The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2013

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

THE EXTERNAL QUALITY ASSURANCE SYSTEM OF THE WHO GLOBAL FOODBORNE INFECTIONS NETWORK YEAR 2013

Rene S. Hendriksen, Susanne Karlsmose, Arne Bent Jensen, Frank M. Aarestrup

1. edition, November 2014

Photo: Mikkel Adsbøl

ISBN: 978-87-93109-33-9

The report is available at

www.food.dtu.dk

National Food Institute

Technical University of Denmark

Kemitorvet

Building 204

DK-2800 Kgs. Lyngby

Denmark

Tel: +45 35 88 70 00

Fax +45 35 88 70 01

Contents

List of Abbreviations

AMP, Ampicillin
AST, Antimicrobial Susceptibility Testing
ATCC, American Type Culture Collection
CAZ, Ceftazidime
CCM, Czech Collection of Micro-organisms
CDC, Centers for Disease Control and Prevention
CHL, Chloramphenicol
CIP, Ciprofloxacin
CLSI, Clinical and Laboratory Standards Institute
CRO, Ceftriaxone
CTX, Cefotaxime
DTU Food, Technical University of Denmark - National Food Institute
EQAS, External Quality Assurance System
ERY, Erythromycin
ESBL, Extended Spectrum Beta-Lactamase
EU, European Union
EUCAST, European Committee on Antimicrobial Susceptibility Testing
GEN, Gentamicin
IATA, International Air Transport Association
INCIENSA, Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud
IP. Institute Pasteur
MDR, Multi-drug resistant
MIC, Minimum Inhibitory Concentration
NAL, Nalidixic Acid
NSSC, National *Salmonella* and *Shigella* Center, Thailand
PHAC, Public Health Agency of Canada
QC, Quality Control
SMX, Sulfamethoxazole
STR, Streptomycin
SXT, Trimethoprim + Sulphonamides
TET, Tetracycline
TMP, Trimethoprim
US, United States
WHO, World Health Organization
WHO GFN, WHO Global Foodborne Infections Network
XDR, Extreme drug resistance

1. Introduction

Since 2000, 12 External Quality Assurance System (EQAS) reports have been issued with this report being the 13th. The WHO Global Foodborne Infections Network (WHO GFN) focuses on enhancing World Health Organization (WHO) Member States' capacity to detect and respond to foodborne disease outbreaks by conducting laboratory-based surveillance of *Salmonella* and other foodborne pathogens. Since its inception, the scope of WHO GFN has expanded to include additional foodborne pathogens like *Shigella* and *Campylobacter*. *Salmonella*, *Campylobacter* and *Shigella* are among the most important foodborne pathogens worldwide and account for millions of cases of diarrheal disease and thousands of deaths per year, impacting both developing and industrialized countries. Furthermore, the increased number of *Salmonella* and *Shigella* isolates which are resistant to antimicrobials is of major concern since these isolates are associated with infections characterized by increased morbidity and mortality.

The EQAS is organized annually by the Technical University of Denmark, 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 Science 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 possibly the respective WHO GFN regional centre. All summary conclusions are made public. The goal set by WHO GFN aim towards having all national reference laboratories perform *Salmonella* serotyping with a maximum of one deviation out of eight strains tested (error rate of 13%) and antimicrobial susceptibility testing (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). No quality threshold has been determined in relation to identification of *Campylobacter* ssp., serotyping and AST of *Shigella*, or identification of the unknown foodborne pathogen.

2. Materials and Methods

2.1 Participants

A pre-notification announcement of the EQAS 2013 was made through the WHO GFN list server on April 26th, 2013 and a reminder was sent on May 28th, 2013 (App. 1). The pre-notification was available in English, Spanish, Portuguese, French, Chinese and Russian, and included invitations to participate in the EQAS 2013 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 2013 from the DTU Food's strain collection. The unknown foodborne pathogen, a verotoxin producing negative *Escherichia coli* O157:H16, was selected by the Laboratory Subcommittee under the WHO GFN Steering Committee, and was selected from the strain collection at DTU Food. Individual sets of *Salmonella*, *Shigella*, and the unknown strain for identification were inoculated as agar stab cultures in nutrient agar. The *Campylobacter* strains were lyophilized in

glass vials by Czech Collection of Micro-organisms (CCM), Czech Republic. The serotype of each *Salmonella* strain was determined based on the O (somatic), phase 1 and phase 2 H (flagellar) antigens according to the scheme of Kaufmann-White (2007) (14). The *Salmonella* serotypes were determined by DTU Food and verified by the CDC and IP prior to distribution. The antimicrobial susceptibility patterns of the *Salmonella*, *Shigella* and *Campylobacter* strains were determined by DTU Food and verified by CDC. The *Shigella* serotypes were performed by PHAC and verified by the NCCS. A final confirmation after production of agar sticks was performed at DTU Food (apart from *Shigella* serotyping which is not routinely performed at DTU Food).

Laboratories which did not formerly participate in the WHO GFN EQAS AST component were provided with lyophilized international reference strains, namely *E. coli* CCM 3954 ~ American Type Culture Collection (ATCC) 25922 and *C. jejuni* CCM 6214 ~ ATCC 33560, purchased from the CCM.

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 for AST of *Salmonella* and *Shigella* strains: ampicillin, AMP; cefotaxime, CTX; ceftazidime, CAZ; ceftriaxone, CRO; chloramphenicol, CHL; ciprofloxacin, CIP; gentamicin, GEN; nalidixic acid, NAL; sulfamethoxazole, SMX; tetracycline, TET; trimethoprim, TMP and trimethoprim + sulphonamides, SXT. In addition, it was possible to confirm the presence of Extended Spectrum Beta-Lactamase (ESBL)-producing strains by using the antimicrobials CTX and CAZ in combination with the inhibitor clavulanic acid. The following antimicrobials were used 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-A9 (2012) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically"; Approved Standard - Ninth Edition (9), M100-S23 (2013) "Performance Standards for Antimicrobial Susceptibility Testing"; Twenty-Third Informational Supplement (8), document VET01-S2 (2013) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated from Animals"; Second Informational supplement (6), and document M45-A2 (2010) "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria"; Approved Guideline – Second Edition (7). CLSI guidelines were used for interpretation of *Salmonella* and *Shigella* AST results, except i) when testing *Shigella* towards ciprofloxacin; for these results, participants were instructed to apply the same interpretative criteria as for *Salmonella*; ii) when performing *Campylobacter* AST, for which EUCAST (European Committee on Antimicrobial Susceptibility Testing; www.eucast.org) epidemiological cut-off values were used. For cefotaxime, ceftazidime and ceftriaxone values listed in CLSI M100-S23, Table 2A Supplemental Table 1 were utilized. All breakpoints are listed in the protocol (App. 3).

2.4 Distribution

Bacterial cultures were enclosed in double pack containers (class UN 6.2) and sent to participating laboratories according to the International Air Transport Association (IATA) regulations as "Biological Substance category B" classified UN3373. Prior to shipping, laboratories were informed about the dispatch date. Import permits were necessary for shipping the parcels to a 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, Srirat Pornruangwong from the National Institute of Health, Thailand, Francois Xavier Weill from IP; France, Elena Campos from Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA); Costa Rica, Ida Luzzi from Istituto Superiore di Sanità, Italy, Rama Murthy from National Institute of Cholera and Enteric Diseases, India and Kan Biao from Institute for Communicable Disease Prevention and Control, Beijing shipped to all Canadian, American, Thai, Francophone African, South American/Caribbean, Italian, Indian and Chinese institutes, respectively. Most parcels were dispatched in the first week of September 2013.

2.5 Procedure

Participants were instructed to download the protocol (App. 3) and additional documents; "Subculture and Maintenance of quality control (QC) strains" and "Instructions for opening and reviving lyophilized cultures" (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 components included serotyping and AST of eight *Salmonella* and four *Shigella* strains, identification and MIC determination of two *Campylobacter* strains, AST of two QC strains (*E. coli* CCM3954 / ATCC25922, *C. jejuni* CCM 6214 / ATCC33560), and identification of an unknown foodborne pathogen (verotoxin producing negative *Escherichia coli* O157:H16). 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 submit i) the obtained results (serogroup and / or serotype, MIC values or zone-diameter in millimeters, and antimicrobial susceptibility categories of the *Salmonella* and *Shigella* strains; ii) identification, MIC values, and antimicrobial susceptibility categories of the *Campylobacter* strains; iii) identification of the unknown strain). The results were to be submitted to 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 5th, 2013 and closed on February 18th, 2014.

The *Salmonella* and *Shigella* strains were categorized as resistant (R), intermediate (I) or susceptible (S) to all tested antimicrobials, whereas the *Campylobacter* strains were categorized as resistant (R) 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 as described above.

Of note, the authors would like to state that 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' (16). 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 according to the values listed in the protocol (App. 3). Participants were instructed to use the *Salmonella / Shigella* antisera and the antimicrobials used in the methods routinely performed. In addition, they were instructed to submit the breakpoints routinely applied in their laboratory for categorizing AST results, if different from those listed in the protocol. All laboratories were requested to enter MIC values for the *C. jejuni* (ATCC 33560) reference strain, and either zone

diameters or MIC values for the *E. coli* (ATCC 25922) reference strain. After submitting the results, participants were instructed to retrieve an instantly generated report from the secure web site. This report was created on an individual basis, and reported all deviations from the expected results and suggestions for solving or investigating the cause of error. Deviations of antimicrobial susceptibility test results from the expected results were categorized as minor, major or very major. Minor deviations are defined as classification of an intermediate strain as susceptible, resistant or vice versa (*i.e.* I ↔ S or I ↔R). Major deviation is the classification of a susceptible strain as resistant (*i.e.* S → R). Very major deviation is the classification of a resistant strain as susceptible (*i.e.* R → S). In this report, the deviations of AST results are divided into two categories, *i.e.* critical deviations which include major and very major deviations, and total deviations which include also the minor deviations.

3. Results

A total of 189 laboratories responded to the pre-notification and were enrolled in the EQAS. When the deadline for submitting results was reached, 174 laboratories in 87 countries had uploaded data. The following countries provided data for at least one of the EQAS components (Figure 1): Albania, Argentina, Australia (3), Bahrain, Barbados, Belarus, Belgium, Belize, Bolivia, Brazil (2), Brunei Darussalam, Bulgaria, Cambodia, Cameroon, Canada (11), Central African Republic, Chile, China (9), Colombia (3), Congo, Democratic Rep. of, Costa Rica (2), Croatia, Cyprus, Czech Republic (2), Denmark (2), Ecuador (2), Egypt (2), El Salvador, Ethiopia, France, Germany (2), Greece (3), Guatemala (2), Honduras, Hungary, India (10), Iran Islamic Republic of (3), Ireland, Israel, Italy (13), Jamaica, Japan (2), Jordan, Kenya (3), Korea Rep. of (2), Kosova, Lao P.´s Dem. Rep., Lithuania, Luxembourg, Madagascar, Malaysia (5), Malta, Mauritius, Mexico (2), Morocco, New Zealand, Nicaragua, Nigeria (2), Norway, Oman, Panama (2), Paraguay (2), Peru (2), Philippines, Poland (4), Senegal, Serbia, Singapore, Slovakia, Slovenia (2), South Africa, Spain, Sri Lanka, Sudan, Suriname, Sweden, Taiwan, Thailand (11), Tunisia, Turkey, Ukraine, United Kingdom, United States of America (4), Uruguay, Venezuela, Viet Nam (3), Zambia.

The participation in the EQAS 2013 decreased compared to the 2012 by 18 institutes and seven countries which can be partly explained by the lack of participation of Russian laboratories.

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

3.1 Methods used by EQAS participants

A total of 182 laboratories received *Salmonella* strains, and 163 (90%) participated in the *Salmonella* serogrouping component of the EQAS, whereas 144 (79%) participated in the serotype module of the EQAS. In addition, 145 (80%) laboratories submitted AST results. Among the laboratories performing AST, 122 (84%) submitted results for the quality control (QC) strain *E. coli* ATCC 25922. The majority (89; 73%) of these laboratories used the disk diffusion method, while a MIC determination method was utilized by a smaller number (33; 27%) of laboratories.

Of 139 laboratories receiving *Shigella* strains, 128 (92%) submitted *Shigella* serogroup results (speciation) and 80 (58%) of these laboratories serogrouping the isolates further analyzed the strains to the serotype level. In addition, *Shigella* AST was performed by 120 (86%) of these laboratories.

All participating laboratories were through the protocol given information regarding the breakpoints used for interpretation when generating the expected interpretation. Expected values were given as

MIC-values only. In addition, all participating laboratories were instructed on interpretation of resistance to third generation cephalosporins and to fluoroquinolones.

Of the 123 laboratories receiving *Campylobacter* strains, all (123; 100%) reported identification results and 47 (38%) submitted AST results for both *Campylobacter* strains.

Of the 144 laboratories receiving the unknown culture for identification, 134 (93%) submitted results.

3.2 Serogrouping and serotyping of *Salmonella* strains

In 2013, the percentage of laboratories reporting complete serotype results for all eight strains decreased to 59% (n=74) which is the second lowest percentage ever reported in the history of this EQAS and a decline of 22% compared to 2012. This is most likely a result of the low number of participants submitting results for all eight isolates increased by 48 participants in 2013 compared to the previous year. However, the proportion of correctly serotyped strains increased from 83% (n=936) in 2012 to 89% (n=812) in 2013 (Table 1).

In Table 2, the number of participating laboratories is reported according to the number of correctly serotyped samples. In 2013, 52 (41%) of the 126 participating laboratories serotyped all eight strains correctly, and 29 (23%) laboratories correctly serotyped seven of the eight strains. In summary, in 2013, a total of 81 (64%) participating laboratories met the threshold for adequate performance of *Salmonella* serotyping, which represents a considerable decrease compared to 2011 where 99 (81%) of the participating laboratories met the performance quality threshold. In addition, 82% of the participating laboratories correctly identified half of the strains, which represents a 1% decrease compared to 2012 (83%). The result of 2011 was poor but this year the result is even poorer compared to previous years. In 2013, all participants had at least one isolate (two laboratories) correctly serotyped.

In Table 3, the number of tested strains reported on a region-based categorization of participating laboratories decreased in all regions except for the Oceanian region were the number of tested strains were unchanged and an increase was observed in the Central Asia & Middle Eastern region compared to 2012. The performance of *Salmonella* serotyping increased in 2013 from the shockingly low performance in regions of developing countries in 2012 except for the Central Asia & Middle Eastern and Russian regions where it further declined to 52.6% and 75.0%, respectively.

In 2013, the overall performance of laboratories performing *Salmonella* serogrouping was poor four isolates seems to cause problems even in serogrouping where WHO S-13.4 (Hvittingfoss; 16:b:e,n,x), WHO S-13.6 (Keurmassar; 35:c:1,2), WHO S-13.7 (Lexington; 3,10:z10:1,5) and WHO S-13.5 (Rubislaw; 11:r:e,n,x) resulted in the following percentage deviations; 22.2%, 15.5%, 8.8%, and 7.4%, respectively (Table 4).

Of 135 laboratories performing serotyping of the internal quality control strain (WHO S13.3, used in EQAS 2000, 2001, 2004, 2006 - 2012), 130 (96%) reported a correct result, thus leading to the same deviation rate of 6% as in 2012 (Table 4, Table 5).

Deviations in *Salmonella* serotyping ranged from 4.4% (WHO S-13.3 internal quality control strain; *S.* Enteritidis) to 30.3% (Hvittingfoss; 16:b:e,n,x) (Table 4). In 2013, all but the internal quality control strain exhibited deviation levels below the magic number of 10% deviations (Table 4).

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

A total of 11,109 antimicrobial susceptibility tests were performed in 2013 by 145 participating laboratories (Table 8). Of the submitted results, 95% were in agreement with the expected result, which is a slight increase compared to 2012 – and the best result ever reported (Table 6). Minor,

major and very major deviations were observed in 3%, 2% and 0% of the submitted results, respectively (Table 6).

Some difficulties in assessing antimicrobial susceptibility were encountered for the tested combinations of strains and antimicrobials. The difficulties were mainly in assessing susceptibility to the usual antimicrobial suspects; CIP and to some degree TET (Table 7).

Major deviations categorized by tested antimicrobial are reported in Table 8. Notably, a large number of total deviations were observed for CIP (18%) most likely due to the often observed double zone when performing disk diffusion (Table 8).

In 2013, the number of laboratories participating in the AST component of EQAS increased in all regions with exception of the Central Asia & Middle Eastern, Oceanic, Southeast Asian, and Latin American regions where participation was unchanged in 2013. In Africa, the number of participants increased by two compared to 2012 (Table 9). Overall, the performance of AST did not differ as much as in previous years ranging from 90.2% correct tests in among three Caribbean countries to 98.4% in seven North American countries (Table 9).

