

The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2016

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The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2016



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





international network



DTU Food National Food Institute

THE EXTERNAL QUALITY ASSURANCE SYSTEM OF THE WHO GLOBAL FOODBORNE INFECTIONS NETWORK YEAR 2016

Susanne Karlsmose Pedersen, Jens-Ole Frimann, Frank M. Aarestrup, Rene Hendriksen

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Revision in January 2019 includes adjustments in the report to correctly reflect the data in the tables.

List of Abbreviations

AGISAR, WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance AST, Antimicrobial Susceptibility Testing ATCC, American Type Culture Collection CAZ, Ceftazidime CDC, Centers for Disease Control and Prevention CRO, Ceftriaxone CTX, Cefotaxime DTU Food, Technical University of Denmark - National Food Institute EQAS, External Quality Assurance System ESBL, Extended Spectrum Beta-Lactamase **GEN**, Gentamicin **IP**, Institute Pasteur MERO, Meropenem MIC, Minimum Inhibitory Concentration NSSC, National Salmonella and Shigella Center, Thailand PHAC, Public Health Agency of Canada QC, Quality Control SMX, Sulfamethoxazole TET, Tetracycline WHO, World Health Organization WHO GFN, WHO Global Foodborne Infections Network

1. Introduction

Since 2000, 15 WHO External Quality Assurance System (EQAS) reports have been issued with this report being the 16th. The WHO Global Foodborne Infections Network (WHO GFN) and the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) focus on enhancing World Health Organization (WHO) Member States' capacity to detect and respond to foodborne disease outbreaks and the emerging of antimicrobial resistance (AMR) bacterial pathogens by conducting laboratory-based surveillance of *Salmonella* and other important foodborne pathogens. Thus, the WHO EQAS align with the 2015 WHO global action plan to target AMR worldwide, objective 2: Strengthen knowledge through surveillance and research, action 2, laboratory capacity.

Since its inception, the scope of WHO EQAS 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*, *Campylobacter* 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 WHO EQAS is organized annually by DTU Food in collaboration with World Health Organization (WHO) in Geneva, Switzerland; Centers for Disease Control and Prevention (CDC) in Atlanta, USA; 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.

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/WHO AGISAR country representative. All summary conclusions are public. The goal set by WHO GFN/AGISAR aims 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 performing antimicrobial susceptibility testing (AST) of Salmonella with a maximum error rate of 10% (either <5% very major / major errors and <5% minor errors, or <10% minor errors). Minor deviations are defined as classification of an intermediate strain as susceptible, resistant or vice versa (*i.e.* $I \leftrightarrow S$ or $I \leftrightarrow R$). Major deviation is the classification of a susceptible strain as resistant (*i.e.* $S \rightarrow R$). Very major deviation is the classification of a resistant strain as susceptible (*i.e.* $R \rightarrow R$). 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. In EQAS 2014, the regions were redefined for all countries worldwide for the analysis of data from the WHO GFN EQAS. This lead to some reorganization of countries into new regions compared to previous years, why interpretation of regional-based results from 2014 and onwards with results from before 2014 should be conducted with care. The countries belonging to each region is listed in Appendix 1.

Appendices 2-5 present additional background information in relation to the WHO EQAS 2016.

2. Summary

The summary report is divided into five sections; the *Salmonella* components, the *Shigella* components, reporting of ESBL *Salmonella* and *Shigella*, the *Campylobacter* components, and identification of the unknown strain. All results reported in the summary can be found in Appendix 1.

Participation

A total of 196 laboratories responded to the pre-notification and were enrolled in the WHO EQAS. When the deadline for submitting results was reached, 182 laboratories in 81 countries had uploaded data.

The following countries provided data for at least one of the EQAS components (Appendix 1): Argentina, Australia (3), Bahrain, Barbados, Belgium, Bolivia, Brazil (2), Brazil, Brunei Darussalam, Bulgaria, Cambodia, Cameroon, Canada (12), Chile (2), China (18), Colombia (3), Congo, Democratic Republic of the, Costa Rica (2), Croatia, Cyprus, Czech Republic (2), Ecuador (2), Egypt, El Salvador, Gambia (2), Germany (2), Greece (2), Guatemala (2), Honduras, Hungary, India (4), Iran, Islamic rep. Of (3), Iraq, Ireland, Israel, Italy (16), Ivory Coast, Jamaica, Japan, Kenya (3), Korea, Rep of (2), Kosovo, Lao PDR, Luxembourg (2), Madagascar, Malaysia (6), Malta (2), Mauritius, Mexico (2), Morocco (2), New Zealand, Nigeria, Norway, Oman, Panama (2), Paraguay, Peru, Philippines, Poland (4), Portugal, Senegal, Serbia (2), Singapore, Slovakia, Slovenia, South Africa, Spain, Sri Lanka (2), Suriname, Sweden, Taiwan, Thailand (15), Trinidad and Tobago, Turkey (2), Ukraine, United Kingdom, United States of America (5), Uruguay, Venezuela (2), Viet Nam (2), Zambia, Zimbabwe.

The level participation in the WHO EQAS 2016 was the same as at the WHO EQAS 2015.

Salmonella EQAS components

The acceptance threshold for the EQAS *Salmonella* serotyping component was met by 73% (n = 106) of the 146 participating laboratories (Table 1). In addition, 89% (n = 130) of the laboratories tested all eight strains with a total at 90% (n = 1.004) of all tests being correct, representing a slight increase compared to 2015 to one of the best performances observed since the initiation of the EQAS (Table 2). The ability to correctly serotype the internal control strain continued to decrease in 2016 to the lowest level since 2001 at 84%, most likely due to many new laboratories participating in 2016. In 2016, the participation in testing the internal control strain increased from 125 to the highest ever recorded, 159 (Table 3). On a region-based categorization of participating laboratories, the Caribbean and Africa both correctly serotyped between 60% and 62% of the test strains whereas Southeast Asia, Latin America, Central Asia & Middle East correctly serotyped between 79% and 88% of the test strains. The performance of correct serotyping in Europe, China, North America was between 93 and 99% but reached 100% correct serotyping of all eight strains in only Oceania.

In 2016, Russia was the only region not participating (Table 4). In all regions, either a marked or consistent improvement was observed and in line with the other data presented.

The main problem regarding the *Salmonella* serotyping appeared to be associated with all strains included the 2016 trial except for the internal control, WHO S-16.3 (Enteritidis; I 9,12:g,m:-).

WHO 2016 S-16.1 (Bovismorbificans / Hindmarsh, I 6,8:r:1,5), WHO 2016 S-16.2 (Infantis, I 6,7:r:1,5), WHO 2016 S-16.4 (Uganda, I 3,10:1,z13;1,5), WHO 2016 S-16.5 (Stanley, I 4,5,12:d;1,2), WHO 2016 S-16.6 (Heidelberg, I 4,12:r:1,2), WHO 2016 S-16.7 (Altendorf, I 4,12,27:c:1,7), and WHO 2016 S-16.8 (Plymouth, I 9,46:d:z6) revealed considerable levels of deviations, 17.9%, 28.0%, 25.5%, 15.7%, 17.6%, 21.0%, and 26.3%, respectively (Table 5). The level of deviation is surprising since the serovars included the 2016 should not pose major difficulties since the somatic O antigen all belong to the major serogroups such as O:4, O:3,10, O:7, O:8, O:9, O:9,46 and the flagella antigens to well know polyvalent antisera HMA, HMB, and HMD, respectively. It is a concern that many laboratories had difficulties at serotyping that many of the major serovars such as Infantis, Stanley, and Heidelberg which are all well-known often to be multidrug resistant.

