. Popoff, M.U., Le Minor, L., 2001. Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

When uploading the data, please fill in the information on the brand of antisera used in the typing.

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.

Antimicrobials	**Reference value, MIC (µg/mL)**		
	Sensitive	**Intermediate**	**Resistant**
Ampicillin, AMP*	≤8	16	≥32
Cefotaxime, CTX**	≤0.5	-	>0.5
Ceftazidime, CAZ**	≤2	-	>2
Ceftriaxone, CRO***	≤0.25	-	>0.25
Chloramphenicol, CHL*	≤8	16	≥32
Ciprofloxacin, CIP**	<0.125	-	≥0.125
Gentamicin, GEN*	≤4	8	≥16
Nalidixic acid, NAL*	≤16	-	≥32
Streptomycin, STR***	≤8	16	≥32
Sulfonamides, SMX*	≤256	-	≥512
Tetracycline, TET*	≤4	8	≥16
Trimethoprim, TMP*	≤8	-	≥16
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT*	≤2/38	-	≥4/76

*CLSI **EUCAST (epidemiological cut off values) ***DTU Food

In this EQAS the breakpoints used as a key to interpreting MIC results are a mixture of reference values from CLSI, EUCAST and DTU Food (see list above). 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. Considering the expected results of this EQAS, microorganisms are considered resistant to ciprofloxacin when showing reduced susceptibility to this antimicrobial.

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

All strains categorized reduced susceptible against cefotaxime (CTX), ceftazidime (CAZ) or ceftriaxone (CRO) could be confirmed by confirmatory tests for ESBL production.

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

Concerning cefotaxime (CTX), ceftazidime (CAZ) and/or ceftriaxone (CRO) used when detecting ESBL-producing strains in the EQAS: If a microorganism is resistant to one or two of these drugs, it should be regarded resistant to all three.

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

The sub-cultured *Campylobacter* should be used for the MIC-testing after incubation at 36ºC 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.

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, 2009**. 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:

Ms. Susanne Karlsmose

National Food Institute, Technical University of Denmark

DTU Food
National Food Institute

WHO Collaborating Centre
for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk

27 Bülowsvej, DK-1790 Copenhagen V - DENMARK

Tel: +45 3588 6601, Fax: +45 3588 6001

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'.
    In the letter following your parcel you can find your username and password.
    Your username and password will be the same in future trials.

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

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

4) In the data entry pages '*Salmonella* strains 1-8', you
    a. SELECT the serogroup (O-group) from the pop-up list, DO NOT WRITE – Wait a few seconds – the page will automatically reload, so that the pop-up in the field "Serotype" only contains serotypes belonging to the chosen serogroup.

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

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

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

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

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

7) The next page is a menu, from where you can review the input pages or approve your input *and finally see and print the evaluated results*
   a. Browse through the input pages and make corrections if necessary. Remember to click on 'save and go to next page' if you make any corrections.
   b. Approve your input. Be sure that you have filled in all the results before approval, as YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database, but allows you to see the evaluated results.
   c. As soon as you have approved your input, an evaluation report will show.

8) When you have seen all pages in the report, you will find a new menu. You can choose 'EQAS 2009 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!

DTU Food
National Food Institute

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk

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.

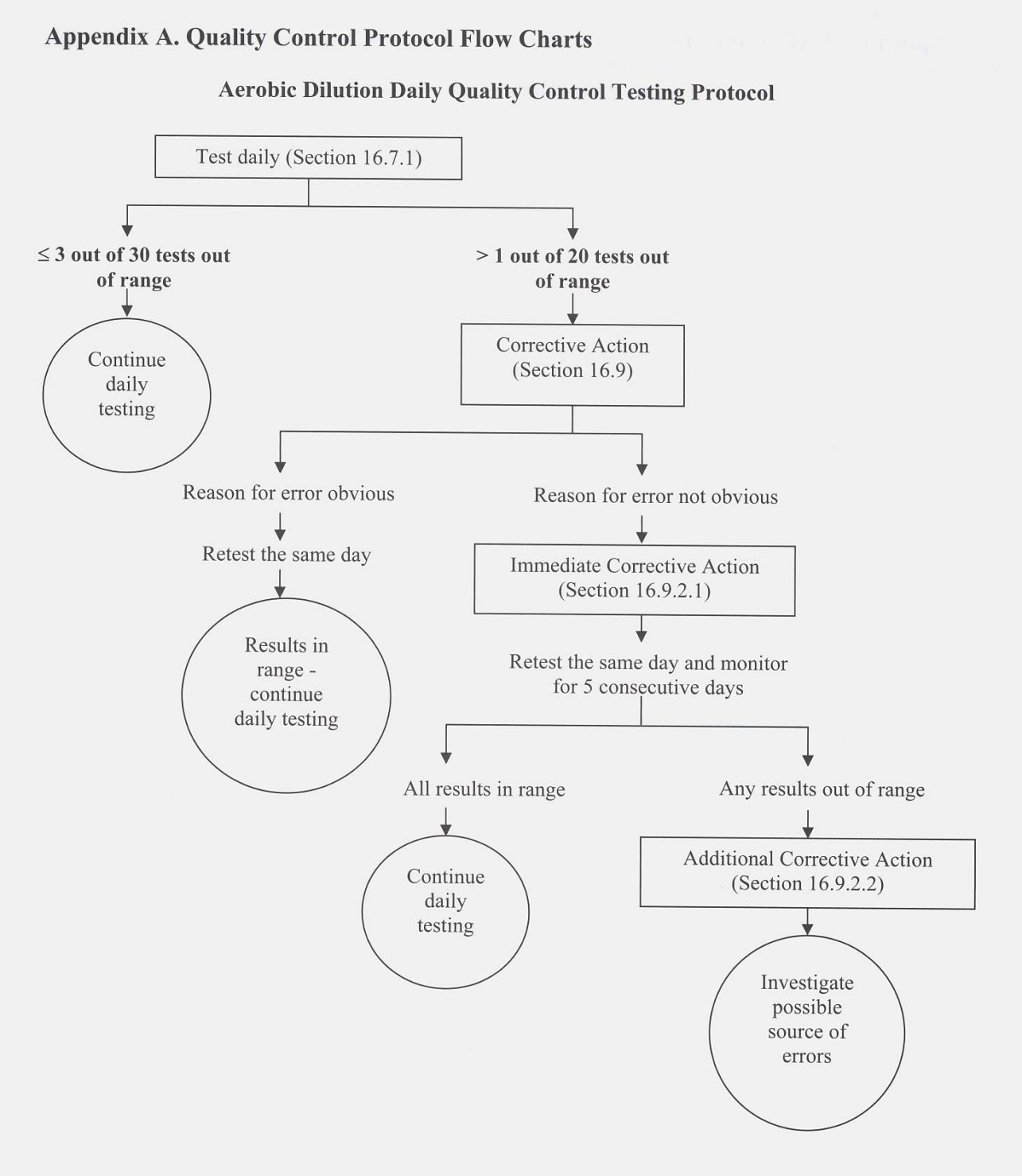
If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.