Antimicrobial susceptibility to *E. coli* ATCC 25922 was tested by 122 laboratories with 33 using the MIC determination method and by 89 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 4% (CAZ and CHL) to 18% (FIS (SMX)) whereas for disk diffusion the values outside the intervals accepted for the QC strain ranged from 4% (GEN) to 13% (AMP) (Table 10). These results indicate that there is no consistency with what cause problems in one year compared to the following years and between methods – some times, on the contrary (Table 10).

3.4 Serogrouping and serotyping of *Shigella* strains

As in previous years, the performance of *Shigella* speciation was excellent in 2013, as the percentages of deviations were very low for all the four test strains, ranging from 0.0% (WHO SH-13.2, WHO SH-13.3) to 0.9% (WHO SH-13.1 and WHO SH-13.4) (Table 11). The deviations observed among laboratories performing full serotyping were excellent compared to 2012 ranging from 3.1% (WHO SH-13.1) to 4.7% (WHO SH-13.4). The strain; WHO SH-13.3 originally expected to be; *Shigella flexneri* serotype 4a resulting in most deviations. However, during analysis of the data the organizers were informed that the expected serotype; 4a was incorrect as the strain was designated a novel serotype; Yv. Thus, all serotyping results of this isolate has been omitted the analysis. The serotype has been thoroughly described by Qiangzheng Sun et al (18).

In Table 12, the performance of *Shigella* serotyping is reported according to geographical distribution of participating laboratories. The number of participating laboratory increased in almost regions compared to 2012 with exception of the Central Asia & Middle Eastern, Oceanic regions where the number of participating laboratories increased with a few and in North America and Africa the number was consistent to the previous year. Unfortunately, there was no participation from laboratories from Caribbean this year. The accuracy of *Shigella* serotyping increased in most regions this year except for the African regions where the performance decreased to 62.5%, respectively.

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

A total of 3,723 antimicrobial susceptibility tests were performed in 2013 by 99 participating laboratories. Agreement with the expected result was achieved in 91% of the reported results, which

is consistent with previous years (Table 13). Minor, major and very major deviations were observed in 6%, 2% and 2% of reported results, respectively (Table 13).

Difficulties in assessing antimicrobial susceptibility to CHL, CIP, and SXT was encountered in isolate WHO SH-13.3 (Table 14). Overall, the percentages of the total deviations were this year excellent ranging from 0.3% (CAZ) to 2.0 (CIP) (Table 15).

In 2013, the majority of participating laboratories was again centered in the European, Latin American, Southeast Asian and African regions (Table 16). By considering participating laboratories in relation to their geographical location, the percentage of correct AST results ranged from 86.2% (Southeast Asia) to 95.5% (Oceanic). The African, Central Asia & Middle Eastern, and Caribbean regions reported results presenting the highest percentages of critical and total deviations (Table 16).

3.6 ESBL-producing *Salmonella* and *Shigella*

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

One of the *Salmonella* test strains (WHO S-13.6) was ESBL-producing. The WHO S-13.6 (*Salmonella* Keurmassar) harboured the *bla*$_{SHV12}$ gene. Uploaded results regarding ESBL-producing strain is listed in Table 17 showing a low deviation level of 1.8%

3.7 Identification of *Campylobacter* strains

Participation in the EQAS 2013 *Campylobacter* component was requested by 123 laboratories, of which all (123; 100%) submitted results within the deadline. Of the participating laboratories, 82% and 84% performed correct species identification for strain #1 (*C. coli*) and #2 (*C. coli*), respectively (Table 18). As expected, a considerable large number of laboratories reported the stains being *C. jejuni.*

In Table 19, the performance of *Campylobacter* identification is reported according to geographical location of participating laboratories. Unfortunately the number of participants from Central Asia & Middle East declined to the same level as in 2011 of one participating country whereas the rest of the regions maintain the same level of participation as in 2012 in the identification of *Campylobacter* strains. The accuracy in *Campylobacter* identification ranged from 41% (Africa) to 100% (Caribbean, North American, Oceanic, Russian regions).

3.8 MIC determination of *Campylobacter* strains

A total of 546 MIC determinations were performed in 2013 by 47 participating laboratories (Table 22). Among the reported results 92.4% were in agreement with the expected result (Table 20). Major and very major deviations were observed in 5.0% and 2.6% of reported results (Table 20).

None of the isolates seemed to created major difficulties in assessing antimicrobial susceptibility (Table 21). For the overall performance by antimicrobial, only STR seems to result in noteworthy deviations; 23.5% (Table 22).

In 2013, MIC values were submitted by almost all laboratories with exception of Oceania and Russia (Table 23). An increase in participation was observed in many of the regions going up with one or two laboratories. Agreement with expected values was observed in percentages ranging from 90.9% (Africa) to 100% (Caribbean and North America) (Table 23). The highest percentages of

critical deviations were reported from laboratories in African and Southeast Asian regions 9.1% and 15.2%, respectively (Table 23).

MIC values of reference strain *C. jejuni* ATCC 33560 were tested by 47 laboratories. Overall, the percentage of laboratories which submitted values within the acceptable interval for the reference strain seemed to experience most problems with ERY and GEN, which showed 83% and 82% results within range, respectively (Table 24).

3.9 Identification of the unknown culture

Identification of the unknown enteric pathogen (verotoxin producing negative *Escherichia coli* O157:H16) was performed by 129 laboratories (Table 25). Overall, 82% of the participating laboratories identified the strain as verotoxin producing negative *Escherichia coli* O157:H16. Only laboratories that determined the strain being non-verotoxin producing *Escherichia coli* were considered correct.

4. Discussion

4.1 Serogrouping and serotyping of *Salmonella* strains

After having conducted the GFN EQAS trials for more than ten years, we have actually covered the more common and frequent reported serovars. This makes it more challenging to find and include appropriate and interesting serovars in the trial panel to facilitate the global assessment of *Salmonella* serotyping capacity. This year, we included *S.* Berta which is associated with poultry. *S.* Berta has never been one of the top serovars in this reservoir but frequently been reported from both the United States (US) and the European union (EU) e.g. ranked 10 among broilers in 2011 from the US (4). In addition, *S.* Berta was reported responsible for a *Salmonella* outbreak in Italy in 2012 due to poor hygiene. The incriminated food source was believed to be dairy products and egg (10). *S.* Kiambu is another poultry related serovar but more infrequent observed. However, the serovar have been reported from different provinces in Canada and listed among top 10 in some provinces in 2008 from chicken isolates from retail meat (3). In Thailand and most likely the entire Southeast Asian region there have been several reports about *S.* Hvittingfoss being responsible for human salmonellosis (5,15,17). Unfortunately, data are scares why the true reservoirs of this serovar haven't been identified. We also included the rare serovar; *S.* Rubislaw reported in pigs from Nigeria (12)which might be a contamination as the serovar has been reported from also amphibians, insects and reptiles (19). In the early 2000s, *S.* Keurmassar emerged in Senegal infected people. It was believed to be associated to a clonal expansion of poultry origin (13). We included in this year's EQAS also this unusual serovar. *S.* Lexington has infrequently been observed in feed why it was selected for this year's EQAS panel. The occurrence of resistance to ciprofloxacin in *S.* Kentucky is emerging in certain animal species and sources; turkeys, broilers, and meat hereof (2) and has been suggested to indicate a clonal expansion of the clone; *S.* Kentucky ST198-X1 why it was included the EQAS panel to allow participants to serotype and susceptibility test this important serovar. (Westrell et al 2014, Le Hello et al 2013, Le Hello et al 2011)

Overall, the panel of 2013 was greatly influenced by rare or infrequently observed serovars associated with poultry but it also as in previous years include *S.* Enteritidis as it serves as internal control but also as it is one of the most frequent serovars worldwide despite a decreasing trend.

The number of laboratories which serotyped all eight *Salmonella* strains decreased in 2013 to unseen level recorded only in 2003 whereas the level of correctly serotyped *Salmonella* strains was maintained compared to recent years. Thus, it is still a satisfactory achievement to have 87% of the participants correctly serotype the *Salmonella* isolates included the 2012 panel. This result might

have been expected due to a general reduction of participation in this year's EQAS including the high performance regions.

The isolates included in this year's EQAS was believed to be as challenging as in recent years where have two, two and three isolates contained G, E, and 1 complexes, respectively which often is a challenge due to the many different antisera needed to pin out the correct antigens. Furthermore, three isolates were of a less common somatic antigen e.g.O:11, O:16, and O35. Similarly, two isolates contained the z_6 and the z_{10} H-antigen; all contributing to an advanced level of difficulty.

Almost 96% of participating laboratories correctly serotyped the internal control strain this year, which was the same as in 2012 but still represent a minor decline in proficiency compared to previous years. This might also be related to the participation of more developing countries which most likely also profit from this participation in highlighting areas for improvement. The quality threshold of correctly serotyping at least seven strains was met by only 64% of participating laboratories, thus demonstrating once again the advanced level of required serotying capacity needed for this year's EQAS.

In general, the obtained results indicate that most laboratories in the developing regions have less capacity to serotype the more challenging *Salmonella* serovars which potentially could be problematic if those become more frequent in the future.

In 2013, the problems in serotyping the isolates are the same as in previous years. The problem is linked to difficulties in the characterization of flagellar antigens but this year also to some of the somatic antigens. In 2013, especially the complexes and somatic antigens related to "higher" serogroups played a significant role in the number of incorrect identification of the serotypes. This most likely is a consequence of a lack of good quality antisera, financial resources, and availability. However, we believe this problem will be diminished with time due to the advancing of new sequence-based molecular techniques and the decreasing price of those methods. In the future, we foresee that multi locus sequence typing (MLST) and whole genome sequencing will replace conventional microbiological techniques such as serotyping and identification of resistance genes, plasmids, virulence genes etc. (1,11).

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

Overall, 95% of the *Salmonella* AST was correctly performed with 2% of critical deviations and only 5% of total deviation. This result is the best ever reported matching in the history of the WHO EQAS. This might be the result of the strengthened global awareness of fighting antimicrobial resistance, the need for surveillance, and the need for performing antimicrobial susceptibility testing accurately due to the emerging of multi-drug resistant (MDR) and extreme-drug resistant (XDR) bacterial pathogens worldwide.

In 2013, we followed the guidelines for MIC breakpoint interpretation as well as the expert guidelines on the interpretation of cephalosporin resistance which was distributed in 2010. Similarly, participating laboratories were asked to utilize EUCAST epidemiologic cut off values for interpretation of CIP susceptibility. The EQAS organizers utilized the lower epidemiologic cut off value 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. Of note, low-level ciprofloxacin-resistant strains (extra-intestinal non-typhoid *Salmonella* and *S.* Typhi) would be interpreted as intermediate according to the CLSI clinical breakpoints available (M100-S24). However, this will not determine plain non-typhoid *Salmonella* or extra-intestinal non-typhoid *Salmonella* and *S.* Typhi as resistant toward fluoroquinolones even by using the CLSI

guidelines of 2014 why we maintain the EUCAST guidelines for interpretation of these compounds. In 2012, CIP seemed to cause some challenges which were linked to detection of a *qnr* gene in one of the isolates and the incorrectly measurement of the double zone when performing disk diffusion. Some participants indicated the isolate harbouring the *qnr* gene incorrectly as intermediate or susceptible for NAL and resistant or susceptible for CIP. One isolate was resistant to both CIP and NAL why the interpretation of this isolate was quite easy and only in few cases mistakes were observed.

Interestingly, TET and SMX susceptibility tests seemed also in 2013 not to create that many deviations compared to previous years. In the case of SMX susceptibility test, we observed a decrease in deviating results since 2010. A pit fall as regards reading the result of this antimicrobial is caused by the fact that it is bacteriostatic, meaning that the zone diameter or the MIC should be read at 80% reduction of growth. A common mistake for this antimicrobial is therefore to register false resistance. This year, two of the test strains were resistant to SMX which might explain the decrease in deviating results.

In general, data from the *Salmonella* AST component of EQAS 2013 demonstrate an excellent performance compared to previous years. Of note, all laboratories performed better compared to 2012.

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. All 47 (100%) participating laboratories submitted AST results of the QC strain. We always encourage laboratories to conduct quality assurance when performing AST. To facilitate the 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. Compared to disk diffusion, similar or worse results were obtained in 2013 as to data outside the QC ranges. These erroneous disk diffusion 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.

4.3 Serogrouping and serotyping of *Shigella* strains

In EQAS 2013, 116 to 119 correctly identified the four *Shigella* isolates resulting in a deviation range of 0.0% to 0.9% showing a high capacity within *Shigella* diagnostics.

The performance in the serotyping the four *Shigella* isolates were lower compared to conducting correct identification. In 2013, only two of the four isolates could be serotyped and caused deviations ranging from 3.1% to 4.7%.

All regions except for African regions encountered an improved performance in serotyping but an overall general decrease in participation. Thus, indicating that the general reduction of country participation and number of tested strains in 2013 might have resulted in a better performance. This might indicate that poor resource countries didn’t participate in this year's EQAS.

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

In EQAS 2013, AST of *Shigella* spp. was performed by 99 laboratories which is a reduction in participation compared to previous years. A total of 91% of the participants obtained a correct AST results which is within the same level previous years and as for AST in *Salmonella*. In comparison with the *Salmonella* results, a few more deviations categorized as critical and total deviations were

observed. Overall, the AST results of the *Shigella* component equal to what was observed in previous years.

No specific isolate caused problems susceptibility testing the *Shigella* isolates. In general, a large proportion of deviations testing CIP were observed associated with all the isolates. None of the isolates were ESBL producers.

The high number of deviations to CIP was most likely related to the reduced susceptibility due to only one point mutation in the gyrase gene, the lack of CLSI breakpoints, and reading difficulties of the zone diameter. All regions submitted results with an overall regional performance similar to the one described for *Salmonella* AST differing with a maximum of 5%.

4.5 Identification of *Campylobacter* strains

In 2013, we selected only *Campylobacter coli* strains. Interestingly, the results from this EQAS support the hypothesis raised in 2011 that correct identification of *C. jejuni* seems to be easier than that of *C. coli* as only 82% and 84% of the participating laboratories obtained a correct identification for the two *C. coli* isolates. One of the explanations may be that when conducting a conventional hippurate hydrolysis test, that some *C. coli* are incorrectly identified based on false positive hippurate hydrolysis test results. The weakness of the conventional hippurate hydrolysis test is that sometimes the test suspensions develop a weak bluish color when testing *C. coli* that for the untrained person often will be mistaken as being positive indicating *C. jejuni.* In contrast, testing *C. jejuni* will provide a strong blue coloration of the suspensions which is easy to interpret. In 2013, the level of participation decreased a bit compared to 2012 but is still better than previous years. Overall, the results related to *Campylobacter* identification were quite satisfactory in all regions in 2013.

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

In EQAS 2013, 47 laboratories participated in the MIC determination and performed overall satisfactorily, since they obtained 92.4% correct test results. In contrast to 2012, only minor problems testing the antimicrobials were observed with most deviations observed to STR. In 2013, no laboratories from Russia and Oceanic region participated.

In 2013, 47 (100%) participating laboratories submitted AST results for the QC strain. The majority of deviations were observed for susceptibility testing by micro-dilution at 42°C. Interestingly, we noticed the same deviations in previous years. Some problems were observed towards testing CIP. In general, AST of the QC strain was satisfactory.

4.8 Identification of the unknown culture

In EQAS 2013, we included a verotoxin producing negative *Escherichia coli* O157:H16strain to see how many of the participating laboratories that would be able to correctly test whether this organism was verotoxin producing; a very important feature in *Escherichia coli* O157. This is the reason why only the following test results are acceptable; *Escherichia coli* non-VTEC, *Escherichia coli* O157 non-VTEC, *Escherichia coli* O157:H16 non-VTEC, *E. coli* O157, *E. coli* O157 non-VTEC, and *E. coli* O157:H16 non-VTEC.

Of 129 laboratories delivering results, 82% identified the strain correctly. This indicates that most of the laboratories in fact are able to detect the verotoxin of *E.* coli.

5. Conclusions

The acceptance threshold for the *Salmonella* serotyping EQAS component was met by 64% (n=81) of the participating laboratories. In addition, 57% of the laboratories tested all eight strains and a total of 87% of all tests were correct, thus representing an increase compared to 2012.The ability in correctly testing the internal QC strain was consistent with 96% obtained in 2012

The obtained results indicate that laboratories in the developing part of the world have lower capacity to serotype the rarer and more infrequent *Salmonella* serovars requiring more advanced sets of antisera.

The main problem as regards serotyping appears to have been linked to difficulties in the characterization of both the somatic and flagellar antigens. In 2013, this especially concerns the complexes G, E, and 1and somatic antigens of higher serogroups which is most likely a consequence of a lack of good quality antisera, financial resources, and availability. In the future, however, it is likely that sequence-based molecular techniques will be competitive with traditional typing methods.

Concerning the *Salmonella* AST component, the EQAS 2013 results as regards AST of *Salmonella* showed the best result ever recorded in the history of the WHO EQAS. Overall, the acceptance threshold was met, and we identified 3% minor, 2% major and 0% very major deviations. CIP was the only antimicrobial that caused the difficulties of the observed deviations. Compared to 2012, the performance of AST did not differ as much between the different regions.