Concerning the Salmonella AST component for the EQAS 2016, the performance slightly increased compared to the EQAS of 2015, with a low deviations of 2% minor, 2% major, and 1% very major deviations. Thus, the percentages of critical deviation was 3% (Table 6). Deviations categorized by the tested antimicrobials revealed that ciprofloxacin (CIP), gentamicin (GEN), meropenem (MERO), sulfamethoxazole (SMX) and tetracycline (TET) caused most of the difficulties observed with the following total percentage deviations: 10%, 6%, 6%, 8% and 6%, respectively (Table 7). The deviation to CIP is most likely due to the often observed double zone when performing disk diffusion and to SMX the bacteriostatic effect. TET, however, also often pose difficulties using disk diffusion whereas this is not reflected conducting MIC determination. For the four antimicrobials, CIP, MERO, SMX and TET the deviations resulted in that less than 90% of the laboratories submitted the correct and expected susceptibility interpretation. Thus, it is a concern that 27 laboratories of 106 incorrectly interpreted WHO 2016 S-16.2 (Infantis, I 6,7:r:1,5) as susceptible to MERO, a carbapenem (Table 8). On a region-based categorization of participating laboratories, Africa obtained the highest percentages of total deviations, 9.9 where as China, Southeast Asia, Latin America, Europe, Central Asia & Middle East, North America, and Oceania obtained a slightly lover percentage of total deviations between 0.9% to 6.5%. The performance of 100% correctly antimicrobial susceptibility testing all eight strains was observed in the Caribbean. Russia did not participate in the 2016 EQAS (Table 9).

For the 150 laboratories performing the *Salmonella* AST component (MIC (n = 30)/Disk diffusion (n = 76)), only 71% (106 laboratories) reported data for AST of the control strain *E. coli* ATCC 25922. This is a very alerting number and an almost 10 percentage-point decrease compared to 2015 (Table 10). It is of extreme importance to once again emphasize that this component represents the true indicator for the laboratory as to the performance of AST. It is noteworthy that the WHO EQAS organizers provide free of charge the control strain *E. coli* ATCC 25922 for all new participants in the AST component, why there should not be any excuses not to test this strain.

Shigella EQAS components

The *Shigella* components included in the WHO EQAS consist of serogrouping (i.e. the identification of the species), serotyping (i.e. the further typing of the species), and AST.

For the *Shigella* serogrouping component in EQAS 2016, the deviations observed ranged from 0.0% to 4.9%, for the four *Shigella* strains. This is an acceptable level as the 4.9% was related to one of the four strains whereas the remaining three isolates revealed a maximum deviation of 1.6% (Table 11).

The serotyping component was performed by a total of 77 laboratories for all of the four strains, WHO 2016 SH-16.1 (*S. flexneri*, 1b), WHO 2016 SH-16.2 (*S. boydii*,4), WHO 2016 SH-16.3 (*S. flexneri*,2b), and WHO 2016 SH-16.4 (*S. flexneri*,3a) with deviating results observed between 36.9% and 43.1%, respectively (Table 11).

According to the geographical distribution of the participating laboratories the results, on a regionbased categorization, ranged from 69.2% (Africa) to 93.3% correctly serotyped strains by the Oceania region. No participation from Russia and the Caribbean in this trial (Table 12).

For the results of the *Shigella* AST component, the number of participating laboratories was somehow at the same level as in previous years, with 112 participating laboratories in EQAS 2016. The results obtained were in 96% of the cases in agreement with the expected results and a slightly better than in previous years. Minor, major and very major deviations were observed in 1%, 1%, and 1% of the reported results, respectively (Table 13). Categorizing the tested antimicrobials according to the deviations revealed again as in 2015 that CIP (7.1%) and (CHL) (7.1%) but also SMX (4.1%) and GEN (4.2%) caused difficulties in the AST component (Table 14). The deviations to CIP and SMX was not surprising as the same explanation given for *Salmonella* also comply to *Shigella* (Table 14). For the four antimicrobials, CAZ, CIP, CHL, and SMX the deviations resulted in that less than 90% of the laboratories submitted the correct and expected susceptibility interpretation (Table 15).

A region-based categorization of the results revealed correct test results between 90.3% (Africa) and 98.7% (North America), with Central Asia & Middle East having most critical deviations (7.2%). No participation from Russian in this trial (Table 16).

ESBL EQAS component

A part of the EQAS is to detect and confirm ESBL production in the *Salmonella* and *Shigella* strains. If participating in this component of the EQAS, all strains showing reduced susceptibility to cefotaxime (CTX), ceftazidime (CAZ) ceftriaxone (CRO) and/or meropenem (MERO) should be tested for ESBL, AmpC and carbapenemase production.

For the EQAS 2016, three AmpC-, ESBL-, carbapenemase-producers were included with two *Salmonella* strains (WHO 2016 S-16.2, Infantis and WHO 2016 S-16.6, Heidelberg) and one *Shigella* isolate (WHO 2016 SH-16.3, *S. flexneri* serovar 2b). The *Salmonella* isolate, WHO 2016

S-16.2, Infantis was a carbapenemase-producer whereas WHO 2016 S-16.6, Heidelberg was an AmpC-phenotype. The *Shigella* strain included was an ESBL-producer (WHO 2016 SH-16.3, *S. flexneri* serovar 2b). For the two *Salmonella* strains, the genes accounting for the phenotypes were: bla_{VIM-1} (WHO 2016 S-16.2) and bla_{CMY-2} (WHO 2016 S-16.6) and the confirmatory tests (CAZ/CI:CAZ and CTX/CI:CTX) showed 32% and 24% of deviations in reporting correct results (based on assigned phenotype), respectively. For the *Shigella* strain; WHO 2016 SH-16.3 (*bla*_{OXA-1} and *bla*_{CTX-M14}), deviations of the confirmatory test result as and ESBL-producer was observed to be 4%.

Campylobacter EQAS components

A total of 95 laboratories participated in the identification of the *C. jejuni* WHO 2016 C-16.1 and *C. coli* WHO 2016 C-16.2 strain with a result of 94% and 91% correct species identification, respectively (Table 18). On a region-based characterization, the accuracy in *Campylobacter* identification ranged from 79% (Southeast Asia) to 100% (Africa, Central Asia & Middle East, Caribbean, Oceania, and China regions). No participation from Russia (Table 19).

Concerning the *Campylobacter* AST component in the EQAS 2016, 49 laboratories participated. The overall performance of the AST showed 4.2% major deviations, and 4.0% very major deviations, summing up to a total of 8.2% critical deviations, a two percent-point decrease compared to 2015 (Table 20). From the categorization of the antimicrobials, the results showed problems when testing all of the antimicrobials with most critical deviations to streptomycin with a level of critical deviations at 17.2% (Table 21). For the three antimicrobials, CIP, NAL, and TET the deviations resulted in less than 90% of the laboratories submitting the correct and expected susceptibility interpretation (Table 22).

On a region-based characterization, the performance in Central Asia & Middle East and Caribbean were noteworthy, with a deviation level of 60.0% (n = 1) and 26.7% (n = 2) critical deviations, respectively. In contrast, the North America and Oceanic region perfectly performed the test without deviations. Latin America, China, Europe, and Southeast Asia reported deviations at 3.1 and 15.8%, respectively. In EQAS 2016, no laboratories in the Africa and the Russian region participated in the *Campylobacter* AST component (Table 23).

For the QC strain *Campylobacter jejuni* ATCC 33560 only 42 laboratories reported AST results. Again, we have to emphasize the importance of including this component as it represents the true indicator for the laboratory's performance of AST. For gentamicin (GEN) which has previously shown to cause problems for the participants, the percentage of laboratories reporting a correct AST result for this compound increased once again from 86% to 93% compared to 2015 (Table 24).

Identification of unknown culture EQAS component

For this part of the EQAS, an unknown culture is provided for identification. In EQAS 2016, the unknown strain was the Gram positive *Listeria monocytogenes*.

A total of 137 laboratories participated in this component, with 86.1% identifying the strain correctly.