DTU Food
National Food Institute

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk

DAILY MIC QC CHART

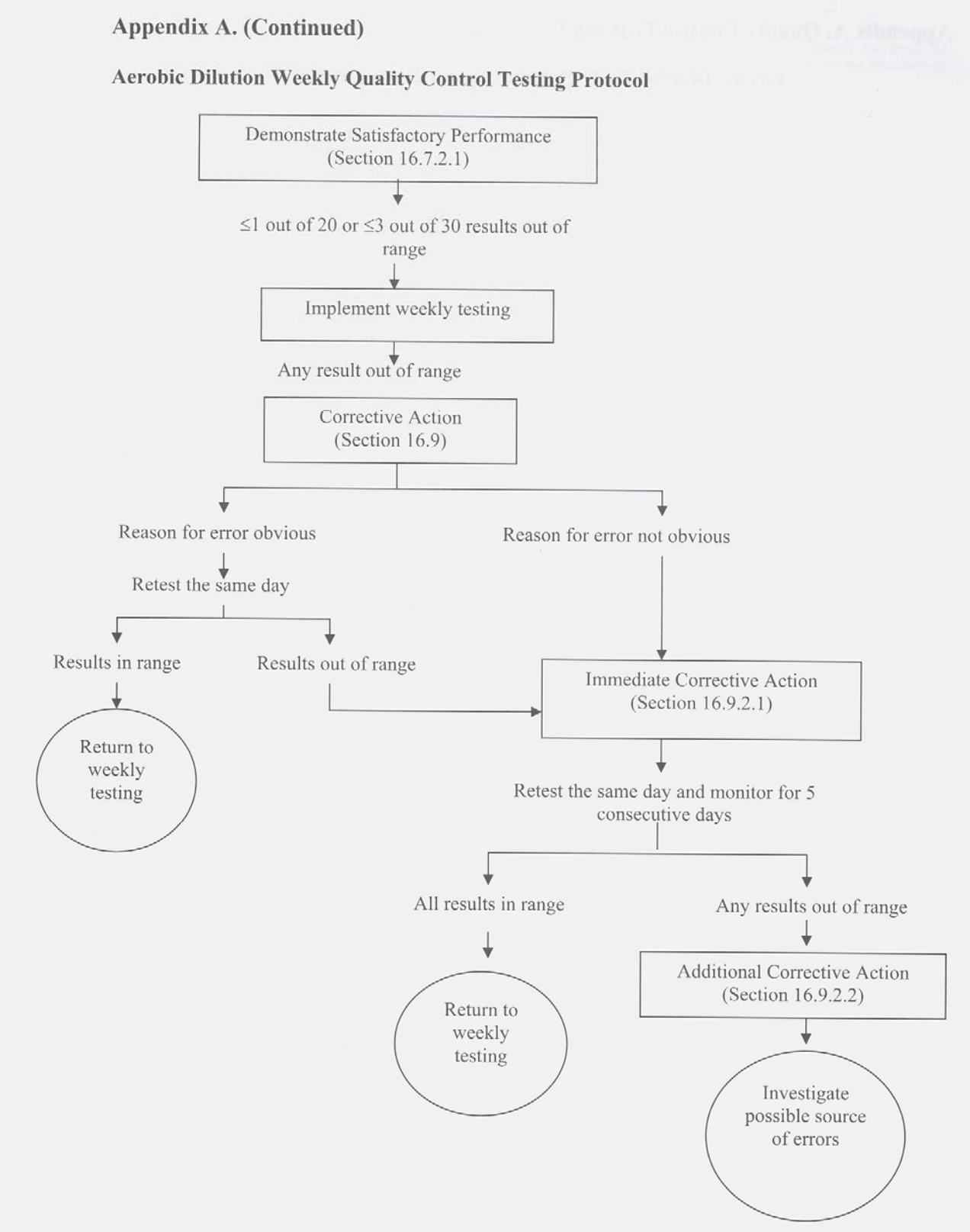


Reference: CLSI M7-A7, page 39

DTU Food
National Food Institute

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk

WEEKLY MIC QC CHART



Reference: CLSI M7-A7, page 40

DTU Food
National Food Institute

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk

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!

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

T: 35 88 70 00
F: 35 88 70 01
www.food.dtu.dk

ISBN: 978-87-92158-88-8