Strengthened awareness of the importance of performing internal quality control is crucial and is introduced in many of the participating laboratories. Eleven (8%) participating laboratories did not report data for AST of the QC strain, though, despite the EQAS organizers' repeated recommendation of the use of such QC strains and the provision of certified strains to new participants. It is important to emphasize that this component represents the true indicator of the quality of AST performance.

For the *Shigella* component in EQAS 2013, consisting of serogrouping, serotyping and AST, most laboratories correctly serogrouped the four *Shigella* strains, and a maximum of 0.9% deviations was observed. A total of 72 laboratories performed serotyping. The number of participating laboratory decreased in almost all regions compared to 2012 The results obtained in the *Shigella* AST were in 91% of the cases in agreement with the expected result which is consistent with previous years.

A total of 123 laboratories received *Campylobacter* for identification, and all of these laboratories uploaded data. Both strains were *C. coli* and resulted in 82% and 84% correct species identification, respectively. The accuracy in *Campylobacter* identification ranged from 41% (Africa) to 100% (Caribbean, North American, Oceanic, and Russian regions). In 2013, the performance increased to levels similar to other years.

EQAS 2013, a total of 47 laboratories participated in MIC determination of *Campylobacter*. The acceptance threshold used for *Salmonella* was applied and was almost met, since we observed 7.6% critical deviations. For the overall performance by antimicrobial, only STR seems to result in noteworthy deviations; 23.5%. Overall, the percentage of laboratories which submitted values within the acceptable interval for the reference strain seemed to experience most problems with GEN and ERY, which showed 82% and 83% results within range, respectively.

The unknown strain; verotoxin producing negative *Escherichia coli* O157:H16, was selected to see the participants could detect the verotoxin. Of 129 laboratories delivering results, 82% identified the strain correctly.

Reference List

1. **Achtman, M., J. Wain, F. X. Weill, S. Nair, Z. Zhou, V. Sangal, M. G. Krauland, J. L. Hale, H. Harbottle, A. Uesbeck, G. Dougan, L. H. Harrison, and S. Brisse**. 2012. Multilocus sequence typing as a replacement for serotyping in Salmonella enterica. PLoS.Pathog. **8**:e1002776.

2. **Anonymous**. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2010. 3 10. 2012. EFSA Journal.

3. **Anonymous**. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2008. Guelph, ON: Public Health Agency of Canada, 2011. 2014. Government of Canada.

4. **Anonymous.** Serotypes Profile of *Salmonella* Isolates from Meat and Poultry Products January 1998 through December 2012 **.** 2014. United States Department of Agriculture.

5. **Bangtrakulnonth, A., S. Pornreongwong, C. Pulsrikarn, P. Sawanpanyalert, R. S. Hendriksen, D. M. Lo Fo Wong, and F. M. Aarestrup**. 2004. Salmonella serovars from humans and other sources in Thailand, 1993-2002. Emerg.Infect.Dis. **10**:131-136.

6. **Clinical and Laboratory Standards Institute**. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated from AnimalsVET01-S2 (2013); Second Informational supplement. 2014.

7. **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.

8. **Clinical and Laboratory Standards Institute**. Performance Standards for Antimicrobial Susceptibility Testing. **M100-S24**. 24th Informational Supplement. 2014. Wayne, PA, USA.

9. 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.
   Ref Type: Generic

10. **Di, G. E., L. Sacchini, T. Persiani, A. Alessiani, F. Marotta, and K. Zilli**. 2012. First outbreak of food poisoning caused by Salmonella enterica subspecies enterica serovar Berta in Italy. Lett.Appl.Microbiol. **55**:122-127.

11. **Didelot, X., R. Bowden, D. J. Wilson, T. E. Peto, and D. W. Crook**. 2012. Transforming clinical microbiology with bacterial genome sequencing. Nat.Rev.Genet. **13**:601-612.

12. **Fashae, K. and R. S. Hendriksen**. 2014. Diversity and antimicrobial susceptibility of Salmonella enterica serovars isolated from pig farms in Ibadan, Nigeria. Folia Microbiol.(Praha). **59**:69-77.

13. **Gassama-Sow, A., A. A. Wane, N. A. Canu, S. Uzzau, A. A. Kane, and S. Rubino**. 2006. Characterization of virulence factors in the newly described Salmonella enterica serotype Keurmassar emerging in Senegal (sub-Saharan Africa). Epidemiol.Infect. **134**:741-743.

14. **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.

15. **Hendriksen, R. S., A. Bangtrakulnonth, C. Pulsrikarn, S. Pornruangwong, G. Noppornphan, H. D. Emborg, and F. M. Aarestrup**. 2009. Risk factors and epidemiology of the ten most common Salmonella serovars from patients in Thailand: 2002-2007. Foodborne.Pathog.Dis. **6**:1009-1019.

16. **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.

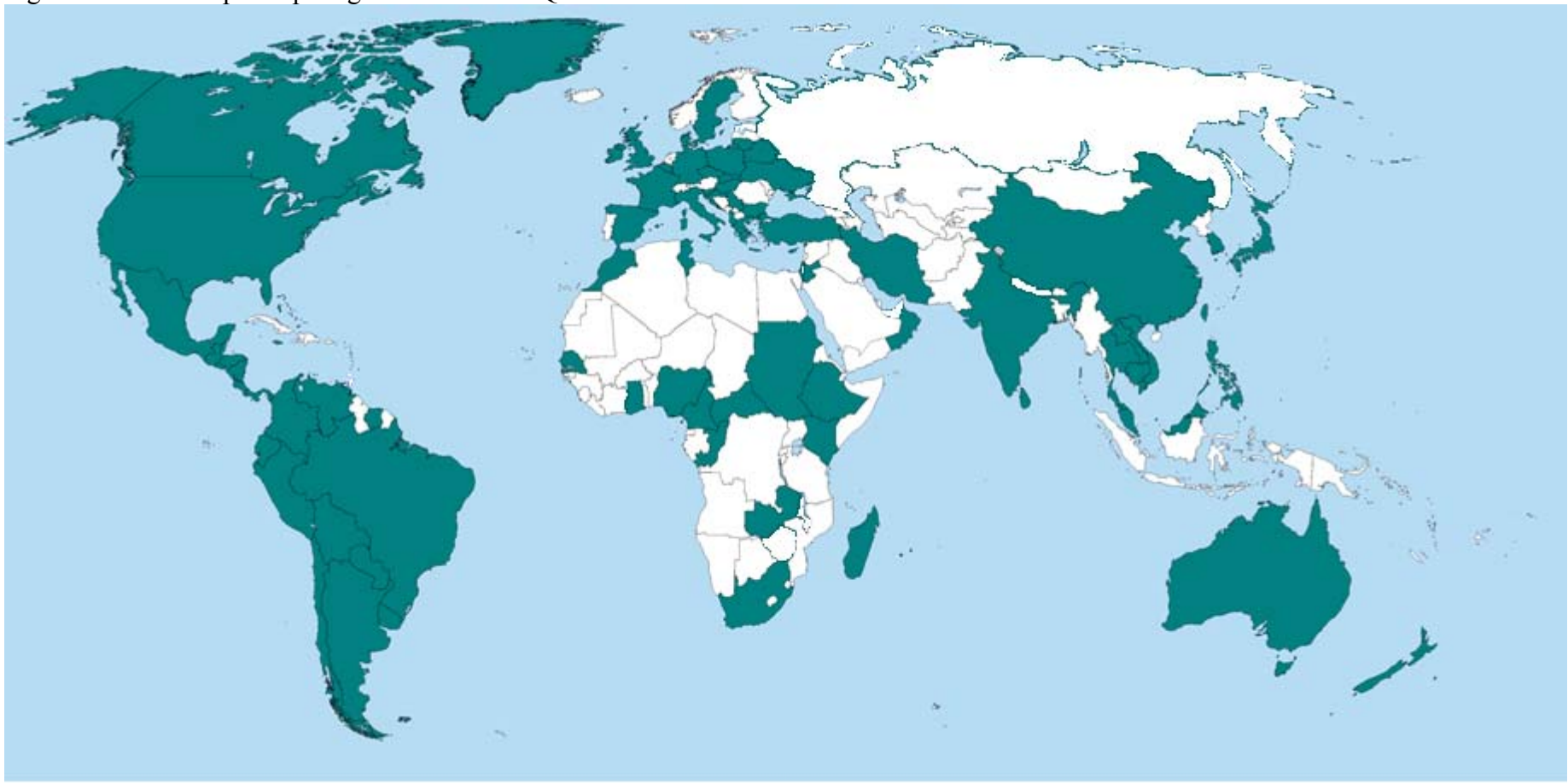
17. **Sirichote, P., A. Bangtrakulnonth, K. Tianmanee, A. Unahalekhaka, A. Oulai, P. Chittaphithakchai, W. Kheowrod, and R. S. Hendriksen**. 2010. Serotypes and antimicrobial resistance of Salmonella enterica ssp in central Thailand, 2001-2006. Southeast Asian J.Trop.Med.Public Health. **41**:1405-1415.

18. **Sun, Q., R. Lan, J. Wang, S. Xia, Y. Wang, Y. Wang, D. Jin, B. Yu, Y. A. Knirel, and J. Xu**. 2013. Identification and characterization of a novel Shigella flexneri serotype Yv in China. PLoS.One. **8**:e70238.

19. **Sylvester, W. R., V. Amadi, R. Pinckney, C. N. Macpherson, J. S. McKibben, R. Bruhl-Day, R. Johnson, and H. Hariharan**. 2014. Prevalence, serovars and antimicrobial susceptibility of Salmonella spp. from wild and domestic green iguanas (Iguana iguana) in Grenada, West Indies. Zoonoses.Public Health. **61**:436-441.

Figure and Tables

Figure 1. Countries participating* in the WHO EQAS 2013



*marked in green

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

EQAS iteration	Labs serotyping all provided strains		Correct test results	
	No.	%	No.	%
2000	34	92	165	76
2001	79	82	513	72
2002	80	81	668	91
2003	69	54	692	80
2004	78	61	701	81
2006	105	81	808	85
2007	109	78	920	88
2008	100	66	888	83
2009	119	83	974	86
2010	129	87	998	89
2011	109	89	878	92
2012	122	81	936	83
2013	74	59	812	89
Average	93	76	766	84

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

Number of strains correctly serotyped	Participating laboratories													
	EQAS 2000		EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004		EQAS 2006		EQAS 2007	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
8	9	24	34	35	52	53	66	47	41	32	42	32	66	47
7	9	24	13	14	19	19	29	21	14	11	35	27	29	21
6	4	11	9	9	12	12	13	9	16	13	19	15	13	9
5	3	8	9	9	4	4	11	8	16	13	12	9	11	8
4	3	8	4	4	1	1	7	5	11	9	7	5	7	5
3	4	11	8	8	4	4	6	4	10	8	5	4	6	4
2	2	5	3	3	5	5	2	1	10	8	3	2	2	1
1	2	5	5	5	1	1	6	4	5	4	4	3	6	4
0	1	3	11	11	1	1	0	0	4	3	3	2	0	0
In total	37	100	96	100	99	100	127	100	127	100	130	100	140	100
Number of strains correctly serotyped	**Participating laboratories**													
	EQAS 2008		EQAS 2009		EQAS 2010		EQAS 2011		EQAS 2012		EQAS 2013		AVERAGE EQAS 2000 - 2013	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
8	50	33	76	50	91	61	82	67	68	47	52	41	695	43
7	36	24	29	19	16	11	17	14	29	20	29	23	290	18
6	11	7	7	5	12	8	10	8	14	10	15	12	160	10
5	14	9	13	8	9	6	2	2	9	6	8	6	133	8
4	12	8	5	3	6	5	4	3	5	3	7	6	86	5
3	9	6	7	5	2	1	4	3	6	4	7	6	85	5
2	8	6	5	3	2	1	1	1	10	7	6	5	61	4
1	9	6	6	4	7	5	3	2	2	1	2	2	57	4
0	2	1	5	3	3	2	0	0	1	1	0	0	34	2
In total	151	100	153	100	148	100	123	100	144	100	126	100	1601	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 2013
Africa	2001	6	37	73.0	Cameroon, Central African Republic, Congo, 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	
	2010	13	83	67.5	
	2011	10	57	79.2	
	2012	10	65	60.0	
	2013	8	51	74.5	
Central Asia & Middle East	2001	10	60	50.0	Egypt (2), Israel, Jordan, Oman,
	2002	5	30	83.3	
	2003	5	35	54.3	
	2004	5	33	54.5	
	2006	5	35	74.3	
	2007	5	40	55.0	
	2008	5	34	61.8	
	2009	5	32	46.9	
	2010	5	22	75.9	
	2011	3	23	95.8	
	2012	4	30	56.7	
	2013	5	38	52.6	
Caribbean	2001	0	0	0	Barbados
	2002	0	0	0	
	2003	3	18	61.1	
	2004	2	8	87.5	
	2006	3	14	78.6	
	2007	2	9	77.8	
	2008	3	14	78.6	
	2009	3	12	83.3	
	2010	2	13	92.9	
	2011	1	7	87.5	
	2012	2	16	62.5	
	2013	1	5	100,0	

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 2013
Europe	2001	43	323	80.5	Albania, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark (2), France, Germany (2), Greece (3), Hungary, Ireland, Italy (12), Lithuania, Luxembourg, Malta, Poland (3), Serbia, Slovakia, Slovenia (2), Spain, Sweden, 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	
	2010	45	332	94.1	
	2011	42	314	94.6	
	2012	47	368	92.9	
	2013	42	309	94.5	
North America	2001	4	32	87.5	Canada (9), United States of America (4)
	2002	2	16	100.0	
	2003	6	41	95.1	
	2004	8	55	81.8	
	2006	10	80	96.3	
	2007	12	94	97.9	
	2008	11	84	95.2	
	2009	12	90	92.2	
	2010	13	103	100.0	
	2011	11	81	97.6	
	2012	14	101	93.1	
	2013	13	92	97.8	
Oceania	2001	4	30	100.0	Australia (3), New Zealand
	2002	6	43	93.0	
	2003	6	46	93.5	
	2004	5	38	97.4	
	2006	5	37	94.6	
	2007	4	32	100.0	
	2008	4	30	93.3	
	2009	4	32	96.9	
	2010	4	32	100.0	
	2011	4	32	100.0	
	2012	4	32	100.0	
	2013	4	31	100.0	
Russia	2001	1	8	12.5	Belarus, Georgia
	2002	1	8	62.5	
	2003	1	7	14.3	
	2004	4	26	69.2	
	2006	5	40	80.0	
	2007	8	51	80.4	
	2008	6	40	90.0	
	2009	7	49	91.8	
	2010	8	54	87.1	
	2011	7	48	87.3	
	2012	6	48	87.5	
	2013	2	16	75.0	

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 2013
Latin America	2001	11	78	57.7	Argentina, Bolivia, Brazil (2), Chile, Colombia (3), Costa Rica (2), Ecuador, Honduras, Mexico (2), Nicaragua, Panama (2), Paraguay, Peru (2), Uruguay, 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	
	2010	22	132	80.0	
	2011	23	144	83.7	
	2012	25	182	73.1	
	2013	22	154	83.1	
Southeast Asia	2001	15	113	54.0	Brunei Darussalam, Cambodia, Japan (2), Korea Rep. of (2), Lao P.´s Dem. Rep., Malaysia (4), Philippines, Singapore, Sri Lanka, Taiwan, Thailand (6), Viet Nam.
	2002	12	90	92.2	
	2003	15	100	81.0	
	2004	17	130	81.5	
	2006	15	117	84.6	
	2007	19	140	91.4	
	2008	18	125	81.6	
	2009	23	180	81.1	
	2010	24	172	90.5	
	2011	23	180	98.4	
	2012	28	207	77.8	
	2013	22	163	89.6	
China	2001	4	32	96.9	China (7)
	2002	3	24	100.0	
	2003	8	60	75.0	
	2004	7	46	78.3	
	2006	6	48	85.4	
	2007	10	80	91.3	
	2008	15	108	94.4	
	2009	16	126	95.2	
	2010	10	74	92.5	
	2012	10	78	80.8	
	2013	7	54	92.6	

Table 4. *Salmonella* serogroups (SG), serotypes (ST) and deviations (D), WHO EQAS 2013