3. List of Appendices

Appendix 1: Figures and Tables

Appendix 2: Prenotification

Appendix 3: Expected results

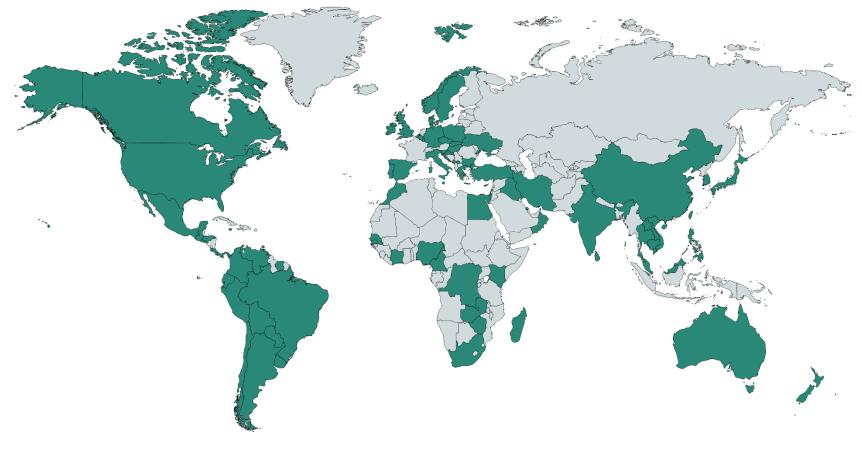
Appendix 4: WHO EQAS 2016 Protocol

Appendix 5a: Subculture and Maintenance of Quality Control Strains

Appendix 5b: Instructions for Opening and Reviving Lyophilized Cultures

Figure and Tables

Figure 1. Countries participating* in the WHO EQAS 2016



*marked in green

List of Countries in the 10 Regions

Africa

Algeria Angola Benin Botswana Burkina Faso Burundi Cameroon Cameroun Cape Verde Central African Republic Chad Comoros Congo (Brazzaville) Congo, Democratic Republic of the Cote d'Ivoire (Ivory Coast) Djibouti Egypt Equatorial Guinea Eritrea Ethiopia

Caribbean

Anguilla Antigua and Barbuda Aruba Bahamas Barbados Bonaire, Saint Eustatius and Saba British Virgin Islands Cayman Islands Cuba Curaçao

Central Asia & Middle East

Afganistan Armenia Azerbaijan Bahrain Bangladesh Bhutan Georgia Hong Kong India Indonesia Iran, Islamic rep. Of Iraq

China

China

Europe

Albania Andorra Austria Gabon Gambia Ghana Guinea Guinea-Bissau Kenva Lesotho Liberia Libyan Arab Jamahiriya Madagascar Malawi Mali Mauritania Mauritius Mayotte Morroco Mozambique Namibia Niger Nigeria

Dominica Dominican Republic Grenada Guadeloupe Haiti Jamaica Martinique Monserrat Puerto Rico Saint Lucia

Israel Jordan Kazakhstan Kuwait Kyrgyzstan Lebanon Macao Maldives Mongolia Myanmar (ex-Burma) Nepal Oman

Guerney and Alderney Hungary Iceland Reunion Rwanda Saint Helena Sao Tome and Principe Senegal Sevchelles Sierra Leone Somalia South Africa South Sudan Sudan Swaziland Tanzania, United Republic of Togo Tunisia Uganda Western Sahara Zambia Zimbabwe

Saint Martin Saint Vincent and the Grenadines Saint-Barthélemy Sint Maarten St. Kitts and Nevis Trinidad and Tobago Turks and Caicos Islands Virgin Islands (US)

Pakistan Palestine Qatar Saudi Arabia Syria Tajikistan Timor Leste (West) Turkmenistan United Arab Emirates Uzbekistan Yemen

Norway Poland Portugal

Romania

Belarus Belgium Bosnia Bulgaria Croatia Cyprus Czech Republic Denmark Estonia European Union Faroe Islands Finland France Germany Gibraltar Greece

Latin America

Argentina Bolivia Brazil Chile Colombia Costa Rica Ecuador

North America

Bermuda Canada

Oceania

Australia Kiribati New Zealand Solomon, Islands Fiji Marshall Islands

Russia

Russia

Southeast Asia

Brunei Darussalam Cambodia Japan Korea, North Korea, Rep of Ireland Italy Jersey Kosovo Latvia Liechtenstein Lithuania Luxembourg Macedonia Malta Man, Island of Moldova Monaco Montenegro Netherlands

El Salvador Falkland Islands (Malvinas) French Guiana Guatemala Guyana Honduras Mexico

Greenland Saint Pierre and Miquelon

Papua New Guinea Tonga French Polynesia Micronesia Samoa Tuvalu

Lao PDR Malaysia Philippines Singapore Sri Lanka San Marino Serbia Slovak Republic Slovakia Slovenia Spain Svalbard and Jan Mayen Islands Sweden Switzerland Turkey Ukraine United Kingdom Vatican City State (Holy See)

Nicaragua Panama Paraguay Peru Suriname Uruguay Venezuela

United States of America

Guam New Caledonia Samoa, American Vanuatu

Taiwan Thailand Viet Nam

Number						Part	icipatir	ng labo	ratorie	s				
of strains correctly serotyped	EQ 20	AS 00	EQ 20		EQ 20		EQ 20		EQ 20		EQ 20			QAS)07
serotypeu	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	3 100	11 96	11 100	1	1	0	0 100	4 127	3 100	3 130	2 100	0	0
In total	37	100	96	100	99	100	127				130	100	140	100
						Par	ticipati	ng labo	oratori	es				
	EQ	AS	EQ	ΔS	EQ	ΔS	EQ	۵S	EQ	۵S	EQ	۵S	EC)AS
		08	20		20		20		20		20)14
0	No. 50	% 33	No.	% 50	No. 91	% 61	No.	% 67	No. 68	% 47	No. 52	% 41	No. 70	% 47
<u>8</u> 7	<u> </u>	33 24	76 29	50 19	91 16	11	82 17	67 14	68 29	20	52 29	41 23	32	21
6	11	7	29 7	5	10	8	17	14 8	 14	10	15	12	32 17	21 11
5	11	9	13	8	9	6	2	8 2	9	6	8	6	6	4
4	14	8	5	8 3	9	5	4	3	5	3	7	6	5	3
4	9	6	7	5	2	1	4	3	6	4	7	6	7	5
2	8	6	5	3	2	1	1	1	10	7	6	5	4	3
1	9	6	6	4	7	5	3	2	2	1	2	2	4	3
0	2	1	5	3	3	2	0	0	1	1	0	0	4	3
In total	151	100	153	100	148	100	123	100	144	100	126	100	149	100
						Part	ticipati	ng labo	oratori	es				
					A		-	0						
	EQ	AS	EQ	AS	Aver EQ									
		15	20		200									
					20									
	No.	%	No.	%	No.	%								
8	65	43	84	58	59	45								
7	25	17	22	15	24	19								
6	17	11	18	12	13	10								
5	22	15	5	3	10	7								
4	5	3	5	3	6	5								
3	2	1	5	3	6	5								
2	4	3	3	2	4	4								
1	7	5	4	3	5	4								
0	4	3	0	0	2	2								
In total	151	100	146	100	128	100								

Table 1. Ability of EQAS participating laboratories to serotype the test Salmonella strains

EQAS iteration		typing all d 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					
2014	85	57	969	92					
2015	104	69	948	87					
2016	130	89	1004	90					
Average	99	76	805	85					

Table 2. EQAS participating laboratories' performance of Salmonella serotyping

Table 3. EQAS participating laboratories' performance of internal quality control strain (WHO S-16.3, *Salmonella* Enteritidis) serotyping).

EQAS iteration		rotyping lis 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
2014	145	98
2015	125	93
2016	159	89
Average	122	94

Region	EQAS iteration	No. of labs	No. of strains serotyped	% strains correctly serotyped	Countries participating in EQAS 2016					
	2001	6	37	73.0						
	2002	9	62	87.1						
	2003	11	70	71.4						
	2004	9	51	62.7						
	2006	16	95	71.6						
_	2007	11	73	80.8	Company Found Made and a					
ica	2008	10	71	49.3	Cameroun, Egypt, Madagascar,					
Africa	2009 2010	15 13	94 83	75.5 67.5	Mauritius, Morocco (2), South					
A	2010	15	83 57	79.2	Africa, The Gambia					
	2011	10	65	60.0						
	2012	8	51	74.5						
	2013	11	63	76.2						
	2015	12	68	61.8						
	2016	8	58	62.1						
	2001	10	60	50.0						
	2002	5	30	83.3						
	2003	5	35	54.3						
	2004	5	33	54.5						
×	2006	5	35	74.3						
Central Asia & Middle East	2007	5	40	55.0						
entral Asia (Middle East	2008	5	34	61.8	Debusin India Inag Isuaal					
ıl ≜ Ile	2009	5	32	46.9	Bahrain, India, Iraq, Israel,					
tra	2010	5	22	75.9	Oman					
Mi	2011	3	23	95.8						
Ŭ	2012	4	30	56.7						
	2013	5	38	52.6						
	2014	7	37	75.7						
	2015	7	44	77.3						
	2016	5	38	78.9						
	2001	0	0	0						
	2002	0	0	0						
	2003	3	18	61.1						
	2004	2 3	8	87.5						
_	2006 2007	2	14 9	78.6 77.8						
Caribbean	2007	3	14	78.6						
pé	2008	3	12	83.3	Barbados, Trinidad and Tobago					
rit	2010	2	13	92.9	Darbados, Timudad and Tobago					
Ca	2011	1	7	87.5						
•	2012	2	16	62.5						
	2013	1	5	100.0						
	2014	3	15	60.0						
	2015	5	24	58.3						
	2016	2	16	60						
	2001	43	323	80.5						
	2002	50	384	90.0						
	2003	60	401	84.8	Belgium, Bulgaria, Croatia,					
	2004	57	392	84.7	Cyprus, Czech Republic (2),					
	2006	52	403	86.4						
c)	2007	54	415	89.4	Germany (2), Greece (3),					
Europe	2008	50	379	82.3	Hungary, Ireland, Italy (16),					
nre	2009	47	362	93.1	Luxembourg (2), Malta, Norway,					
Ē	2010	45 42	332	94.1	Poland (3), Portugal, Serbia (2),					
	2011	42	314	94.6 92.9	Slovak Republic, Spain, Sweden,					
	2012 2013	47 42	368 309	92.9 94.5	Turkey (2), Ukraine, United					
	2013 2014	42 52	309 391	94.5 96.2	Kingdom					
	2014 2015	48	371	93.8	Tringdom					
	201 5 201 6	46 46	362	93.6 93.4						
	2010	40	502	73.4						