Strain ID	Correct serotype		No. of labs reporting SG	% D_{SG}	No. of labs reporting ST	% D_{ST}	Deviating results (*)
WHO S-13.1	Berta	9,12:f,g,t:-	150	4,7	133	10,5	Wernigerode (3), Enteritidis (2), II .1.,9,12:g,m,[s],t:[1,5,7]:[z42] (2), Dublin, II 9,12:g,s,t:e,n,x, II 9,46:g,m,s,t:e,n,x, Manica, Pensacola, Regent, Typhi
WHO S-13.2	Kiambu	4,12:z:1,5	155	1,9	129	10,9	Shubra (2), Berta, Bury, Heidelberg, Indiana, Kingston, Koenigstuhl, Neftenbach, Preston, Reading, S.Paratyphi B, Shubra, Tudu
WHO S-13.3	Enteritidis	9,12:g,m:-	151	1,3	135	4,4	Blegdam, Eastbourne, Gueuletapee, Macclesfield, Typhi
WHO S-13.4	Hvittingfoss	16:b:e,n,x	126	22,2	99	30,3	Rhydyfelin (2), Sangera (2), Abony, Annedal, Battle, Bellville, Blegdam, Braenderup, Hull, IV .1.,44:z4,z32:-, Parathypi B "java" n, Rottnest, Rovaniemi, S. Paratyphi B, Shamba, Typhimurium, Urbana, VI 11:b:e,n,x, Vancouver, Kentucky, Worb
WHO S-13.5	Rubislaw	11:r:e,n,x	148	7,4	130	14,6	Clanvillian (3), Euston (3), Woumbou (2), Adamstua, Brijbhumi, Chingola, Eko, Okatie, Papuana, Redhill, Simi, Adamstua
WHO S-13.6	Keurmassar	35:c:1,2	129	15,5	107	17,8	Gouloumbo (3), Adelaide, Choleraesuis, Colindale, Enschede, Haga, Hissar, Jericho, Meleagridis, Paratyphi C, Typhimurium, Woodinville, Worthington, Anatum,
WHO S-13.7	Lexington	3,10:z10:1,5	148	8,8	124	16,1	Biafra (2), Orion (3), Assinie, Coquilhatville, Eppendorf, Gbadago, Haifa, Ituri, Kiambu, Muenster, Okerara, Oritamerin, Portland, Regent, Shangani, Ughelli, Yalding
WHO S-13.8	Kentucky	8,20:i:z6	153	3,3	132	3,0	Bargny (2), S.Paratyphi C, Warnow

*number of participants reporting the specified deviating result

Table 5. EQAS participating laboratories' performance of internal quality control strain (WHO S-13.3, *Salmonella* Enteritidis) serotyping

EQAS iteration	Labs serotyping *S.* Enteritidis correctly	
	No.	%
2000	34	92
2001	64	84
2004	113	95
2006	116	94
2007	135	96
2008	139	96
2009	141	93
2010	138	97
2011	128	98
2012	139	96
2013	130	96
Average	116	94

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

EQAS iteration	**No. of EQAS participating laboratories**	**% correct test results**	**% minor deviations** (S ↔ I or I ↔ R)^	**% major deviations** (S → R)^	**% very major deviations** (R→ S)^	**% critical deviations** (R→ S & S → R)^	**% total deviations** (S → R & R → S & S ↔ I or I ↔ R)^
2000	44	92	4	4	0	4	8
2001	108	91	6	2	1	3	9
2002	119	92	6	2	1	3	9
2003*	147	93	4	3	0	3	7
2004	152	93	4	2	1	3	7
2006	143	88	8	3	1	4	12
2007	143	93	4	2	1	3	7
2008	168	91	4	2	3	5	9
2009	153	94	3	2	1	3	6
2010	152	92	4	3	2	5	8
2011	127	91	4	2	3	5	9
2012	159	94	3	2	1	3	6
2013	145	95	3	2	0	2	5
Average*	134	92	4	2	1	4	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 2013 *Salmonella* strains*

Strain	Antimicrobial^											
	AMP	**CTX**	**CAZ**	**CRO**	**CHL**	**CIP**	**GEN**	**NAL**	**SMX**	**SXT**	**TET**	**TMP**
WHO S-13.1	**138**/1/1	4/3/**119**	3/1/**109**	1/0/**95**	1/0/**127**	30/**61**/50	**132**/1/3	**100**/14/14	4/2/**55**	2/0/**116**	2/2/**128**	2/0/**67**
WHO S-13.2	3/0/**138**	3/1/**124**	3/2/**108**	1/1/**94**	0/1/**128**	1/13/**128**	3/3/**130**	2/1/**128**	4/1/**57**	0/0/**119**	2/2/**129**	0/0/**68**
WHO S-13.3	10/5/**126**	7/5/**115**	3/0/**110**	2/0/**94**	0/5/**124**	4/14/**124**	**131**/1/4	1/2/**126**	**62**/0/0	1/0/**118**	8/9/**116**	1/0/**68**
WHO S-13.4	2/3/**136**	5/1/**121**	3/1/**109**	0/0/**95**	0/0/**128**	0/9/**133**	4/2/**131**	1/1/**126**	3/2/**56**	2/0/**117**	3/3/**127**	1/0/**67**
WHO S-13.5	2/3/**136**	4/0/**123**	3/1/**108**	1/0/**94**	1/0/**128**	0/7/**136**	4/3/**129**	0/1/**127**	4/2/**55**	3/1/**115**	3/2/**128**	2/0/**66**
WHO S-13.6	**139**/0/1	**113**/12/1	**110**/0/2	**86**/8/2	**127**/0/1	10/45/**86**	**132**/2/1	3/8/**116**	**62**/0/0	**116**/0/2	**129**/0/3	**67**/0/1
WHO S-13.7	6/1/**131**	5/2/**118**	6/0/**105**	0/0/**95**	1/2/**125**	4/10/**127**	4/2/**129**	3/5/**119**	5/0/**56**	2/2/**113**	6/3/**123**	3/0/**64**
WHO S-13.8	**139**/0/0	6/1/**118**	3/0/**108**	0/0/**94**	0/2/**126**	**136**/5/1	**109**/13/13	**128**/0/1	**61**/0/0	3/1/**113**	**131**/0/1	2/1/**65**

^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	-	3,193
		% 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	-	7,706
		% 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	-	8,499
		% 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	-	1,019	-	996	995	-			993	738	947	615	768	929	995	582	-	9,577
		% 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	1,178	-	1,159	1,162	-	-	995	1,201	-	1,130	734	947	1051	1,122	729	-	12,381
		% 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	1,092	769	1,060	1,110	305	-	956	1,078	-	1,035	649	896	996	1,054	607	225	12,782
		% 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	1,114	830	1,105	1,101	389	-	914	1,111	-	1,092	678	875	971	1,047	583	258	12,976
		% 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	-	1,331	961	1,226	1,307	-	791	1,104	1,265	-	1,168	718	867	1,155	1,249	696	-	13,858
		% 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	-	1,206	921	1,108	1,190	-	775	1,009	1,143	-	1,095	624	864	1,042	1,114	616	-	12,707
		% critical deviations*	-	3	1	1	8	-	0	1	2	-	1	7	9	3	4	1	-	3
		% total deviations^	-	6	1	2	10	-	1	2	3	-	3	9	30	4	10	1	-	6
2010	152	No. of tests	-	1,173	937	1,118	1,194	-	787	1,026	1,133	-	1,096	566	800	1,012	1,134	604	-	12,580
		% critical deviations*	-	4	2	1	3	-	4	4	5	-	1	14	19	4	5	1	-	5
		% total deviations^	-	5	3	2	3	-	8	8	6	-	2	17	55	4	9	1	-	9

Table 8 (continued). 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
2011	127	No. of tests	-	1099	829	988	1070	-	744	909	999	-	993	542	682	988	1017	493	-	11,353
		% critical deviations*	-	5	3	2	20	-	3	4	4	-	7	4	3	3	4	1	-	5
		% total deviations^	-	6	4	2	21	-	3	6	5	-	15	5	42	3	10	2	-	9
2012	159	No. of tests	-	1228	993	1159	1245	-	834	1058	1161	-	1136	584	814	1054	1163	613	-	13,042
		% critical deviations*	-	3	2	1	11	-	2	4	3	-	2	5	2	1	2	1	-	3
		% total deviations^	-	5	2	2	12	-	3	5	4	-	4	7	35	2	5	1	-	7
2013	145	No. of tests	-	1121	898	1027	1134	-	763	1011	1086	-	1027	491	-	946	1060	545	-	11,109
		% critical deviations*	-	2	3	0	2	-	1	3	3	-	2	4	-	2	3	2	-	2
		% total deviations^	-	3	3	1	18	-	2	6	6	-	6	5	-	2	5	2	-	5
Average•	134	No. of tests	944	426	892	480	53	347	782	650	486	588	465	567	769	702	480	560	242	2739
		% critical deviations*	7	3	3	2	5	3	2	3	3	3	2	5	6	3	5	1	1	3
		% total deviations^	17	5	4	4	8	21	4	7	5	10	5	7	31	4	11	2	8	8

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

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

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

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

-, not determined

Table 9. Region-based categorization of EQAS participants' performance of *Salmonella* AST

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 2013 iteration
Africa	2001	7	80.1	9.6	7.7	2.5	10.2	19.8	Cameroon, Central African Republic, Congo, Rep. of, Kenya (3), Madagascar, Mauritius, Morocco, Nigeria (2), Senegal, South Africa, Sudan, Tunisia, Zambia
	2002	10	94.3	4.1	1.0	0.6	1.6	5.7	
	2003	13	86.9	6.6	2.8	3.7	6.5	13.1	
	2004	11	85.7	7.2	5.2	1.9	7.1	14.3	
	2006	20	85.8	7.5	4.1	2.7	6.8	14.3	
	2007	16	90.7	4.4	4.0	0.9	4.9	9.3	
	2008	19	83.8	6.5	5.5	4.2	9.7	16.2	
	2009	22	90.1	4.5	3.6	1.8	5.4	9.9	
	2010	22	84.7	6.0	6.5	2.8	9.3	15.3	
	2011	17	87.0	5.0	4.7	3.3	8.0	13.0	
	2012	18	89.4	5.3	3.5	1.9	5.4	10.6	
	2013	16	92.0	3.2	4.0	0.9	4.9	8.0	
Central Asia & Middle East	2001	10	87.7	6.3	5.2	0.8	6.0	12.3	Bahrain, Egypt (2), Iran Islamic Republic of (2), Israel, Jordan, Oman
	2002	6	83.4	9.8	6.6	0.2	6.8	16.6	
	2003	8	89.9	4.5	4.0	1.6	5.6	10.1	
	2004	10	87.5	6.7	5.5	0.3	5.8	12.5	
	2006	7	79.2	10.5	9.8	0.5	10.3	20.8	
	2007	8	87.8	5.0	6.2	1.1	7.3	12.2	
	2008	12	86.1	6.5	4.0	3.4	7.4	13.9	
	2009	6	93.7	4.3	0.9	1.1	2.0	6.3	
	2010	7	95.8	2.6	0.2	1.4	1.6	4.2	
	2011	4	91.8	4.1	1.8	2.3	4.1	8.2	
	2012	8	92.8	4.4	1.6	0.7	2.3	6.6	
	2013	8	93.6	5.2	1.0	0.1	1.2	6.4	
Caribbean	2001	2	83.5	9.5	7.0	0.0	7.0	16.5	Barbados, Jamaica (2)
	2002	1	95.8	4.2	0.0	0.0	0.0	4.2	
	2003	8	91.7	6.4	1.5	0.5	2.0	8.4	
	2004	8	94.1	3.1	1.9	0.9	2.8	5.9	
	2006	5	92.1	5.4	1.6	1.0	2.6	8.0	
	2007	4	95.0	3.1	0.9	0.9	1.8	5.0	
	2008	5	90.7	5.5	0.9	2.9	3.8	9.3	
	2009	4	93.2	1.8	3.2	1.8	5.0	6.8	
	2010	4	90.9	5.4	2.7	0.7	3.4	8.8	
	2011	2	96.5	1.4	0.0	2.1	2.1	3.5	
	2012	4	91.1	1.5	6.7	0.7	7.4	8.9	
	2013	3	90.2	2.6	7.3	0.0	7.3	9.8	

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 2013 iteration
Europe	2001	47	91.3	5.7	2.7	0.3	3.0	8.7	Albania, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark (2), France, Greece (3), Hungary, Ireland, Italy (9), Kosova, Lithuania, Luxembourg, Malta, Norway, Poland (3), Serbia, Slovakia, Slovenia Spain, 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	
	2010	39	92.4	4.1	1.2	2.3	3.5	7.6	
	2011	36	92.5	4.5	1.7	1.3	3.0	7.5	
	2012	40	95.5	2.8	1.2	0.4	1.7	4.5	
	2013	37	95.7	2.5	1.4	0.3	1.7	4.2	
North America	2001	4	95.8	3.8	0.0	0.4	0.4	4.2	Canada (5), United States of America (2)
	2002	3	90.5	6.9	0.6	2.0	2.6	9.5	
	2003	7	93.4	5.2	0.0	1.4	1.4	6.6	
	2004	9	94.2	4.2	1.8	0.0	1.8	6.0	
	2006	8	94.8	2.9	1.0	1.3	2.3	5.2	
	2007	10	95.4	2.9	0.8	0.8	1.6	4.6	
	2008	14	96.4	0.6	0.4	2.6	3.0	3.6	
	2009	10	98.7	0.0	0.4	0.9	1.3	1.3	
	2010	11	94.8	2.6	0.2	2.4	2.6	5.2	
	2011	9	92.1	2.6	1.5	3.8	5.3	7.9	
	2012	10	96.0	2.1	1.0	0.9	1.9	4.0	
	2013	7	98.4	1.3	0.0	0.2	0.2	1.6	
Oceania	2001	6	91.8	4.7	2.7	0.9	3.6	8.2	Australia (3). New Zealand
	2002	7	91.7	6.2	0.0	2.0	2.0	8.3	
	2003	9	94.3	2.5	1.2	2.0	3.2	5.7	
	2004	11	97.1	2.5	0.3	0.1	0.4	2.9	
	2006	7	93.4	4.6	0.9	1.1	2.0	6.6	
	2007	1	98.9	1.1	0.0	0.0	0.0	1.1	
	2008	4	93.9	3.8	0.0	2.3	2.3	6.1	
	2009	4	95.9	3.2	0.3	0.6	0.9	4.1	
	2010	4	92.5	4.6	0.6	2.3	2.9	7.5	
	2011	4	93.8	5.6	0.6	0.0	0.6	6.2	
	2012	4	95.5	3.1	0.6	0.9	1.4	4.5	
	2013	4	96.8	2.9	0.0	0.3	0.3	3.2	

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 2013 iteration
Russia	2001	1	81.9	15.3	2.8	0.0	2.8	18.1	Belarus, Ukraine
	2002	1	84.5	9.9	5.6	0.0	5.6	15.5	
	2003	1	100.0	0.0	0.0	0.0	0.0	0.0	
	2004	4	91.2	6.6	1.5	0.7	2.2	8.8	
	2006	5	87.4	8.2	2.7	1.7	4.4	12.6	
	2007	8	88.9	5.8	4.8	0.4	5.2	11.0	
	2008	6	92.2	4.7	1.4	1.7	3.1	7.8	
	2009	6	93.8	2.1	3.3	0.8	4.1	6.2	
	2010	8	94.3	3.3	1.3	1.1	2.4	5.7	
	2011	7	90.0	4.8	3.2	2.0	5.2	10.0	
	2012	6	97.4	2.0	0.0	0.6	0.6	2.6	
	2013	2	98.2	1.8	0.0	0.0	0.0	1.8	
Latin America	2001	11	90.8	6.9	1.4	1.0	2.4	9.2	Argentina, Belize, Bolivia, Brazil (3), Chile, Colombia (3), Costa Rica, Ecuador (2), El Salvador, Guatemala (2),Honduras, Mexico (2), Nicaragua, Panama, Paraguay, Peru, Suriname, Uruguay, Venezuela
	2002	13	93.7	4.6	0.7	1.0	1.7	6.3	
	2003	12	90.8	4.2	2.0	3.0	5.0	9.2	
	2004	17	94.4	4.7	0.8	0.1	0.9	5.6	
	2006	16	88.7	6.3	4.5	0.6	5.1	11.3	
	2007	17	94.9	1.8	1.9	1.4	3.3	5.0	
	2008	20	93.0	3.4	1.5	2.1	3.6	7.0	
	2009	20	95.6	2.1	1.1	1.2	2.3	4.4	
	2010	23	90.8	2.1	5.6	1.4	7.1	9.2	
	2011	22	90.8	2.8	3.1	3.3	6.4	9.2	
	2012	25	94.4	1.6	3.0	1.0	4.0	5.6	
	2013	25	95.5	2.6	1.2	0.3	1.5	4.2	
China	2001	4	98.9	0.8	0.0	0.3	0.3	1.1	China (6)
	2002	3	96.0	4.0	0.0	0.0	0.0	4.0	
	2003	8	90.1	3.6	2.8	3.6	6.4	10.0	
	2004	8	96.0	3.2	0.7	0.1	0.8	4.0	
	2006	6	89.6	7.0	2.9	0.5	3.4	10.4	
	2007	10	98.3	1.1	0.3	0.2	0.5	1.6	
	2008	18	92.8	3.7	0.8	2.7	3.5	7.2	
	2009	14	94.8	2.2	2.1	0.8	2.9	5.1	
	2010	9	92.1	4.5	1.6	1.8	3.4	7.9	
	2012	9	95.3	3.0	0.5	1.2	1.6	4.7	
	2013	8	96.9	2.0	0.5	0.5	1.0	3.1	