Table 4. 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 2016
North America	2001 2002 2003 2004 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016	4 2 6 8 10 12 11 12 13 11 14 13 13 13 13 13	32 16 41 55 80 94 84 90 103 81 101 92 84 93 100	87.5 100.0 95.1 81.8 96.3 97.9 95.2 92.2 100.0 97.6 93.1 97.8 100.0 100.0 99.0	Canada (9), United States of America (4)
Oceania	2001 2002 2003 2004 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016	4 6 5 5 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	30 43 46 38 37 32 30 32 32 32 32 32 31 32 31 32	100.0 93.0 93.5 97.4 94.6 100.0 93.3 96.9 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0	Australia (3), New Zealand
Russia	2001 2002 2003 2004 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016	1 1 4 5 8 6 7 8 7 6 2 4 3	8 8 7 26 40 51 40 49 54 48 48 16 30 24	12.5 62.5 14.3 69.2 80.0 80.4 90.0 91.8 87.1 87.3 87.5 75.0 93.3 100.0	- none -
Latin America	2001 2002 2003 2004 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016	11 11 13 15 13 15 17 21 22 23 25 22 24 20 23	78 82 83 88 84 107 120 150 132 144 182 154 166 133 165	57.7 87.8 75.9 79.5 84.5 88.8 71.7 77.3 80.0 83.7 73.1 83.1 84.9 84.2 87.9	Argentina, Bolivia, Brazil (2), Chile (2), Colombia (3), Costa Rica (2), Ecuador (2), Honduras, Mexico (2), Panama (2), Paraguay, Peru, Uruguay, Venezuela (2)

Table 4 (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 2016					
	2001	15	113	54.0						
	2002	12	90	92.2						
	2003	15	100	81.0						
	2004	17	130	81.5						
ia	2006	15	117	84.6	Brunei Darussalam, Cambodia,					
As	2007	19	140	91.4						
st	2008	18	125	81.6	Japan, Korea, Rep of (2), LAO					
lea	2009	23	180	81.1	PDR, Malaysia (5), Philippines,					
Southeast Asia	2010	24	172 180	90.5	Singapore, Sri Lanka, Taiwan,					
00	2011 2012	23		98.4 77.8	Thailand (11), Viet Nam (2)					
0 2	2012	28 22	207 163	77.8 89.6						
	2013	22	165	89.0 94.6						
	2014 2015	22	179	94.0 88.3						
	2013 2016	24 28	211	88.5 87.7						
	2010	4	32	96.9						
	2001	3	24	100.0						
	2002	8	60	75.0						
	2003	7	46	78.3						
	2004	6	48	85.4						
	2007	10	80	91.3						
a	2008	15	108	94.4						
China	2009	16	126	95.2	China (17)					
C	2010	10	74	92.5						
	2012	10	78	80.8						
	2012	7	54	92.6						
	2013	9	71	93.0						
	2014	15	118	78.0						
	2015	17	136	95.6						

Table 4 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* serotyping

Table 5. Salmonella serogroups (SG), serotypes (ST) and deviations (D), WHO EQAS 2016

Strain ID	Correct s	erotype	No. of labs reporting SG	% D _{SG}	No. of labs reporting ST	% D _{ST}	Deviating results (*)
WHO 2016 S-16.1	Bovismorbificans / Hindmarsh	I 6,8:r:1,5	156	5.8	156	17.9	Bsilla (2), Chailey, Diogoye, Goldcoast, Hidalgo, Haardt, Infantis (2), Takoradi, Utah
WHO 2016 S-16.2	Infantis	I 6,7:r:1,5	157	1.9	157	28.0	Aequatoria, Austin, Bulovka, Escanaba, Grampian (2), I 6,7:-:-, IV 6,7:z36:-, Lille (2), Nigeria (3), Oranienburg, Othmarschen, Papuana (2), Paratyphi C, Rumford, Thompson (2), Virchow (3)
WHO 2016 S-16.3	Enteritidis	I 9,12:g,m;-	159	1.3	159	10.7	Dublin, Essen, Hillingdon
WHO 2016 S-16.4	Uganda	I 3,10:1,z13;1,5	157	5.1	157	25.5	Assinie, Butantan, Chittagong, Freiburg, Harrisonburg, Joal , Langensalza, Lexington, Parkroyal, Sinstorf (5), Stuivenberg (2), Tyresoe, Uganda var. 15+ (3), Ughelli
WHO 2016 S-16.5	Stanley	I 4,5,12:d;1,2	159	0.6	159	15.7	Brezany, Bury, Eppendorf (2), Paratyphi B, Typhimurium
WHO 2016 S-16.6	Heidelberg	I 4,12:r:1,2	159	0.0	159	17.6	Albert, Altendorf, Ball, Bochum, Bradford, Fyris, Saintpaul, Southampton, Typhimurium (2)
WHO 2016 S-16.7	Altendorf	I 4,12,27:c:1,7	157	0.6	157	21.0	Abony, Arechavaleta, Haifa, Indiana (2), Kubacha (2), Kaapstad, Lagos, Legon, Schwarzengrund, Tafo, Togo, Travis
WHO 2016 S-16.8	Plymouth	I 9,46:d:z6	156	23.7	156	26.3	Niloese, Tarshyne (3), Typhi (2), Zega (18)

*number of participants reporting the specified deviating result

EQAS iteration	No. of EQAS participating laboratories	% correct test results	% minor deviations (S \leftrightarrow I or I \leftrightarrow R)^	% major deviations $(S \rightarrow R)^{\wedge}$	% very major deviations (R→ S)^	% critical deviations $(R \rightarrow S \& S \rightarrow R)^{\wedge}$	% total deviations $(S \rightarrow R \& R \rightarrow S \& S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$
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
2014	155	95	3	1	1	2	5
2015	155	92	4	2	1	4	8
2016	150	95	2	2	1	3	5
Average*	139	93	4	2	1	3	7

Table 6. EOAS participating laboratories ²	performance of antimicrobial susceptibility testing of <i>Salmonella</i> strains

*Data do not include one strain which may have lost resistance due to transport or storage stress ^S, susceptible; I, intermediate; R, resistant

EQAS	No.						Antimicrobial [∞]														
iteration	of labs	Performance	AMC	AMP	CAZ	CHL	CIP	POD	CRO	СТХ	GEN	KAN	NAL	SMX	MER	STR	SXT	TET	ТМР	XNL	OVERALL average
		No. of tests	-	343	-	343	334	-			343	312	328	248		312	-	335	295	-	798
2000	44	% critical deviations*	-	6	-	4	1	-			4	4	1	3		4	-	6	1	-	6
		% total deviations^	-	8	-	7	6	-			5	16	4	5		12	-	13	1	-	14
		No. of tests	-	822	-	814	813	-			821	623	726	431		679	757	804	416	-	1778
2001	108	% critical deviations*	-	4	-	2	1	-			2	2	2	6		7	2	7	1	-	б
		% total deviations^	-	7	-	3	4	-			4	7	8	9		27	5	18	2	-	15
		No. of tests	-	918	-	903	911	-			905	680	885	495		718	724	861	499	-	1961
2002	119	% critical deviations*	-	2	-	2	0	-			2	2	2	4		4	7	3	3	-	5
		% total deviations^	-	3	-	3	2	-			16	10	4	4		34	10	7	3	-	15
		No. of tests	-	1019	-	996	995	-			993	738	947	615		768	929	995	582	-	2210
2003•	147	% critical deviations*	-	2	-	1	0	-			2	2	1	4		9	2	4	1	-	5
		% total deviations^	-	4	-	2	1	-			2	6	4	5		39	2	11	1	-	12
		No. of tests	973	1178	-	1159	1162	-	-	995	1201	-	1130	734		947	1051	1122	729	-	2653
2004	152	% critical deviations*	6	3	-	2	0	-	-	0	2	-	1	5		1	3	5	2	-	5
		% total deviations^	12	5	-	2	1	-	-	14	3	-	4	8		21	4	11	2	-	13
		No. of tests	950	1092	769	1060	1110	305	-	956	1078	-	1035	649		896	996	1054	607	225	2256
2006	143	% critical deviations*	9	2	7	3	2	1	-	7	3	-	2	6		5	3	9	1	2	8
		% total deviations^	22	3	11	15	6	26	-	15	7	-	6	7		22	5	20	2	9	21
		No. of tests	908	1114	830	1105	1101	389	-	914	1111	-	1092	678		875	971	1047	583	258	2290
2007	143	% critical deviations*	6	5	1	0	1	4	-	1	3	-	2	5		4	3	4	1	0	5
		% total deviations^	17	7	1	6	1	16	-	2	4	-	3	6		26	3	11	2	6	13
		No. of tests	-	1331	961	1226	1307	-	791	1104	1265	-	1168	718		867	1155	1249	696	-	2769
2008	168	% critical deviations*	-	3	3	1	19	-	3	3	4	-	2	4		7	3	6	2	-	8
		% total deviations^	-	8	6	11	21	-	6	6	6	-	4	5		25	4	13	2	-	16
		No. of tests	-	1206	921	1108	1190	-	775	1009	1143	-	1095	624		864	1042	1114	616	-	2541
2009	153	% critical deviations*	-	3	1	1	8	-	0	1	2	-	1	7		9	3	4	1	-	6
		% total deviations^	-	6	1	2	10	-	1	2	3	-	3	9		30	4	10	1	-	11
		No. of tests	-	1173	937	1118	1194	-	787	1026	1133	-	1096	566		800	1012	1134	604	-	2516
2010	152	% critical deviations*	-	4	2	1	3	-	4	4	5	-	1	14		19	4	5	1	-	9
		% total deviations^	-	5	3	2	3	-	8	8	6	-	2	17		55	4	9	1	-	17

Table 7. EQAS participants' performance of Salmonella strains antimicrobial susceptibility testing categorized by antimicrobial

EQAS	No. of										An	timicro	bial∞								
iteration	labs	Performance	AMC	AMP	CAZ	CHL	CIP	POD	CRO	СТХ	GEN	KAN	NAL	SMX	MER	STR	SXT	TET	TMP	XNL	OVERALL Average
		No. of tests	-	1099	829	988	1070	-	744	909	999	-	993	542	-	682	988	1017	493	-	2271
2011	127	% critical deviations*	-	5	3	2	20	-	3	4	4	-	7	4	-	3	3	4	1	-	9
		% total deviations^	-	6	4	2	21	-	3	6	5	-	15	5	-	42	3	10	2	-	17
		No. of tests	-	1228	993	1159	1245	-	834	1058	1161	-	1136	584	-	814	1054	1163	613	-	2608
2012	159	% critical deviations*	-	3	2	1	11	-	2	4	3	-	2	5	-	2	1	2	1	-	5
		% total deviations^	-	5	2	2	12	-	3	5	4	-	4	7	-	35	2	5	1	-	12
		No. of tests	-	1121	898	1027	1134	-	763	1011	1086	-	1027	491	-	-	946	1060	545	-	2381
2013	145	% critical deviations*	-	2	3	0	2	-	1	3	3	-	2	4	-	-	2	3	2	-	4
		% total deviations^	-	3	3	1	18	-	2	6	6	-	6	5	-	-	2	5	2	-	9
		No. of tests	-	1176	1003	1072	1161	-	817	1014	1147	-	1078	561	-	-	1039	1107	541	-	2511
2014	155	% critical deviations*	-	3	3	1	3	-	1	2	3	-	1	5	-	-	2	3	2	-	4
		% total deviations^	-	4	4	2	19	-	2	3	5	-	2	6	-	-	3	5	2	-	9
		No. of tests	-	1176	1010	1064	1172	-	787	1018	1145	-	1010	514	611	-	1034	1077	591	-	2468
2015	155	% critical deviations*	-	3	9	2	1	-	3	5	3	-	4	7	1	-	2	2	2	-	6
		% total deviations^	-	5	11	22	14	-	4	6	5	-	10	9	1	-	3	5	2	-	13
		No. of tests	-	1133	988	1020	1100	-	800	968	1104	-	959	529	838	-	953	1042	599	-	2407
2016	150	% critical deviations*	-	4	4	1	1	-	2	4	4	-	1	7	5	-	2	3	2	-	8
		% total deviations^	-	5	4	2	10	-	3	4	6	-	3	8	6	-	2	6	2	-	12
		No. of tests	944	1071	922	1010	1062	347	789	999	1040	588	982	561	725	769	977	1011	563	242	1354
Average•	139	% critical deviations*	7	3	3	2	5	3	2	3	3	3	2	6	3	6	3	4	2	1	3
		% total deviations^	17	5	5	4	9	21	4	6	5	10	5	7	4	31	4	10	2	8	9

Table 7 (continued). EQAS participants' performance of *Salmonella* strains antimicrobial susceptibility testing categorized by antimicrobial.

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

*R \rightarrow S & S \rightarrow R (R, resistant; S, susceptible)

^S \rightarrow R & R \rightarrow S & S \leftrightarrow I or I \leftrightarrow R (I, intermediate)

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

-, not determined

Strain	Antimicrobial^													
	AMP	СТХ	CAZ	CRO	CHL	CIP	GEN	MER	NAL	SMX	TET	TMP	SXT	
WHO S- 16.1	139 /2/3	6/2/113	5/0/ 119	1/1/ 98	1/1/ 125	1/12/ 125	4/3/132	3/0/103	1/1/ 117	65 /0/2	129 /0/2	77/0/0	120 /0/1	
WHO S- 16.2	140 /1/1	122/0/0	124/0/0	100 /1/0	127/0/0	1/9/ 128	7/5/126	74 /5/27	0/1/ 119	62 /0/3	4/3/ 121	73 /0/1	117 /0/2	
WHO S- 16.3	10/6/ 125	9/1/ 111	8/3/113	3/1/ 96	3/4/121	1/18/ 119	132 /4/3	4/0/101	1/2/ 117	58 /0/9	7/2/123	1/1/ 72	4/0/116	
WHO S- 16.4	5/0/136	3/0/118	5/0/ 119	1/1/ 98	2/1/ 125	0/8/131	6/2/130	2/0/102	0/2/119	9/1/ 57	2/2/ 127	4/0/ 71	1/1/ 117	
WHO S- 16.5	7/1/ 134	3/0/118	5/0/ 119	2/0/98	124 /1/3	0/8/129	6/4/ 128	2/0/102	3/2/115	62 /0/4	104/ 13/14	70 /0/4	115/0/4	
WHO S- 16.6	135 /1/3	114 /1/5	120 /0/1	96 /0/3	125 /1/1	2/16/ 120	5/1/ 129	4/0/102	3/2/113	62 /2/3	128 /2/0	0/0/75	3/2/112	
WHO S- 16.7	6/0/ 136	4/2/115	8/1/ 114	3/0/98	3/0/125	1/19/ 116	4/2/ 132	1/0/102	1/5/ 115	4/2/60	5/1/ 125	1/0/ 74	3/0/117	
WHO S- 16.8	5/1/ 136	5/0/116	5/1/ 118	2/1/ 96	2/2/123	1/10/ 125	4/2/133	2/0/102	1/2/ 117	1/2/ 61	2/2/ 124	1/0/ 74	0/0/118	

Table 8. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2016 Salmonella strains*

^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.