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

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 2013 iteration
Southeast Asia	2001	16	88.1	7.7	2.3	1.9	4.2	11.9	Brunei Darussalam, Cambodia, India (9), Japan (2), Korea Rep. Of (2), Lao P.´s Dem. Rep., Malaysia (5), Philippines, Sri Lanka, Taiwan, Thailand (9), Viet Nam (2)
	2002	18	89.0	8.1	1.4	1.6	3.0	11.0	
	2003	17	87.4	5.2	4.7	2.7	7.4	12.6	
	2004	16	92.8	4.4	2.3	0.5	2.8	7.2	
	2006	15	90.0	8.1	1.2	0.8	2.0	10.0	
	2007	20	93.9	4.0	1.4	0.7	2.1	6.1	
	2008	19	90.5	4.7	2.2	2.6	4.8	9.5	
	2009	27	91.8	4.1	3.0	1.2	4.2	8.3	
	2010	25	92.8	3.8	1.5	1.9	3.4	7.2	
	2011	26	90.5	3.5	2.4	3.5	5.9	9.5	
	2012	35	91.7	3.9	3.5	0.9	4.4	8.3	
	2013	35	93.4	3.2	2.5	0.7	3.2	6.4	

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

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

		Method	Perfor-mance[5.6]	AMC	AMP	CAZ	CHL	CIP	POD	CRO	CTX	ENR[2]	FFN[2]	FIS (SMX)[3]	GEN	NAL	STR	SXT	TET	TMP	XNL[2]
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	8-32	0.25-1	1-4	4-16[4]	≤0.5/9.5	0.5-2	0.5-2	0.25-1
		Disks (mm)		18-24	16-22	25-32	21-27	30-40	23-28	29-35	29-35	32-40	22-28	15-23	19-26	22-28	12-20	23-29	18-25	21-28	26-31
EQAS iteration (total no. of participants)	**2000** (44)	MIC & Disk	No.[5]	-	37	-	38	35	-	-	-	-	-	19	39	37	36	-	42	31	-
			%[6]	-	27	-	37	20	-	-	-	-	-	53	23	35	22	-	42	30	-
	2001 (107)	MIC & Disk	No.[5]	-	97	-	97	97	-	-	-	-	-	53	99	74	81	90	96	50	-
			%[6]	-	19	-	20	14	-	-	-	-	-	34	12	14	12	14	22	22	-
	2002 (114)	MIC & Disk	No.[5]	-	109	-	107	108	-	-	-	-	-	57	108	102	82	102	102	66	-
			%[6]	-	16	-	15	14	-	-	-	-	-	26	12	14	11	12	13	11	-
	2003 (144)	MIC & Disk	No.[5]	-	140	-	137	138	-	-	-	-	-	82	138	132	105	129	137	79	-
			%[6]	-	14	-	22	9	-	-	-	-	-	17	9	16	9	14	19	14	-
	2004 (140)	MIC & Disk	No.[5]	117	132	-	128	132	-	-	111	-	-	84	134	126	110	120	129	87	-
			%[6]	13	10	-	13	8	-	-	18	-	-	16	10	9	6	11	13	9	-
	2006 (137)	MIC & Disk	No.[5]	116	133	96	126	127	39	-	115	19	-	74	131	122	106	122	125	74	32
			%[6]	9	14	15	18	8	12	-	21	63	-	29	14	20	11	19	12	17	22
	2007 (126)	MIC & Disk	No.[5]	102	124	92	123	121	47	-	104	-	13	64	124	120	97	107	117	67	35
			%[6]	8	11	9	14	12	9	-	16	-	0	22	6	7	6	13	7	10	11
	2008 (147)	MIC & Disk	No.[5]	-	147	111	135	144	-	-	124	-	-	71	145	136	101	129	139	79	-
			%[6]	-	12	9	10	8	-	-	14	-	-	14	8	8	12	13	7	13	-
		MIC	No.[5]	-	33	23	24	33	-	-	23	-	-	18	31	23	19	22	28	16	-
			%[6]	-	0	5	0	6	-	-	9	-	-	11	0	0	11	9	0	13	-
		Disk	No.[5]	-	114	89	112	111	-	-	101	-	-	53	114	113	82	107	111	63	-
			%[6]	-	16	10	12	8	-	-	15	-	-	15	11	10	12	14	9	13	-
	2009 (129)	MIC & Disk	No.[5]	-	128	100	121	124	-	88	107	-	-	63	123	117	98	113	122	70	-
			%[6]	-	16	13	15	7	-	16	10	-	-	11	18	13	10	14	14	11	-
		MIC (27)	No.[5]	-	27	19	24	26	-	20	20	-	-	14	25	24	19	21	27	25	-
			%[6]	-	11	11	8	8	-	15	15	-	-	21	12	8	5	19	11	13	-
		Disk (102)	No.[5]	-	101	81	97	98	-	68	87	-	-	49	98	93	79	92	95	55	-
			%[6]	-	16	14	16	6	-	16	9	-	-	10	18	14	11	12	15	11	-
	2010 (116)	MIC & Disk	No.[5]	-	114	97	108	115	-	79	100	-	-	51	112	104	84	101	110	63	-
			%[6]	-	11	9	9	6	-	10	14	-	-	11	11	5	5	12	5	15	-
		MIC (25)	No.[5]	-	25	15	21	25	-	15	17	-	-	12	24	19	17	17	24	11	-
			%[6]	-	12	20	10	8	-	7	18	-	-	8	13	16	18	18	17	36	-
		Disk (91)	No.[5]	-	89	82	87	90	-	64	83	-	-	39	88	85	67	84	86	52	-
			%[6]	-	9	6	8	4	-	9	11	-	-	10	9	2	1	10	1	8	-

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

		Method	Perfor-mance[5.6]	AMC	AMP	CAZ	CHL	CIP	POD	CRO	CTX	ENR[2]	FFN[2]	FIS (SMX)[3]	GEN	NAL	STR	SXT	TET	TMP	XNL[2]
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	8-32	0.25-1	1-4	4-16[4]	≤0.5/9.5	0.5-2	0.5-2	0.25-1
		Disks (mm)		18-24	16-22	25-32	21-27	30-40	23-28	29-35	29-35	32-40	22-28	15-23	19-26	22-28	12-20	23-29	18-25	21-28	26-31
EQAS iteration (total no. of participants)	**2011** (112)	MIC & Disk	No.[5]	-	111	89	102	109	-	76	96	-	-	50	103	103	72	99	107	51	-
			%[6]	-	17	4	11	7	-	7	9	-	-	8	11	8	4	16	7	14	-
		MIC (23)	No.[5]	-	23	15	18	22	-	16	15	-	-	13	22	19	17	16	21	11	-
			%[6]	-	4	7	0	9	-	6	0	-	-	8	9	0	6	6	5	0	-
		Disk (89)	No.[5]	-	88	74	84	87	-	60	81	-	-	37	81	84	55	83	86	40	-
			%[6]	-	20	4	13	7	-	7	11	-	-	8	11	10	4	18	8	18	-
	2012 (135)	MIC & Disk	No.[5]	-	134	111	121	131	-	90	115	-	-	53	127	121	89	112	129	66	-
			%[6]	-	13	12	7	6	-	11	10	-	-	11	9	9	8	13	10	21	-
		MIC (37)	No.[5]	-	37	26	31	35	-	23	28	-	-	19	35	31	26	23	35	22	-
			%[6]	-	3	4	0	3	-	0	4	-	-	5	3	3	8	0	0	9	-
		Disk (98)	No.[5]	-	97	85	90	96	-	67	87	-	-	34	92	90	63	89	94	44	-
			%[6]	-	16	14	9	7	-	15	11	-	-	15	11	11	8	16	14	27	-
	2013 (122)	MIC & Disk	No.[5]	-	117	100	112	119	-	82	107	-	-	44	113	113	-	101	114	59	-
			%[6]	-	12	7	5	7	-	4	8	-	-	10	6	11	-	8	8	11	-
		MIC (33)	No.[5]	-	31	25	28	32	-	19	27	-	-	17	32	28	-	22	32	22	-
			%[6]	-	6	4	4	13	-	5	11	-	-	18	9	11	-	5	6	5	-
		Disk (89)	No.[5]	-	86	75	84	87	-	63	80	-	-	27	81	85	-	79	82	37	-
			%[6]	-	13	8	6	5	-	5	6	-	-	7	4	9	-	10	7	8	-

[0]For antimicrobial abbreviations: see List of Abbreviations page 1
[1]CLSI standard. Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 22nd Informational supplement. CLSI document M100-S22. 2012 Wayne. PA. USA
[2]CLSI standard. 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]FIS (sulfisoxazole) covers the group of SMX (sulfonamides)
[4]Quality control range developed by the manufacturer of Sensititre®
[5]No.. number of laboratories performing the analysis
[6]%. percentage of laboratories reporting erroneous results
-. not determined

Table 11. *Shigella* serotypes (ST) and deviations (D). WHO EQAS 2013

Strain	Correct serotype	No. of labs reporting correct identification	D (%)	Deviating results	No. of labs reporting correct ST	D (%)	Deviating results (*)
WHO SH-13.1	*S. boydii* serotype 2	116	0.9	1	62	3.1	1 (2)
WHO SH-13.2	*S. sonnei*	119	0.0	0	NA	NA	NA
WHO SH-13.3	*S. flexneri* serotype ~~4a~~ Yv**	118**	0.0**	0**	59**	14.5**	2a (4), var. Y (3), 4b, 4c, 5**
WHO SH-13.4	*S. boydii* serotype 2	116	0.9	1	61	4.7	1 (3)

*number of participants reporting deviating result

**It has come to our attention that the correct serotype for WHO 2013 SH-13.3 is *Shigella flexneri* Yv. It is a novel *Shigella* serotype which has been described in 'Identification and characterization of a novel *Shigella flexneri* serotype Yv in China' by Sun Q1, Lan R, Wang J, Xia S, Wang Y, Wang Y, Jin D, Yu B, Knirel YA, Xu J. PLoS One. 2013 Jul 30;8(7):e70238. doi: 10.1371/journal.pone.0070238.

.

Table 12. Region-based categorization of laboratories performing *Shigella* serotyping in 2013

Region	Year	No. of laboratories	No. of strains serotyped	Strains serotyped correctly (%)	Countries participating in the 2013 iteration
Africa	2009	8	18	72.2	Kenya, Mauritius, Senegal, South Africa, Tunisia
	2010	7	16	62.5	
	2011	4	10	100.0	
	2012	5	18	90.0	
	2013	5	8	62.5	
Central Asia & Middle East	2009	3	5	100.0	Egypt, Israel, Jordan, Oman
	2010	3	6	83.3	
	2011	2	6	100.0	
	2012	3	9	81.8	
	2013	4	8	100.0	
China	2009	13	35	100.0	China (6)
	2010	9	23	91.3	
	2011	-	-	-	
	2012	8	29	90.6	
	2013	6	11	100.0	
Caribbean	2009	-	-	-	-
	2010	-	-	-	
	2011	-	-	-	
	2012	1	1	33.3	
	2013	-	-	-	
Europe	2009	15	40	92.5	Albania, Belgium, Czech Republic, Denmark, Germany (2), Greece, Ireland, Italy, Lithuania, Luxembourg, Malta, Serbia, Slovenia, Spain, Sweden, Turkey, United Kingdom
	2010	15	35	85.7	
	2011	16	42	92.9	
	2012	19	63	86.3	
	2013	18	31	96.8	
North America	2009	7	18	100.0	Canada (6), United States of America (2)
	2010	7	20	100.0	
	2011	6	16	100.0	
	2012	8	25	80.6	
	2013	8	14	100.0	
Oceanic	2009	3	8	100.0	Australia (3), New Zealand
	2010	3	8	100.0	
	2011	3	8	100.0	
	2012	3	12	100.0	
	2013	4	10	100.0	

Table 12 (continued). Region-based categorization of laboratories performing *Shigella* serotyping in 2013

Region	Year	No. of laboratories	No. of strains serotyped	Strains serotyped correctly (%)	Countries participating in the 2013 iteration
Russia	2009	6	18	83.3	Belarus, Ukraine
	2010	7	20	75.0	
	2011	6	18	88.9	
	2012	5	16	80.0	
	2013	2	4	100.0	
Latin America	2009	16	40	97.5	Argentina, Brazil (2), Chile, Colombia, Costa Rica, Ecuador (2), Honduras, Mexico, Nicaragua, Panama (2), Paraguay, Peru, Venezuela
	2010	13	33	78.8	
	2011	15	37	94.6	
	2012	19	58	80.6	
	2013	16	30	93.3	
Southeast Asia	2009	11	30	90.0	Korea Rep. of, Lao P.´s Dem. Rep., Malaysia, Philippines, Taiwan, Thailand (4)
	2010	14	32	87.5	
	2011	13	33	84.8	
	2012	14	47	90.4	
	2013	9	17	100.0	

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

EQAS iteration	No. of participating laboratories	% correct test results	% minor deviations (S ↔ I or I ↔ R)^	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S → R & R → S & S ↔ I or I ↔ R)^
2008	15	95	2	2	1	3	5
2009	111	96	2	1	1	2	4
2010	114	91	2	1	6	7	9
2011	107	92	2	1	4	5	7
2012	120	91	3	1	5	6	9
2013	99	91	6	2	2	4	10

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

Table 14. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2013 *Shigella* strains*

Strain	Antimicrobial∞											
	AMP	**CTX**	**CAZ**	**CRO**	**CHL**	**CIP**	**GEN**	**NAL**	**SMX**	**SXT**	**TET**	**TMP**
WHO SH-13.1	9/3/**90**	3/1/**92**	0/1/**86**	1/0/**77**	1/1/**94**	16/**40**/49	3/1/**94**	**88**/11/0	**41**/0/0	**84**/0/2	5/0/**93**	**47**/0/1
WHO SH-13.2	6/1/**95**	3/0/**93**	0/1/**87**	0/0/**77**	1/0/**93**	2/8/**95**	2/2/**94**	4/2/**91**	**41**/0/0	**84**/0/3	**95**/1/2	**46**/0/2
WHO SH-13.3	**99**/2/1	4/2/**90**	3/0/**85**	1/1/**75**	**75**/14/6	**34**/38/33	2/2/**94**	**94**/0/4	3/0/**38**	12/3/**72**	**95**/1/2	**45**/1/2
WHO SH-13.4	5/2/**95**	3/1/**92**	1/2/**85**	1/0/**76**	0/1/**94**	12/**45**/48	2/0/**96**	**86**/8/5	**41**/0/0	**82**/1/3	2/0/**96**	**48**/0/1

∞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	1.5
		% total deviations^	1	2	1	-	2	1	-	-	9	2	8	-	2	2.2
2009	111	No. of tests	423	358	388	426	372	396	388	211	293	388	386	218	301	4.548
		% critical deviations*	2.4	0.3	2.1	0.2	1.1	2.5	0.5	3.8	5.8	2.3	2.8	1.8	0.3	1.9
		% total deviations^	3.8	0.3	4.6	0.9	1.1	3.5	1.5	3.8	18.1	3.6	7.5	1.8	0.6	3.8
2010	114	No. of tests	424	344	402	434	377	403	382	194	275	363	410	218	291	4.517
		% critical deviations*	1.7	0.6	3.5	40.8	2.4	3.5	2.1	4.6	8.0	8.3	4.4	3.7	0.0	6.4
		% total deviations^	1.9	1.2	9.2	77.9	3.0	5.5	3.0	6.0	14.6	13.8	5.9	3.8	0.0	11.2
2011	107	No. of tests	403	322	353	396	343	359	369	179	246	371	376	178	289	4.184
		% critical deviations*	5.5	5.2	2.2	38.9	2.7	3.3	4.0	1.7	3.6	3.2	2.7	2.2	2.0	5.5
		% total deviations^	7.7	12.0	4.2	40.7	2.7	4.4	11.0	1.7	10.5	3.2	3.5	2.2	2.0	7.7
2012	120	No. of tests	462	376	427	464	400	430	442	196	291	396	426	215	337	4.862
		% critical deviations*	2.6	0.8	5.6	35.3	2.0	4.9	1.6	1.5	9.3	6.3	5.4	1.9	0.9	6.0
		% total deviations^	3.9	0.8	11.5	38.6	3.8	6.3	3.2	2.0	27.1	8.1	7.5	4.2	2.1	9.2
2013	99	No. of tests	-	351	379	420	384	392	393	164	-	346	392	193	309	3723
		% critical deviations*	-	1.1	2.1	8.3	3.4	2.3	3.3	1.8	-	5.8	2.8	3.1	1.0	3.4
		% total deviations^	-	0.3	0.6	2.0	0.9	0.6	0.8	1.1	-	1.7	0.7	1.6	0.3	9.5