Region	EQAS iteration	No. of labs	% correct test result	% minor deviations $(S \leftrightarrow I \text{ or} I \leftrightarrow R)^{\wedge}$	% major deviations $(S \rightarrow R)^{\wedge}$	% very major deviations $(R \rightarrow S)^{\wedge}$	% critical deviations $(S \rightarrow R \& R \rightarrow S)^{\wedge}$		Countries participating in the 2016 iteration
	2001	7	80.1	9.6	7.7	2.5	10.2	19.8	
	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	Cameroun, Congo,
	2007	16	90.7	4.4	4.0	0.9	4.9	9.3	Democratic Republic of
g	2008	19	83.8	6.5	5.5	4.2	9.7	16.2	the, Egypt, Ivory Coast,
Africa	2009	22	90.1	4.5	3.6	1.8	5.4	9.9	Kenya (3), Madagascar, Mauritius, Morocco (2),
A	2010	22	84.7	6.0	6.5	2.8	9.3	15.3	Nigeria, Senegal, South
	2011	17	87.0	5.0	4.7	3.3	8.0	13.0	Africa, The Gambia (2),
	2012	18	89.4	5.3	3.5	1.9	5.4	10.6	Zambia, Zimbabwe
	2013	16	92.0	3.2	4.0	0.9	4.9	8.0	,
	2014	20	92.5	3.8	2.0	1.7	3.7	7.5	
	2015	22	86.7	7.3	4.1	1.9	6.0	13.3	
	2016	18	90.1	4.6	4.2	1.1	5.3	9.9	
	2001	10	87.7	6.3	5.2	0.8	6.0	12.3	
	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	
Cast	2004	10	87.5	6.7	5.5	0.3	5.8	12.5	
le F	2006	7	79.2	10.5	9.8	0.5	10.3	20.8	
lbb	2007	8	87.8	5.0	6.2	1.1	7.3	12.2	
Mi	2008	12	86.1	6.5	4.0	3.4	7.4	13.9	Bahrain, India (4), Iran,
Ś	2009	6	93.7	4.3	0.9	1.1	2.0	6.3	Islamic rep. Of (3), Iraq,
vsia	2010	7	95.8	2.6	0.2	1.4	1.6	4.2	Israel, Oman
Central Asia & Middle East	2011	4	91.8	4.1	1.8	2.3	4.1	8.2	
ntra	2012	8	92.8	4.4	1.6	0.7	2.3	6.6	
Cei	2013	8	93.6	5.2	1.0	0.1	1.2	6.4	
-	2014	17	91.0	4.2	2.9	2.0	4.9	9.0	
	2015	14	91.4	4.3	2.3	2.1	4.4	8.6	
	2016	11	95.5	0.9	1.8	1.8	3.6	4.5	
	2001	2	83.5	9.5	7.0	0.0	7.0	16.5	
	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	
E	2007	4	95.0	3.1	0.9	0.9	1.8	5.0	
Caribbean	2008	5	90.7	5.5	0.9	2.9	3.8	9.3	
ibł	2009	4	93.2	1.8	3.2	1.8	5.0	6.8	Barbados, Jamaica
Car	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	
	2014	4	78.3	4.7	9.4	7.6	17.0	21.7	
	2015	4	87.5	6.6	3.7	2.2	5.9	12.5	
	2016	2	100.0	0.0	0.0	0.0	0.0	0.0	

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

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

			public te	Ŭ	0 (A (A (~
Region	EQAS iteration	No. of labs	% correct test result	% minor deviations $(S \leftrightarrow I \text{ or} I \leftrightarrow R)^{\wedge}$	% major deviations $(S \rightarrow R)^{\wedge}$	% very major deviations $(R \rightarrow S)^{\wedge}$	% critical deviations $(S \rightarrow R \& R \rightarrow S)^{\wedge}$	% total deviations $(S \rightarrow R \& R \rightarrow S \& S \leftrightarrow I \text{ or} I \leftrightarrow R)^{\wedge}$	Countries participating in the 2016 iteration
	2001	47	91.3	5.7	2.7	0.3	3.0	8.7	
	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	Belgium, Bulgaria,
	2006	54	88.7	7.0	3.8	0.6	4.4	11.3	Croatia, Cyprus, Czech Republic, Greece (3),
	2007	49	94.2	3.7	1.6	0.4	2.0	5.7	Hungary, Ireland, Italy
ЭС	2008	51	91.2	4.4	2.5	1.9	4.4	8.8	(9), Kosova,
Europe	2009	40	95.1	2.6	1.3	0.9	2.2	4.8	Luxembourg (2), Malta
Eu	2010	39	92.4	4.1	1.2	2.3	3.5	7.6	(2), Norway, Poland (2),
	2011	36	92.5	4.5	1.7	1.3	3.0	7.5	Portugal, Serbia (2),
	2012	40	95.5	2.8	1.2	0.4	1.7	4.5	Slovak Republic, Spain,
	2013	37	95.7	2.5	1.4	0.3	1.7	4.2	Turkey (2), Ukraine,
	2014	40	96.6	2.1	0.8	0.5	1.3	3.4	United Kingdom
	2015	38	93.4	4.1	1.3	1.2	2.5	6.6	
	2016	36	96.9	1.5	1.2	0.5	1.6	3.1	
	2001	4	95.8	3.8	0.0	0.4	0.4	4.2	
	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	
ica	2007	10	95.4	2.9	0.8	0.8	1.6	4.6	
North America	2008	14	96.4	0.6	0.4	2.6	3.0	3.6	
An	2009	10	98.7	0.0	0.4	0.9	1.3	1.3	Canada (5), United States of America (3)
rth	2010	11	94.8	2.6	0.2	2.4	2.6	5.2	of America (5)
10N	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	
	2014	8	96.9	2.2	0.4	0.6	0.9	3.1	
	2015	8	94.5	2.0	0.8	2.8	3.6	5.5	
	2016	8	99.1	0.2	0.0	0.7	0.7	0.9	
	2001	6	91.8	4.7	2.7	0.9	3.6	8.2	
	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	
nia	2008	4	93.9	3.8	0.0	2.3	2.3	6.1	
Oceania	2009	4	95.9	3.2	0.3	0.6	0.9	4.1	Australia (2), New Zealand
ŏ	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	
	2014	5	97.4	2.0	0.0	0.6	0.6	2.6	
	2015	5	95.3	3.8	0.5	0.5	1.0	4.8	
	2016	3	98.1	0.0	0.5	1.4	1.9	1.9	

Region	EQAS	No.	% correct	% minor	% major	% very	% critical	% total	Countries participating
Kegion	iteration	of labs	test result	deviations (S \leftrightarrow I or I \leftrightarrow R)^	deviations $(S \rightarrow R)^{\wedge}$	$\begin{array}{c} \textbf{major} \\ \textbf{deviations} \\ (R \rightarrow S)^{\wedge} \end{array}$	deviations $(S \rightarrow R \& R \rightarrow S)^{\wedge}$	$\frac{\text{deviations}}{(S \rightarrow R \& R \rightarrow S \& S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}}$	in the 2016 iteration
	2001	1	81.9	15.3	2.8	0.0	2.8	18.1	
	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	
đ	2008	6	92.2	4.7	1.4	1.7	3.1	7.8	
Russia	2009	6	93.8	2.1	3.3	0.8	4.1	6.2	- none -
Ru	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	
	2014	4	98.2	0.3	0.9	0.6	1.5	1.8	
	2015	4	98.7	1.0	0.0	0.3	0.3	1.3	
	2016	-	-	-	-	-	-	-	
	2001	11	90.8	6.9	1.4	1.0	2.4	9.2	
	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	Argentina, Bolivia, Brazil
ca	2007	17	94.9	1.8	1.9	1.4	3.3	5.0	(2), Chile (2), Colombia
eri	2008	20	93.0	3.4	1.5	2.1	3.6	7.0	(3), Costa Rica (2),
- m	2009	20	95.6	2.1	1.1	1.2	2.3	4.4	Ecuador (2), El Salvador, Guatemala (2), Honduras,
in /	2010	23	90.8	2.1	5.6	1.4	7.1	9.2	Mexico, Panama,
Latin America	2011	22	90.8	2.8	3.1	3.3	6.4	9.2	Paraguay, Peru, Suriname,
-	2012	25	94.4	1.6	3.0	1.0	4.0	5.6	Uruguay, Venezuela
	2013	25	95.5	2.6	1.2	0.3	1.5	4.2	
	2014	24	96.5	1.9	1.1	0.6	1.7	3.5	
	2015	20	94.9	3.8	0.6	0.7	1.3	5.1	
	2016	24	95.6	2.5	1.4	0.5	1.9	4.4	
	2001	4	98.9	0.8	0.0	0.3	0.3	1.1	
	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	
ina	2008	18	92.8	3.7	0.8	2.7	3.5	7.2	Ching(1C)
China	2009	14	94.8	2.2	2.1	0.8	2.9	5.1	China (16)
	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	
	2014	8	97.0	1.2	0.1	1.6	1.8	3.0	
	2015	15	92.8	2.0	4.0	1.1	5.1	7.2	
	2016	16	96.7	0.4	1.8	1.1	2.9	3.3	
AC			iate: R. resist	ant					

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

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

Region	EQAS iteration	No. of labs	% correct test result	% minor deviations $(S \leftrightarrow I \text{ or} I \leftrightarrow R)^{\wedge}$	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations $(S \rightarrow R \& R \rightarrow S)^{\wedge}$	% total deviations $(S \rightarrow R \& R \rightarrow S \& S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$	Countries participating in the 2015 iteration
	2001	16	88.1	7.7	2.3	1.9	4.2	11.9	
	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	
Asia	2007	20	93.9	4.0	1.4	0.7	2.1	6.1	Cambodia, Japan,
t A	2008	19	90.5	4.7	2.2	2.6	4.8	9.5	Korea, Rep of (2), LAO
Southeast	2009	27	91.8	4.1	3.0	1.2	4.2	8.3	PDR, Malaysia (5), Philippines, Sri Lanka
Ithe	2010	25	92.8	3.8	1.5	1.9	3.4	7.2	(2), Taiwan, Thailand
Sot	2011	26	90.5	3.5	2.4	3.5	5.9	9.5	(10), Viet Nam
	2012	35	91.7	3.9	3.5	0.9	4.4	8.3	(10), 1001100
	2013	35	93.4	3.2	2.5	0.7	3.2	6.4	
	2014	8	97.0	1.2	0.1	1.6	1.8	3.0	
	2015	25	89.9	6.0	2.6	1.5	4.1	10.1	
	2016	30	93.5	2.2	3.5	0.8	4.3	6.5	

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

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

		Method	Perfor- mance ^{4.5}	AMP	CAZ	CHL	CIP	CRO	СТХ	FIS (SMX) ²	FOX	GEN	MER	NAL	STR	SXT	ТЕТ	ТМР
Ac	cepted	MIC (µg/ml)		2-8	0.06-0.5	2-8	0.004-0.016	0.03-0.12	0.03-0.12	8-32	2-8	0.25-1	0.008-0.06	1-4	4-16 ³	≤0.5/9.5	0.5-2	0.5-2
	erval ¹	Disks (mm)		15-22	25-32	21-27	30-40	29-35	29-35	15-23	23-29	19-26	28-34	22-28	12-20	23-29	18-25	21-28
	2000 (44)	MIC & Disk	No.4 % ⁵	37 27	-	38 37	35 20	-	-	19 53	-	39 23	-	37 35	36 22	-	42 42	31 30
	2001	MIC & Disk	No. ⁴	97	-	97	97	-	-	53	-	99	-	74	81	90	96	50
	(107) 2002	MIC & Disk	% ⁵ No. ⁴	19 109	-	20 107	14 108	-	-	34 57	-	12 108	-	14 102	12 82	14 102	22 102	22 66
	(114) 2003		% ⁵ No. ⁴	16 140	-	15 137	14 138	-	-	26 82	-	12 138	-	14 132	11 105	12 129	13 137	11 79
	(144)	MIC & Disk		140	-	22	9	-	-	<u>82</u> 17	-	9	-	152	9	129	137	14
(s	2004 (140)	MIC & Disk	<u>No.</u> ⁴ % ⁵	132 10	-	128 13	132 8	-	111 18	84 16	-	134 10	-	126 9	110 6	120 11	129 13	87 9
of participants)	2006	MIC & Disk	No. ⁴	133	96	126	127	-	115	74	-	131	-	122	106	122	125	74
icip	(137) 2007		% ⁵ No. ⁴	14 124	15 92	18 123	<u>8</u> 121	-	21 104	29 64	-	14 124	-	20 120	<u>11</u> 97	<u>19</u> 107	<u>12</u> 117	<u>17</u> 67
arti	(126)	MIC & Disk	% ⁵	124	92	125	121	-	104	22	-	6	-	7	6	107	7	10
of p		MIC & Disk	<u>No.</u> ⁴ % ⁵	147 12	<u>111</u> 9	135 10	<u>144</u> 8	-	124 14	71 14	-	145 8	-	136 8	101	129 13	139 7	<u>79</u> 13
	2008	MIC	No. ⁴	33	23	24	33	-	23	14	-	31	-	23	12	22	28	16
(total no.	(147)	WIIC	% ⁵	0	5	0	6	-	9	11	-	0	-	0	11	9	0	13
tota		Disk	<u>No.4</u> % ⁵	114 16	<u>89</u> 10	112 12	111 8	-	101 15	53 15	-	<u>114</u> 11	-	113 10	<u>82</u> 12	107 14	<u>111</u> 9	<u>63</u> 13
		MIC & Disk	No. ⁴	128	100	121	124	88	107	63	-	123	-	117	98	113	122	70
iteration	2009		% ⁵ No. ⁴	16 27	13 19	15 24	7 26	16 20	10 20	11 14	-	18 25	-	13 24	10 19	14 21	<u>14</u> 27	11 25
.at	(129)	MIC (27)	<u>No.</u>	11	19	<u></u> 8	20	15	20 15	21	-	12		24 8	19 5	19	11	13
teı	(12))	Disk (102)	No. ⁴	101	81	97	98	68	87	49	-	98	-	93	79	92	95	55
		DISK (102)	% ⁵	16	14	16	6	16	9	10	-	18	-	14	11	12	15	11
EQAS		MIC & Disk	No.4	114	97	108	115	79	100	51	-	112	-	104	84	101	110	63
Õ	2010		% ⁵ No. ⁴	11 25	<u>9</u> 15	<u>9</u> 21	<u>6</u> 25	10	14	11	-	11 24	-	5 19	5	12	5 24	<u>15</u> 11
E	(116)	MIC (24)	<u>No.</u> % ⁵	12	20	10	<u> </u>	<u>15</u> 7	17	8	-	13	-	19	17	17	<u></u> 17	36
	(110)		70 No. ⁴	89	82	87	90	64	83	39	-	88		85	67	84	86	52
		Disk (91)	% ⁵	9	6	8	4	9	11	10	-	9	-	2	1	10	1	8
		MIC & Disk	No. ⁴	111	89	102	109	76	96	50	-	103	-	103	72	99	107	51
		WIIC & DISK	% ⁵	17	4	11	7	7	9	8	-	11	-	8	4	16	7	14
	2011	MIC (23)	No. ⁴	23	15	18	22	16	15	13	-	22	-	19	17	16	21	11
	(112)	MIC (23)	% ⁵	4	7	0	9	6	0	8	-	9	-	0	6	6	5	0
		Disk (89)	No.4	88	74	84	87	60	81	37	-	81	-	84	55	83	86	40
			% ⁵	20	4	13	7	7	11	8	-	11	-	10	4	18	8	18