∞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

Region	Year	No. of labs	% correct test result	% minor deviations (S↔I or I↔R)^	% major deviations (S→R)^	% very major deviations (R→ S)^	% critical deviations (R→ S & S → R)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2013 iteration
Africa	2009	17	93.3	2.4	3.5	0.8	4.3	6.8	Cameroon, Central African Republic, Congo, Rep. of, Kenya (3), Madagascar, Mauritius, Nigeria, Senegal, South Africa, Sudan, Tunisia, Zambia
	2010	16	84.8	2.5	2.7	10.0	12.7	15.2	
	2011	16	86.0	1.8	3.6	8.3	11.9	13.7	
	2012	17	82.6	4.2	2.5	10.7	13.2	17.4	
	2013	14	87,6	7,2	2,5	2,7	5,2	12,4	
Central Asia & Middle East	2009	5	94.8	0.9	3.0	1.3	4.4	5.2	Egypt, Iran Islamic Republic of (2). Israel, Jordan, Oman
	2010	6	90.6	1.2	1.6	6.7	8.3	9.4	
	2011	4	92.9	1.6	0.5	4.9	5.4	7.1	
	2012	6	92.3	4.0	2.0	1.3	3.4	7.4	
	2013	6	86,9	8,5	3,9	0,8	4,6	13,1	
Caribbean	2009	4	95.6	1.5	0.7	2.2	2.9	4.4	Barbados, Jamaica,
	2010	4	88.5	1.5	3.8	6.2	10.0	11.5	
	2011	1	97.7	2.3	0.0	0.0	2.3	2.3	
	2012	3	84.6	1.9	7.7	5.8	13.5	15.4	
	2013	2	87,5	9,4	0,0	3,1	3,1	12,5	
Europe	2009	22	98.1	1.1	0.7	0.1	0.8	1.9	Albania, Belgium, Croatia, Cyprus, Denmark (2), Greece (2), Ireland, Italy (4), Lithuania, Luxembourg, Malta, Poland (2), Serbia, Slovenia, Spain, Turkey, United Kingdom
	2010	27	93.6	1.5	0.9	3.9	4.8	6.4	
	2011	24	94.8	2.2	0.5	2.5	3.0	5.1	
	2012	24	96.6	1.7	0.4	1.4	1.7	3.4	
	2013	23	93,6	4,8	1,2	0,3	1,5	6,4	
North America	2009	6	100.0	0.0	0.0	0.0	0.0	0.0	Canada (3). United States of America
	2010	7	95.0	0.0	0.0	5.0	5.0	5.0	
	2011	4	90.1	0.7	3.3	5.9	9.2	9.9	
	2012	6	89.5	0.0	2.1	8.4	10.5	10.5	
	2013	4	95,2	3,2	0,0	1,6	1,6	4,8	

Table 16 (continued) Region-based categorization of EQAS participating laboratories' performance of antimicrobial susceptibility tests for *Shigella* strains

Region	Year	No. of labs	% correct test result	% minor deviations (S↔I or I↔R)^	% major deviations (S→R)^	% very major deviations (R→ S)^	% critical deviations (R→ S & S → R)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2013 iteration
Oceanic	2009	-	-	-	-	-	-	-	Australia
	2010	1	90.0	10.0	0.0	0.0	0.0	10.0	
	2011	1	92.5	5.0	0.0	2.5	2.5	7.5	
	2012	1	90.0	7.5	0.0	2.5	2.5	10.0	
	2013	1	95,5	4,5	0,0	0,0	0,0	4,5	
Russia	2009	6	95.5	1.6	1.6	1.3	2.9	4.6	Belarus, Ukraine
	2010	7	92.1	2.9	1.5	3.5	5.0	7.9	
	2011	6	94.4	3.6	0.0	2.0	2.0	5.6	
	2012	5	96.8	1.4	0.5	1.4	1.8	3.2	
	2013	2	95,2	4,8	0,0	0,0	0,0	4,8	
Latin America	2009	20	98.3	1.1	0.4	0.3	0.7	1.7	Argentina, Belize, Bolivia, Brazil (2), Chile, Colombia, Costa Rica, Ecuador (2), El Salvador, Guatemala (2), Honduras, Mexico (2), Nicaragua, Panama, Paraguay, Peru, Suriname, Uruguay, Venezuela
	2010	22	92.1	1.3	2.1	4.5	6.6	7.9	
	2011	20	94.0	1.5	1.3	3.2	4.5	6.0	
	2012	24	91.7	1.3	0.6	6.5	7.1	8.3	
	2013	23	94,1	3,9	1,2	0,8	2,0	5,9	
Southeast Asia	2009	18	94.1	3.9	0.3	1.7	2.0	5.9	Cambodia, India (8), Korea Rep. Of, Lao P.´s Dem. Rep., Malaysia, Philippines, Sri Lanka, Taiwan, Thailand (3), Viet Nam
	2010	16	90.5	2.4	0.7	6.4	7.1	9.5	
	2011	19	90.0	2.1	0.8	6.1	6.9	9.0	
	2012	27	87.1	5.1	1.9	5.6	7.6	12.7	
	2013	19	86,2	7,5	2,9	3,1	6,0	13,5	
China	2009	12	96.3	2.2	1.0	0.5	1.5	3.7	China (5)
	2010	8	92.7	1.2	0.6	5.5	6.1	7.3	
	2011	-	-	-	-	-	-	-	
	2012	7	90.3	2.9	0.0	6.8	6.8	9.7	
	2013	5	92,7	3,4	0,4	3,4	3,9	7,3	

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

Table 17. Proportion of laboratories that obtained the expected result. Number (n/N) and percentages of laboratories which correctly detected and confirmed the ESBL and non ESBL producing *Salmonella* and *Shigella* strains.

Isolate no.	Expected interpretation	Confirmatory tests	
		CAZ/Cl:CAZ	CTX/Cl:CTX
WHO S-13.1	non ESBL	30/31 (97%)	34/35 (97%)
WHO S-13.2	non ESBL	29/29 (100%)	33/33 (100%)
WHO S-13.3	non ESBL	29/30 (97%)	34/34 (100%)
WHO S-13.4	non ESBL	29/29 (100%)	33/33 (100%)
WHO S-13.5	non ESBL	29/30 (97%)	33/34 (97%)
WHO S-13.6	ESBL-producer	58/60 (97%)	64/66 (97%)
WHO S-13.7	non ESBL	29/30 (97%)	33/34 (97%)
WHO S-13.8	non ESBL	30/31 (97%)	34/35 (97%)
WHO SH-13.1	non ESBL	26/26 (100%)	29/29 (100%)
WHO SH-13.2	non ESBL	26/26 (100%)	29/29 (100%)
WHO SH-13.3	non ESBL	27/27 (100%)	30/30 (100%)
WHO SH-13.4	non ESBL	26/27 (96%)	29/30 (97%)

Table 18. 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	93	88%	*C. coli* (9) *C. lari* (3)
	97	*C. coli*	# 2	93	84%	*C. jejuni* (7) *C. lari* (4) *C. upsaliensis* (4)
2004	109	*C. lari*	# 1	97	79%	*C. coli* (11) *C. jejuni* (8)
	109	*C. jejuni*	# 2	109	87%	*C. coli* (8) *C. lari* (4) *C. upsaliensis* (2)
2006	99	*C. jejuni*	# 1	87	90%	*C. lari* (3) *C. coli* (3) *C. upsaliensis* (3)
	99	*C. coli*	# 2	95	65%	*C. lari* (19) *C. jejuni* (11) *C. upsaliensis* (2)
2007	142	*C. lari*	# 1	98	74%	*C. jejuni* (10) *C. coli* (9) *C. upsaliensis* (7)
	142	*C. coli*	# 2	102	76%	*C. lari* (3) *C. jejuni* (20) *C. upsaliensis* (2)
2008	154	*C. lari*	# 1	109	62%	*C. coli* (14) *C. jejuni* (18) *C. upsaliensis* (7)
	154	*C. lari*	# 2	109	62%	*C. coli* (10) *C. jejuni* (19) *C. upsaliensis* (13)
2009	131	*C. coli*	# 1	87	77%	*C. upsaliensis* (10) *C. jejuni* (9) *C. lari* (1)
	131	*C. jejuni*	# 2	87	95%	*C. upsaliensis* (3) *C. lari* (1)
2010	130	*C. jejuni*	# 1	88	92%	*C. coli* (4) *C. lari* (3) *C. upsaliensis* (1)
	130	*C. coli*	# 2	84	85%	*C. jejuni* (11) *C. lari* (2) *C. upsaliensis* (2)
2011	132	*C. coli*	# 1	81	59%	*C. jejuni* (19) *C. lari* (13) *C. upsaliensis* (1)
	132	*C. coli*	# 2	79	70%	*C. jejuni* (17) *C. lari* (5) *C. upsaliensis* (2)
2012	135	*C. jejuni*	# 1	112	96%	*C. coli* (4)
	135	*C. jejuni*	# 2	103	85%	*C. coli* (10) *C. lari* (5) *C. upsaliensis* (1)
2013	123	*C. coli*	# 1	95	82%	*C. jejuni (13)* *C. lari* (3) *C. upsaliensis* (1)
	123	*C. coli*	# 2	92	84%	*C. jejuni* (9) *C. lari* (4) *C. upsaliensis* (2)

*number of participants reporting the specified deviating result

Table 19. Region-based categorization of EQAS 2013 participating laboratories' performance of *Campylobacter* strains identification

Region	Year	No. of labs	No. of strains identified	% strains correctly identified	Countries participating in the 2013 iteration
Africa	2009	9	15	53	Cameroon, Central African Republic, Kenya (3), Mauritius, South Africa, Sudan, Tunisia
	2010	7	13	77	
	2011	10	19	32	
	2012	9	17	82	
	2013	9	17	41	
Central Asia & Middle East	2009	14	27	85	Oman
	2010	13	26	89	
	2011	2	4	50	
	2012	11	22	96	
	2013	1	8	50	
Caribbean	2009	2	4	100	Barbados, Jamaica
	2010	3	6	67	
	2011	1	2	0	
	2012	4	7	57	
	2013	2	4	100	
Europe	2009	29	55	89	Croatia, Cyprus, Czech Republic, Denmark, Germany (2), Greece, Hungary, Italy (8), Lithuania, Luxembourg, Malta, Poland (2), Serbia, Slovenia (2), Spain, Turkey
	2010	29	57	97	
	2011	25	48	85	
	2012	29	56	95	
	2013	26	51	88	
North America	2009	10	19	90	Canada (7), United States of America (3)
	2010	11	22	86	
	2011	9	18	78	
	2012	13	26	96	
	2013	10	18	100	
Oceania	2009	2	4	100	Australia, New Zealand
	2010	2	3	100	
	2011	2	4	100	
	2012	2	4	100	
	2013	2	4	100	
Russia	2009	2	4	100	Belarus,
	2010	2	4	100	
	2011	2	4	50	
	2012	5	10	80	
	2013	1	2	100	
Latin America	2009	14	26	89	Argentina, Brazil (2), Chile, Colombia (3), Costa Rica, Ecuador, El Salvador, Guatemala (2), Mexico, Panama, Paraguay (2), Peru, Suriname, Uruguay,Venezuela
	2010	19	37	78	
	2011	19	37	49	
	2012	22	40	95	
	2013	20	36	83	

Table 19 (continued). Region-based categorization of EQAS 2013 participating laboratories' performance of *Campylobacter* strains identification

Region	Year	No. of labs	No. of strains identified	% strains correctly identified	Countries participating in the 2013 iteration
Southeast Asia	2009	10	20	90	Brunei Darussalam, Cambodia, India, Japan (2), Korea Rep. of (2), Lao P.´s Dem. Rep., Malaysia, Philippines, Taiwan, Thailand (3), Viet Nam
	2010	14	27	93	
	2011	12	24	67	
	2012	17	33	85	
	2013	15	28	89	
China	2009	12	24	92	China (5)
	2010	10	20	85	
	2011	-	-	-	
	2012	-	-	-	
	2013	5	10	90	

Table 20. EQAS participants' performance of *Campylobacter* strains antimicrobial susceptibility testing

EQAS iteration	No. of labs	% correct test results	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (R → S & S → R)^
2009	25	91.4	4.5	4.1	8.6
2010	37	91.3	4.2	4.5	8.7
2011	38	93.8	2.8	3.4	6.2
2012	47	93.6	5.0	1.5	6.4
2013	47	92.4	5.0	2.6	7.6

^S. susceptible; R. resistant

Table 21. Antimicrobial susceptibility test results (number of R/S) for the EQAS 2013 *Campylobacter* strains*

Strain	Antimicrobial^						
	CHL	CIP	ERY	GEN	NAL	STR	TET
WHO C-13.1	3/0/**32**	4/0/**40**	**39**/0/3	0/0/**40**	5/0/**34**	4/0/**21**	**39**/0/3
WHO C-13.2	1/0/**35**	2/0/**44**	4/0/**41**	0/0/**42**	2/0/**38**	**18**/0/8	4/0/**40**

^For antimicrobial abbreviations. see List of Abbreviations page 1

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

§ Results for the combination WHO C-12.1 and NAL were disregarded due to conflicting results that indicated a problem with the expected result.

Table 22. 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	10.9	2.3	9.8	11.8	11.1
2010	37	No. of tests	44	70	71	59	53	39	68
		% critical deviations*	4.5	7.1	11.3	10.2	7.5	10.3	8.8
2011	38	No. of tests	41	67	62	65	62	30	60
		% critical deviations*	0.0	6.0	6.5	3.1	8.1	13.3	8.3
2012	47	No. of tests	70	84	81	81	39	53	74
		% critical deviations*	4.3	6.0	6.2	7.4	5.1	11.3	5.4
2013	47	No. of tests	71	90	87	82	79	51	86
		% critical deviations*	5.6	6.7	8.0	0.0	8.9	23.5	8.1

^For antimicrobial abbreviations. see List of Abbreviations page 1

*R→ S & S → R (R. resistant; S. susceptible

Table 23. Region-based categorization of EQAS 2013 participants' performance of antimicrobial susceptibility testing of *Campylobacter* strains

Region	Year	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 2013 iteration
Africa	2009	2	75.0	10.7	14.3	25.0	Cameroon, Central African Republic, Kenya, Sudan, Tunisia
	2010	2	95.2	0.0	4.8	4.8	
	2011	7	85.0	3.3	11.7	15.0	
	2012	4	94.3	0.0	5.7	5.7	
	2013	5	90.9	5.5	3.6	9.1	
Central Asia & Middle East	2009	0	-	-	-	-	Israel, Iran Islamic Republic of, Oman
	2010	0	-	-	-	-	
	2011	1	75.0	0.0	25.0	25.0	
	2012	2	93.8	6.3	0.0	6.3	
	2013	3	93.3	3.3	3.3	6.7	
China	2009	2	95.2	4.8	0.0	4.8	China (3),
	2010	1	100.0	0.0	0.0	0.0	
	2011	0	-	-	-	-	
	2012	2	88.5	7.7	3.8	11.5	
	2013	3	95.2	2.4	2.4	4.8	
Caribbean	2009	0	-	-	-	-	Jamaica
	2010	0	-	-	-	-	
	2011	0	-	-	-	-	
	2012	1	75.0	25.0	0.0	25.0	
	2013	1	100	0	0	0.0	

^S. susceptible; R. resistant

Table 23 (continued).. Region-based categorization of EQAS 2013 participants' performance of antimicrobial susceptibility testing of *Campylobacter* strains

Region	Year	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 2013 iteration
Europe	2009	10	94.8	3.0	2.2	5.2	Cyprus, Denmark (2), Greece, Hungary, Italy (2), Lithuania, Luxembourg, Malta, Poland (2), Slovenia, Slovenia, Spain, Turkey
	2010	13	100.0	0.0	0.0	0.0	
	2011	11	100.0	0.0	0.0	0.0	
	2012	16	97.3	1.6	1.1	2.7	
	2013	16	94.9	3.5	1.5	5.1	
North America	2009	2	100.0	0.0	0.0	0.0	Canada, United States of America (2)
	2010	5	93.8	6.3	0.0	6.3	
	2011	5	100.0	0.0	0.0	0.0	
	2012	5	100.0	0.0	0.0	0.0	
	2013	3	100.0	0.0	0.0	0.0	
Oceania	2009	0	-	-	-	-	- none -
	2010	0	-	-	-	-	
	2011	1	100.0	0.0	0.0	0.0	
	2012	0	-	-	-	-	
	2013	0	-	-	-	-	
Russia	2009	0	-	-	-	-	- none -
	2010	1	78.6	7.1	14.3	21.4	
	2011	1	100.0	0.0	0.0	0.0	
	2012	0	-	-	-	-	
	2013	0	-	-	-	-	
Latin America	2009	5	93.2	6.8	0.0	6.8	Argentina, Brazil, Chile, Costa Rica, Ecuador, Paraguay, Peru
	2010	8	89.6	6.0	4.5	10.4	
	2011	7	96.8	0.0	3.2	3.2	
	2012	7	95.2	3.2	1.6	4.8	
	2013	7	92.4	4.5	3.0	7.6	
Southeast Asia	2009	4	84.4	4.4	11.1	15.6	India (2), Japan, Korea Rep. of (2), Philippines, Thailand (3)
	2010	7	77.2	9.8	13.0	22.9	
	2011	5	85.1	9.0	6.0	14.0	
	2012	10	85.8	13.3	0.9	14.2	
	2013	9	84.8	10.7	4.5	15.2	

^S. susceptible; R. resistant

Table 24. EQAS 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 2010 (N=20)	Microdilution	42°C / 24h	**No.[1]**	3	6	6	6	4	6
			%[2]	67	83	100	83	75	83
	Microdilution	36-37°C / 48h	**No.[1]**	5	8	8	8	7	8
			%[2]	80	88	88	75	86	88
	Agardilution	42°C / 24h	**No.[1]**	-	6	6	6	-	-
			%[2]	-	100	83	83	-	-
	Agardilution	36-37°C / 48h	**No.[1]**	-	0	0	0	-	-
			%[2]	-	0	0	0	-	-
	Overall	Overall	**No.[1]**	8	20	20	20	11	14
			%[2]	75	90	90	80	82	86
EQAS 2011 (N=26)	Microdilution	42°C / 24h	**No.[1]**	4	9	9	8	7	9
			%[2]	100	67	100	88	100	67
	Microdilution	36-37°C / 48h	**No.[1]**	6	8	6	8	7	7
			%[2]	83	88	100	75	86	86
	Agardilution	42°C / 24h	**No.[1]**	-	8	8	8	-	-
			%[2]	-	88	63	100	-	-
	Agardilution	36-37°C / 48h	**No.[1]**	-	1	1	1	-	-
			%[2]	-	0	0	100	-	-
	Overall	Overall	**No.[1]**	10	26	24	25	14	16
			%[2]	90	77	83	88	93	75
EQAS 2012 (N=34)	Microdilution	42°C / 24h	**No.[1]**	9	12	12	12	10	12
			%[2]	67	75	83	83	80	75
	Microdilution	36-37°C / 48h	**No.[1]**	7	9	8	8	8	8
			%[2]	100	89	100	63	88	88
	Agardilution	42°C / 24h	**No.[1]**	-	9	7	9	-	-
			%[2]	-	89	86	89	-	-
	Agardilution	36-37°C / 48h	**No.[1]**	-	4	4	4	-	-
			%[2]	-	50	100	100	-	-
	Overall	Overall	**No.[1]**	34	80	75	78	43	50
			%[2]	82	81	88	83	86	80
EQAS 2013 (N=47)	Microdilution	42°C / 24h	**No.[1]**	6	8	8	8	7	8
			%[2]	83	88	100	88	86	100
	Microdilution	36-37°C / 48h	**No.[1]**	8	12	12	11	11	12
			%[2]	88	92	83	73	91	75
	Agardilution	42°C / 24h	**No.[1]**	-	9	9	8	-	-
			%[2]	-	89	67	75	-	-
	Agardilution	36-37°C / 48h	**No.[1]**	-	7	7	6	-	-
			%[2]	-	86	86	100	-	-
	Overall	Overall	**No.[1]**	14	36	36	33	18	20
			%[2]	86	89	83	82	89	85

[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 25. EQAS participating laboratories' performance of unknown strain identification

EQAS iteration	Strain ID	No. of participating labs	Percentage (%) 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
2010	*Citrobacter spp.*	115	90
2011	*Aeromonas hydrophila*	106	83
2012	*Salmonella* Paratyphi B var. Java	134	23% (*Salmonella* spp) 7% (*Salmonella* O:B) 24% (*Salmonella* Paratyphi B var. java. In total 54% Deviations: *Citrobacter freundii* (1), *Edwardsiella* sp (1), *Escherichia fergusonii* (1), *Proteus mirabilis* (1), *Salmonella* serovar X* (24), *Salmonella* serovar Paratyphi B (34) * incorrect serovar
2013	*E. coli* O157:H16 non-VTEC	129	82% including: *Escherichia coli* non-VTEC *Escherichia coli* O157 non-VTEC *Escherichia coli* O157:H16 non-VTEC *E. coli* non-VTEC *E. coli* O157 non-VTEC *E. coli* O157:H16 non-VTEC Deviations: *Escherichia coli* O157 H7 (9), *Escherichia hermannii* (2), *Shigella sonnei* (2), *E.coli* EHEC, *Escherichia coli* O114: nonmotile, *Escherichia coli* O157:H12, *Escherichia coli* O157:H16, Stx1+, *Escherichia coli* O157:H45, *Escherichia coli* O157:H7/ Verotoxin negative, *Escherichia fergusonii*, *Esherichia coli* STEC, *Vibrio mimicus*, *Citrobacter amalonaticus*

M00-06-001/01.12.2011

Kgs. Lyngby, Denmark, April 2013

SIGN-UP FOR EQAS 2013

Greetings to the 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 and regional reference laboratories that attended WHO GFN training courses to participate in the External Quality Assurance System (EQAS). The 2012 EQAS cycle is completed, and we are pleased to announce the launch of the 2013 EQAS cycle.

WHY PARTICIPATE IN EQAS?

EQAS provides the opportunity for proficiency testing which is considered an important tool for the production of reliable laboratory results of consistently good quality.

WHAT IS OFFERED IN EQAS?

This year, WHO EQAS offers the following components:

- 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 isolate.

WHO SHOULD PARTICIPATE IN EQAS 2013?

All national and regional reference laboratories which perform analysis on *Salmonella*, *Shigella* and/or *Campylobacter* and are interested in participating in an external quality assurance program are invited to participate.
We expect that all national and regional reference laboratories that 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 laboratories that sign up for EQAS 2013. Laboratories which signed up and received bacterial isolates in year 2012 but did not submit any result should provide a consistent explanation for this if they want to participate in 2013.

COST FOR PARTICIPATING IN EQAS

There is no participation fee in EQAS 2013. Laboratories should, however, cover the expenses for parcel shipment if they can afford it. If FedEx has ‘Dangerous Goods-service’ in your country or if you have a DHL-account no, please provide your FedEx or DHL import account number (for import of UN3373 Biological Substance Category B) in the sign-up form or, alternatively, to the EQAS Coordinator (please find contact information below). We need this information at this stage to save time and resources. Participating laboratories are responsible for paying any expenses related to taxes or custom fees applied by their country.

HOW TO SIGN- UP FOR EQAS 2013

This link will open a sign-up webpage: http://thor.dfvf.dk/signup

In this webpage, you will be asked to provide the following information:

- Name of institute, department, laboratory, and contact person
- Complete mailing address for shipment of bacterial isolates (no post-office box number)
- Telephone and fax number, e-mail address
- FedEx or DHL import account number (if available)
- Approximate number of *Salmonella* isolates annually serogrouped/serotyped
- Approximate number of *Salmonella* isolates annually tested for antimicrobial susceptibility
- Availability of ATCC reference strains
- Components of EQAS 2013 you plan to participate in
- Level of reference function in your country

If you experience any problem in the sign-up webpage, please try again a few days later. If problems persist after several attempts, please contact the EQAS Coordinator Susanne Karlsmose: E-mail suska@food.dtu.dk; fax +45 3588 6341.

TIMELINE FOR SHIPMENT OF ISOLATES AND AVAILABILITY OF PROTOCOLS

Due to increased number of participants in WHO EQAS, a number of different institutions will ship the bacterial isolates, and you will receive information concerning the institution shipping your parcel. The bacterial isolates will be shipped between August and September 2013.

In order to minimize delays, **please send a valid import permit to the EQAS coordinator**. Please apply for a permit to receive the following (according to your level of participation): "UN3373, Biological Substance Category B": eight *Salmonella* strains, four *Shigella* strains, two *Campylobacter*, one *Campylobacter* reference strain (for new participants performing antimicrobial susceptibility testing on *Campylobacter*), one *Escherichia coli* reference strain (for new participants performing antimicrobial susceptibility testing on *Salmonella* and/or *Shigella*) and an unknown isolate (enteric bacteria) between August and September 2013.

Protocols and all relevant information will be available for download from the website http://www.antimicrobialresistance.dk/233-169-215-eqas.htm.

DEADLINE FOR SUBMITTING RESULTS TO THE NATIONAL FOOD INSTITUTE

Results must be submitted to the National Food Institute (DTU Food) by **31st December 2013** through the password-protected website. An evaluation report will be generated upon submission of results. Full anonymity is ensured, and only DTU Food and the WHO GFN Regional Centre in your region will have access to your results.

Deadline for sign-up for EQAS 2013 is 30th May 2013

			Ampicillin AMP		Cefotaxime CTX		Ceftazidime CAZ		Ceftriaxone CRO		Chloramphenicol CHL		Ciprofloxacin CIP		Gentamicin GEN		Nalidixic acid NAL		Sulfonamides SMX		Tetracycline TET		Trimethoprim TMP		Trim/Sulfa SXT	
WHO 2013 S-13.1	*Salmonella* Berta	9,12:f,g,t:-	> 32	RESIST	<= 0.12	SUSC	= 0.25	SUSC	= 0.06	SUSC	= 8	SUSC	= 0.5	RESIST	> 16	RESIST	= 32	RESIST	<= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.06	SUSC
WHO 2013 S-13.2	*Salmonella* Kiambu	4,12:z:1,5	<= 1	SUSC	<= 0.12	SUSC	= 0.5	SUSC	= 0.125	SUSC	= 8	SUSC	= 0.03	SUSC	<= 0.5	SUSC	= 4	SUSC	<= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.06	SUSC
WHO 2013 S-13.3	*Salmonella* Enteritidis	9,12:g,m:-	= 4	SUSC	= 0.25	SUSC	= 1	SUSC	= 0.5	SUSC	= 8	SUSC	= 0.03	SUSC	> 16	RESIST	= 4	SUSC	> 1024	RESIST	<= 2	SUSC	<= 1	SUSC	= 0.06	SUSC
WHO 2013 S-13.4	*Salmonella* Hvittingfoss	16:b:e,n,x	<= 1	SUSC	<= 0.12	SUSC	= 0.25	SUSC	<= 0.06	SUSC	= 8	SUSC	= 0.03	SUSC	= 0.5	SUSC	<= 4	SUSC	<= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.03	SUSC
WHO 2013 S-13.5	*Salmonella* Rubislaw	11:r:e,n,x	<= 1	SUSC	<= 0.12	SUSC	= 0.125	SUSC	= 0.06	SUSC	= 8	SUSC	<= 0.015	SUSC	<= 0.5	SUSC	<= 4	SUSC	<= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.03	SUSC
WHO 2013 S-13.6	*Salmonella* Keurmassar	35:c:1,2	> 32	RESIST	> 4	RESIST	> 256	RESIST	= 32	RESIST	> 64	RESIST	= 0.06	SUSC	> 16	RESIST	= 4	SUSC	> 1024	RESIST	> 32	RESIST	> 32	RESIST	> 32	RESIST
WHO 2013 S-13.7	*Salmonella* Lexington	3,10:z10:1,5	<= 1	SUSC	<= 0.12	SUSC	= 0.25	SUSC	= 0.125	SUSC	= 8	SUSC	= 0.03	SUSC	<= 0.5	SUSC	= 4	SUSC	<= 64	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.06	SUSC
WHO 2013 S-13.8	*Salmonella* Kentucky	8,20:i:z6	> 32	RESIST	<= 0.12	SUSC	= 0.125	SUSC	= 0.125	SUSC	= 8	SUSC	> 4	RESIST	= 16	RESIST	> 64	RESIST	> 1024	RESIST	> 32	RESIST	<= 1	SUSC	= 1	SUSC
WHO 2013 SH-13.1	*Shigella* boydii 2		= 2	SUSC	<= 0.12	SUSC	= 0.125	SUSC	= 0.03	SUSC	<= 2	SUSC	= 0.12	RESIST	= 1	SUSC	= 64	RESIST	> 1024	RESIST	<= 2	SUSC	> 32	RESIST	> 32	RESIST
WHO 2013 SH-13.2	*Shigella* sonnei		= 2	SUSC	<= 0.12	SUSC	= 0.06	SUSC	= 0.03	SUSC	= 4	SUSC	<= 0.015	SUSC	<= 0.5	SUSC	<= 4	SUSC	> 1024	RESIST	> 32	RESIST	> 32	RESIST	> 32	RESIST
WHO 2013 SH-13.3	*Shigella* flexneri 4a		> 32	RESIST	<= 0.12	SUSC	= 0.125	SUSC	= 0.06	SUSC	= 64	RESIST	= 1	RESIST	= 1	SUSC	> 64	RESIST	<= 16	SUSC	> 32	RESIST	> 32	RESIST	= 0.5	SUSC
WHO 2013 SH-13.4	*Shigella* boydii 2		= 2	SUSC	<= 0.12	SUSC	= 0.125	SUSC	= 0.06	SUSC	<= 2	SUSC	= 0.12	RESIST	<= 0.5	SUSC	= 32	RESIST	> 1024	RESIST	<= 2	SUSC	> 32	RESIST	> 32	RESIST

			Chloramphenicol CHL		Ciprofloxacin CIP		Erythromycin ERY		Gentamicin GEN		Nalidixic acid NAL		Streptomycin STR		Tetracycline TET	
WHO 2013 C-13.1	*C. coli*		= 8	SUSC	= 0.5	SUSC	> 32	RESIST	= 0.5	SUSC	= 8	SUSC	= 2	SUSC	> 16	RESIST
WHO 2013 C-13.2	*C. coli*		= 8	SUSC	= 0.12	SUSC	= 4	SUSC	= 0.25	SUSC	= 8	SUSC	> 16	RESIST	= 0.5	SUSC

WHO B-13.1	*Escherichia coli* O157:H16 non-VTEC

WHO Collaborating Centre
External Quality Assurance System (EQAS) 2013

DTU Food
National Food Institute

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

PROTOCOL for

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

---

1 INTRODUCTION

In 2000, the Global Foodborne Infections Network (formerly 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 for the subcontractor's work.

The WHO EQAS 2013 includes

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

All participants will receive the strains according to the information they reported in the sign-up form.

The above-mentioned reference strains are included in the parcel only for new participants of the EQAS who did not receive them previously. The reference strains are original CERTIFIED cultures provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The reference strains will not be included in the years to come. Therefore, please take proper care of these 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 antimicrobial susceptibility testing of enteric human pathogens, especially *Salmonella*. A further objective is to assess and improve the comparability of surveillance data on *Salmonella* serotypes and antimicrobial susceptibility reported by different laboratories. Therefore, the laboratory work for this EQAS should be done by using the methods routinely used in your laboratory.

3 OUTLINE OF THE EQAS 2013

3.1 Shipping, receipt and storage of strains

In September 2013, around 200 laboratories located worldwide will receive a parcel containing eight *Salmonella* strains, four *Shigella* strains, two *Campylobacter* strains and one 'unknown' bacterial isolate (according to information reported in the sign-up form). An *E. coli* ATCC 25922 reference strain and a *C. jejuni* ATCC 33560 reference strain will be included for participants who signed up to perform antimicrobial susceptibility testing (AST) and did not receive them previously.

All provided strains belong to UN3373, Biological substance category B. ESBL-producing strains could be included in the selected material.

Please confirm receipt of the parcel through the confirmation form enclosed in the shipment

The *Salmonella* and *Shigella* strains, and the 'unknown' bacterial isolate are shipped as agar stab cultures whereas the reference strains and the *Campylobacter* strains are shipped lyophilised. On arrival, the agar stab cultures must be subcultured and prepared for storage in your strain collection (e.g. in a -80°C freezer). This set of cultures should serve as reference if discrepancies are detected during the testing (e.g. they can be used to dectect errors such as mis-labelling or contamination). Lyophilised strains must be reconstituted, and you can find below a suggested procedure.

3.2 Serotyping of *Salmonella*

The eight *Salmonella* strains should be serotyped by using the method routinely used in the laboratory. If you do not have all the necessary antisera please go as far as you can in the identification and report the serogroup, since also serogroup results will be evaluated. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (Grimont and Weill, 2007. 9th ed. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

Please fill in information concerning the brand of antisera used for typing in the fields available in the database for entering results. In addition, we kindly ask you to report which antisera you think are required to complete the serotyping, if relevant.

3.3 Antimicrobial susceptibility testing of *Salmonella, Shigella* and *Escherichia coli* ATCC 25922

The *Salmonella* and *Shigella* strains as well as the *E. coli* ATCC 25922 reference strain should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods routinely used in your 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 susceptibility may be valuable for monitoring purposes. Therefore we kindly ask you to disregard, for the purpose of this proficiency trial, that the Clinical and Laboratory Standards Institute (CLSI) guidelines state that *Salmonella* and *Shigella* should not be reported as susceptible to aminoglycosides.

The breakpoints used in this EQAS for interpreting MIC results are in accordance with CLSI values (Table 1). Consequently, interpretation of MIC results will lead to categorization of strains into

three categories: resistant (R), intermediate (I) and susceptible (S). In the evaluation report you receive upon result submission, you can find that obtained interpretations in accordance with the expected interpretation will be defined as 'correct', whereas deviations from the expected interpretation will be defined as 'minor' (I ↔ S or I ↔ R), 'major' (S interpreted as R) or 'very major' (R interpreted as S).

Please report the breakpoints that you routinely use in your laboratory for interpretation of antimicrobial susceptibility test results in the fields available in the database (or in the test forms).

Table 1. Interpretive breakpoint for *Salmonella* and *Shigell*a antimicrobial susceptibility testing

Antimicrobials	Reference value, MIC (µg/mL)			Reference value, Disk diffusion (mm)		
	Sensitive	Intermediate	Resistant	Resistant	Intermediate	Sensitive
Ampicillin, AMP	≤8	16	≥32	≤13	14-16	≥17
Cefotaxime, CTX	≤1	-	>1	≤27	-	>27
Ceftazidime, CAZ	≤1	-	>1	≤22	-	>22
Ceftriaxone, CRO	≤1	-	>1	≤25	-	>25
Chloramphenicol, CHL	≤8	16	≥32	≤12	13-17	≥18
Ciprofloxacin, CIP	≤0.06*	0.12-0.5*	≥1*	<23mm (1µg)** or ≤20mm (5µg)*	- (1µg)** or 21-30 (5µg)*	≥23mm (1µg)** or ≥31mm (5µg)*
Gentamicin, GEN	≤4	8	≥16	≤12	13-14	≥15
Nalidixic acid, NAL	≤16	-	≥32	≤13	14-18	≥19
Sulfonamides, SMX	≤256	-	≥512	≤12	13-16	≥17
Tetracycline, TET	≤4	8	≥16	≤11	12-14	≥15
Trimethoprim, TMP	≤8	-	≥16	≤10	11-15	≥16
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT	≤2/38	-	≥4/76	≤10	11-15	≥16

Reference values used in this EQAS are according to CLSI, with the following exceptions:
* These breakpoints should also be applied for *Shigella* test strains for interpretation of AST results in this EQAS
** The publication by Cavaco LM and Aarestrup FM (J. Clin. Microbiol. 2009. Sep;47(9):2751-8) provides the background for these interpretative criteria in the WHO GFN EQAS. In the publication, *Shigella* was not included, however, these interpretative criteria are also applied for *Shigella* test strains for interpretation of AST results in this EQAS

Concerning ciprofloxacin susceptibility tests, please note that for results obtained in this proficiency test, the breakpoints for *Salmonella* are applied for *Shigella* also. These breakpoints for

ciprofloxacin correspond to the EUCAST (European Committee on Antimicrobial Susceptibility Testing; www.eucast.org) epidemiological cut-off value, and take into consideration mechanisms of resistance like qnr-genes or one point-mutation in the gyrase gene.

Important notes*: beta-lactam resistance*

The following tests for detection of Extended-Spectrum Beta-Lactamase (ESBL) production are optional.

All strains showing reduced susceptibility to cefotaxime (CTX), ceftazidime (CAZ) and/or ceftriaxone (CRO) could be tested for ESBL production by confirmatory test. Confirmatory test for ESBL production requires use of both cefotaxime (CTX) and ceftazidime (CAZ) alone, and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC CTX : CTX/CL or CAZ : CAZ/CL ratio ≥ 8) or ii) a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production.

Of note, MIC values and relative interpretation of cefotaxime (CTX), ceftazidime (CAZ) and/or ceftriaxone (CRO) used for detection of beta-lactamase-producing strains in this EQAS should be reported as found.

3.4 Handling the *Campylobacter* strains

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule, and 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. Transfer the rest of the content of 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. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding
j. Inoculate a second agar plate from the liquid media with a 10µl loop or a cotton swab if the initial plate had inadequate growth.
k. Select a pure culture with vigorous growth from the agar plate for further work.

Please note that:

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

For reconstitution of *C. jejuni* ATCC33560 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 Antimicrobial susceptibility testing of *Campylobacter* and *Campylobacter jejuni* ATCC 33560

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

In this EQAS, the breakpoints used for interpretation of MIC results for *Campylobacter* are epidemiological cut-off values according to EUCAST (www.eucast.org; Table 2). Consequently, only two categories of characterisation (resistant, R or susceptible, S) are allowed. In the evaluation report that you receive upon result submission, you can find that obtained interpretations in agreement with the expected interpretation, will be categorised as 'correct', whereas deviations from the expected interpretation will be categorizes as 'incorrect'.

Please report the breakpoints that you routinely use in your laboratory for interpretation of antimicrobial susceptibility test results, in the fields available in the database (or in the test form).

Note that the interpretation of antimicrobial susceptibility test results for *Campylobacter* requires knowledge of the *Campylobacter* species. If you did not sign-up for *Campylobacter* identification, but perform AST on *Campylobacter*, you are welcome to contact the EQAS Coordinator to obtain information regarding the identity of the *Campylobacter* test strains.

Table 2. Interpretive criteria for *Campylobacter* antimicrobial susceptibility testing

Antimicrobials for *Campylobacter*	MIC (µg/mL) **R is >** *C. jejuni*	MIC (µg/mL) **R is >** *C. coli*
Chloramphenicol, CHL	16	16
Ciprofloxacin, CIP	0.5	0.5
Erythromycin, ERY	4	8
Gentamicin, GEN	2	2
Nalicixic acid, NAL	16	16
Streptomycin, STR	4	4
Tetracycline, TET	1	2

Reference values for interpretation of *Campylobacter* AST results according to EUCAST

The sub-cultured *Campylobacter* strains should be used for MIC-testing after incubation at 36-37ºC for 48 hours or at 42ºC for 24 hours. Likely, two subcultures are needed prior to MIC-testing to ensure optimal growth.

3.7 Identification of the unknown enteric bacterium

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

4 REPORTING OF RESULTS AND EVALUATION

Please write your results in the enclosed test forms and enter your results into the interactive web database.

We recommend reading carefully the description in paragraph 5 before entering your results in the web database. For entering your results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print a report evaluating your results. Results in agreement with the expected interpretation are categorised as ‘correct’, while results deviating from the expected interpretation are categorised as ‘incorrect’.

Results must be submitted no later than 31 December 2013.

If you do not have access to the Internet, or if you experience difficulties in entering your results, please return the completed test forms by e-mail, fax or mail to the National Food Institute, Denmark.

All results will be summarized in a report which will be publicly available. Individual results will be anonymous and will only be forwarded to the official GFN Regional Centre in your region.

We are looking forward to receiving your results.

If you have any questions or concerns, please do not hesitate to contact the EQAS Coordinator:

Susanne Karlsmose

National Food Institute, Technical University of Denmark

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

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

E-mail: suska@food.dtu.dk

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

5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read these instructions before entering the web page. Remember that you need by your side the completed test forms and the breakpoint values you used.

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 lower-case letters and click on ‘Login’.
    You can find your username and password in the letter accompanying your parcel.
    Your username and password will remain unchanged in future trials.

2) Click on ‘Materials and methods’
    a. Fill in the fields relative to brand of antisera (very important because we would like to compare results obtained with different brands of antisera)
    b. Fill in the fields relative to the method used for antimicrobial susceptibility testing
    c. Enter the brand of materials, e.g. Oxoid

d. Fill in the field asking whether your institute serves as a national reference laboratory
e. In the comment field, report which antisera you think is required to complete your serotyping, if relevant
f. Click on 'Save and go to next page' – REMEMBER TO SAVE EACH PAGE BEFORE LEAVING IT!

3) In the data entry page 'Routinely used breakpoints'
   a. Fill in the fields relative to the breakpoints used routinely in your laboratory to determine the antimicrobial 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',
   a. SELECT the serogroup (O-group) from the drop-down list, DO NOT WRITE – Wait a few seconds – the page will automatically reload, so that the drop-down list in the field "Serotype" only contains serotypes belonging to the chosen serogroup.
   b. SELECT the serotype from the drop-down 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 zone diameters 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 (resistant), I (intermediate) or S (susceptible)
   e. If you performed confirmatory tests for ESBL production, please choose the appropriate result from the pick list.
   f. If relevant, fill in the field related to comments (e.g. which antisera you miss for complete serotyping)
   g. Click on 'Save and go to next page'

   If you did not perform these tests, please leave the fields empty

5) In the data entry page '*E. coli* reference strain':
   a. Enter the zone diameters 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 field concerning species and type of the unknown bacterial isolate, and report the method used for identification
   c. Click on 'Save and go to next page'

   If you did not perform these tests, please leave the fields empty

WHO Collaborating Centre
External Quality Assurance System (EQAS) 2013

DTU Food
National Food Institute

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

7) The next page is a menu that allows you to review the input pages and 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 into the interactive database, but allows you to see the evaluated results.
   c. As soon as you have approved your input, an evaluation report will appear.

8) After browsing all pages in the report, you will find a new menu. You can choose 'EQAS 2013 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!

Technical University of Denmark
DTU

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-S21, January 2011 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A8, January 2009 (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% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen. (Alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

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.

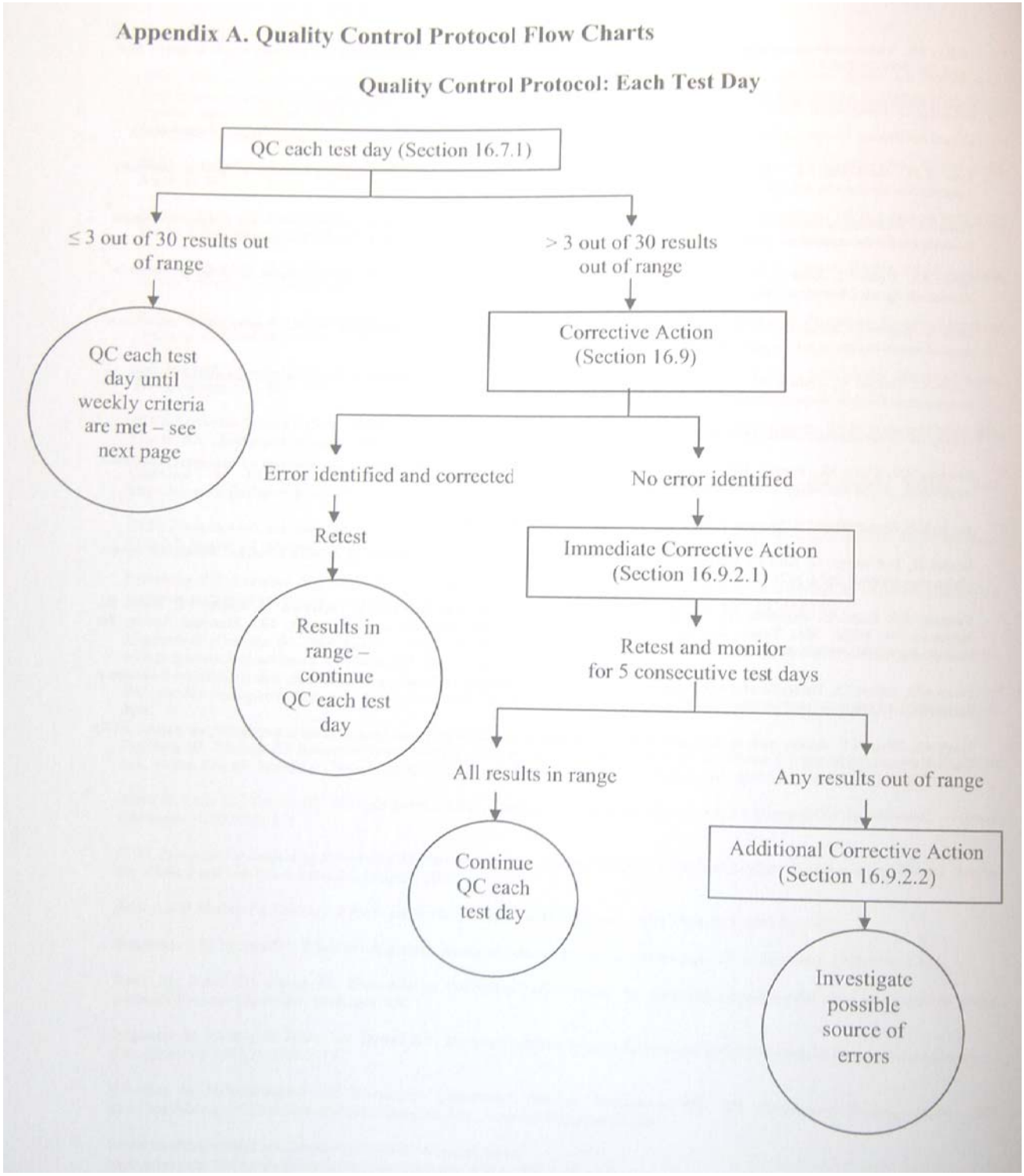
If the problem cannot be resolved, continue daily testing until the errors are identified.

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



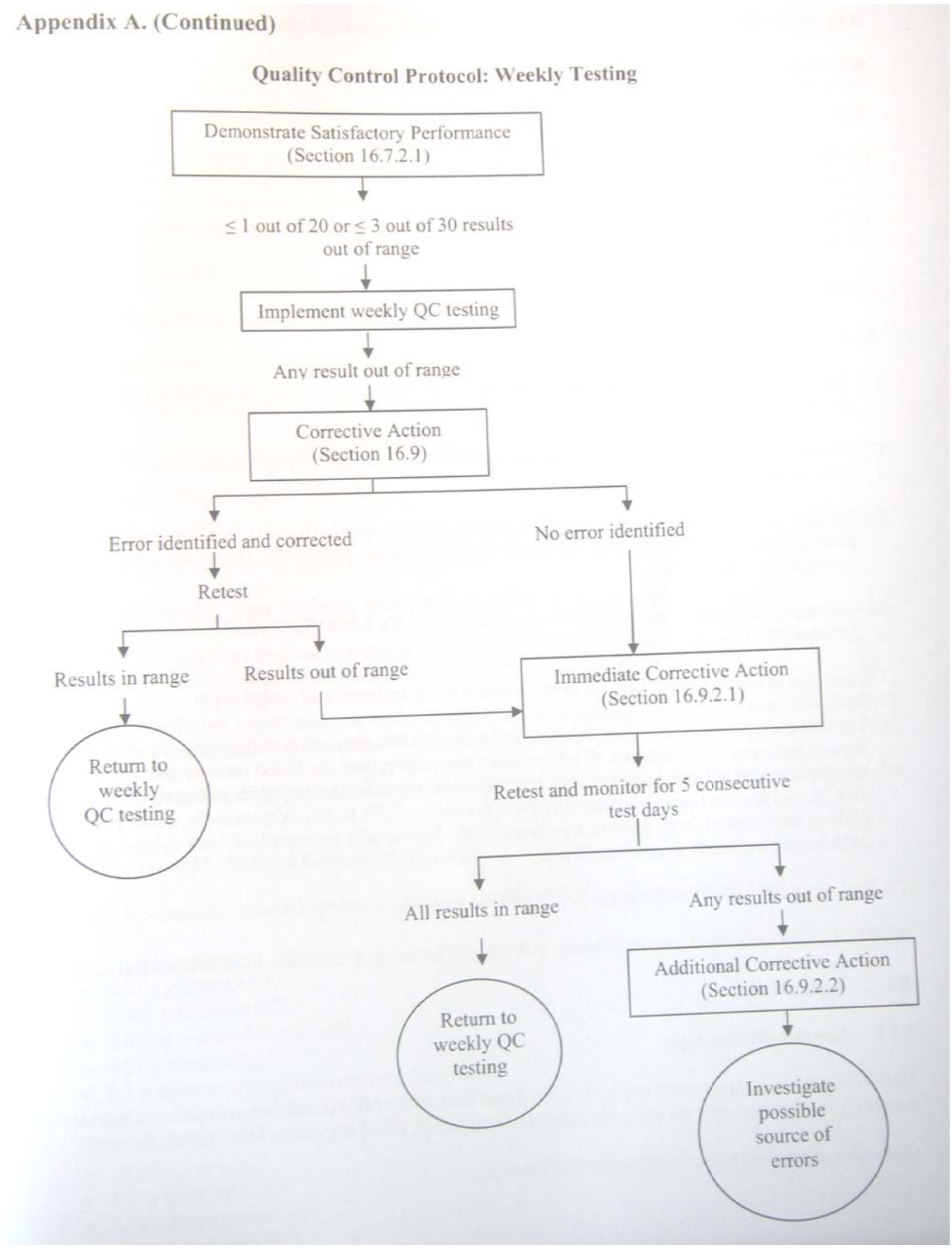
Reference: CLSI M7-A8, page 44

DTU Food
National Food Institute

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

WEEKLY MIC QC CHART

Appendix A. (Continued)



Reference: CLSI M7-A8, page 45

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

Tel. 35 88 70 00
Fax 35 88 70 01

www.food.dtu.dk

ISBN: 978-87-93109-33-9