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

		Method	Perfor- mance ^{4.5}	AMP	CAZ	CHL	CIP	CRO	СТХ	FIS (SMX) ²	FOX	GEN	MER	NAL	STR	SXT	ТЕТ	ТМР
Acc	epted	MIC (µg/ml)		2-8	0.06-0.5	2-8	0.004-0.016	0.03-0.12	0.03-0.12	8-32	2-8	0.25-1	0.008-0.06	1-4	4-16 ³	≤0.5/9.5	0.5-2	0.5-2
inte	rval ¹	Disks (mm)		15-22	25-32	21-27	30-40	29-35	29-35	15-23	23-29	19-26	28-34	22-28	12-20	23-29	18-25	21-28
		MIC & Disk	No. ⁴	134	111	121	131	90	115	53	-	127	-	121	89	112	129	66
		MIC & DISK	% ⁵	13	12	7	6	11	10	11	-	9	-	9	8	13	10	21
	2012	MIC (37)	No. ⁴	37	26	31	35	23	28	19	-	35	-	31	26	23	35	22
	(135)		% ⁵	3	4	0	3	0	4	5	-	3	-	3	8	0	0	9
		Disk (98)	No.4	97	85	90	96	67	87	34	-	92	-	90	63	89	94	44
		Disk (90)	% ⁵	16	14	9	7	15	11	15	-	11	-	11	8	16	14	27
ts)		MIC & Disk	No.4	117	100	112	119	82	107	44	-	113	-	113	-	101	114	59
participants)			% ⁵	12	7	5	7	4	8	10	-	6	-	11	-	8	8	11
üp.	2013	MIC (33)	No.4	31	25	28	32	19	27	17	-	32	-	28	-	22	32	22
Ţ.	(122)	- ()	% ⁵	6	4	4	13	5	11	18	-	9	-	11	-	5	6	5
pai		Disk (89)	No.4	86	75	84	87	63	80	27	-	81	-	85	-	79	82	37
of]			% ⁵	13	8	6	5	5	6	7	-	4	-	9	-	10	7	8
		MIC & Disk	No.4 % ⁵	111	99 7	101	108	75	97	49	-	111	-	103	-	102	104	50
no.	2014			5	/	,	6	/	14	14	-	8	-	8	-	8		2
(total	2014 (115)	MIC (28)	No.4 % ⁵	27 4	21 5	24 4	27	16	22	16	-	28	-	24	-	21	25 0	12
(to	(115)		7.0		-		15	<u>6</u> 59	14	0	-	14	-	8 79	-	14	0 79	
		Disk (87)	No.4 % ⁵	84 6	78 8	77 8	<u>81</u> 4	59 7	75	33	-	83	-	79 8	-	81 6	<u> </u>	<u>38</u> 3
i 0			No.4	113	101	8 101	4 112	78	15 99	21 54	- 75	6 112	- 74	100	-	104	9 106	<u> </u>
iteration		MIC&Disk		8	5	7	7	9	6	11	9	9	12	100	-	104	8	9
ter	2015		70 No. ⁴	30	26	25	30	16	25	11	20	30	12	24	-	24	27	16
÷	(117)	MIC (31)	<u>%</u> 5	30	8	4	13	0	12	7	10		19	4	-	8	7	13
EQAS	(117)		No.4	83	75	76	82	62	74	39	55	82	55	76		80	79	41
Ò		Disk (85)	% ⁵	10	4	8	5	11	4	13	9	10	13	8	_	14	8	7
E			No.4	101	93	95	101	76	94	54	84	99	88	91	_	91	97	59
		MIC&Disk	% ⁵	101	5	13	9	16	15	24	7	8	10	9	_	8	10	14
	2016		⁷⁰ No. ⁴	27	24	24	27	10	24	13	22	29	25	20	-	20	25	
	2016	MIC (30)					27	-	1			-			-		-	16
	(106)		% ⁵	4	4	0	/	12	4	23	0	3	4	0	-	0	8	13
		Disk (76)	No.4	74	69	71	74	59	70	41	62	70	63	71	-	71	72	43
		· 1 11 ·	% ⁵	14	6	17	9	17	19	24	10	10	13	11	-	10	11	14

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

^oFor antimicrobial abbreviations: see List of Abbreviations page 1

¹CLSI standard. Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 22nd Informational supplement. CLSI document M100-S22. 2012 Wayne. PA. USA ²FIS (sulfisoxazole) covers the group of SMX (sulfonamides)

³Quality control range developed by the manufacturer of Sensititre® ⁴No.. number of laboratories performing the analysis

⁵%. percentage of laboratories reporting erroneous results

-. not determined

Strain	Correct sero	type	No. of labs reporting correct identification	D (%)	Deviating results	No. of labs reporting correct ST	D (%)	Deviating results (*)
WHO 2016 SH-16.1	S. flexneri	1b	120	1.6	2	77	36.9	6
WHO 2016 SH-16.2	S. boydii	4	117	4.9	6	70	43.1	1(2), 2, 9
WHO 2016 SH-16.3	S. flexneri	2b	121	1.6	2	75	39.0	
WHO 2016 SH-16.4	S. flexneri	3a	123	0.0	0	71	42.3	6(2)

Table 11. Shigella serotypes (ST) and deviations (D). WHO EQAS 2016

*number of participants reporting deviating result

•

Region	Year	No. of laboratories	No. of strains serotyped	Strains serotyped correctly (%)	Countries participating in the 2016 iteration
	2009	8	18	72.2	
	2010	7	16	62.5	
	2011	4	10	100.0	
	2012	5	18	90.0	
Africa	2013	5	8	62.5	Ivory Coast, Kenya, Mauritius, Senegal, South Africa, Zimbabwe
	2014	6	9	55.6	
	2015	8	22	68.2	
	2016	6	13	69.2	
	2009	3	5	100.0	
	2010	3	6	83.3	
	2011	2	6	100.0	
Central Asia &	2012	3	9	81.8	Bahrain, India (2), Iraq, Israel, Oman
Middle East	2013	4	8	100.0	Damani, mula (2), naq, israel, Oman
	2014	5	10	80.0	
	2015	6	24	100.0	
	2016	6	22	90.9	
	2009	13	35	100.0	
	2010	9	23	91.3	
	2011	-	-	-	
China	2012	8	29	90.6	China (17)
Cillia	2013	6	11	100.0	
	2014	9	18	94.4	-
	2015	14	55	87.3	-
	2016	17	68	91.2	
	2009	-	-	-	
	2010	-	-	-	
	2011	-	-	-	-
Caribbean	2012	1	1	33.3	- none -
Curibbeun	2013	-	-	-	
	2014		1	0.0	-
	2015	1	3	100.0	-
	2016	-	-	•	
	2009	15	40	92.5	
	2010	15	35	85.7	4
	2011	16	42	92.9	Belgium, Bulgaria, Czech Republic, Germany (2), Greece, Ireland,
Europe	2012 2013	19 18	63 31	86.3 96.8	Luxembourg, Malta, Norway, Portugal, Serbia (2), Slovenia, Spain, Sweden,
- T	2013	20	31 36	96.8 86.1	Turkey, Ukraine, United Kingdom
	2014	20	74	93.2	Turkey, Oktuile, Olited Kingdoli
					4
	2016	19	73	91.8	

Table 12. Region-based categorization of laboratories performing *Shigella* serotyping in 2016

Region	Year	No. of laboratories	No. of strains serotyped	Strains serotyped correctly (%)	Countries participating in the 2016 iteration
	2009	7	18	100.0	
	2010	7	20	100.0	
	2011	6	16	100.0	
North America	2012	8	25	80.6	Canada (5), United States of America (2)
North America	2013	8	14	100.0	Canada (5), United States Of America (2)
	2014	6	11	100.0	
	2015	7	26	100.0	
	2016	7	25	92.0	
	2009	3	8	100.0	
	2010	3	8	100.0	
	2011	3	8	100.0	
Ossan's	2012	3	12	100.0	Australia (3), New Zealand
Oceania	2013	4	10	100.0	Australia (5), New Zealand
	2014	4	7	100.0	
	2015	4	15	86.7	
	2016	4	15	93.3	
	2009	6	18	83.3	
	2010	7	20	75.0	
	2011	6	18	88.9	
Derasta	2012	5	16	80.0	
Russia	2013	2	4	100.0	- none -
	2014	3	6	100.0	
	2015	3	12	100.0	
	2016	-	-	-	
	2009	16	40	97.5	
	2010	13	33	78.8	
	2011	15	37	94.6	
T	2012	19	58	80.6	Argentina, Brazil (2), Chile (2), Costa Rica, Ecuador (2), Guatemala,
Latin America	2013	16	30	93.3	Honduras, Mexico (2), Paraguay, Peru, Uruguay, Venezuela (2)
	2014	17	29	86.2	(
	2015	13	45	88.9	
	2016	17	62	83.9	
	2009	11	30	90.0	
	2010	14	32	87.5	
	2011	13	33	84.8	
a	2012	14	47	90.4	Japan, Korea, Rep of, LAO PDR, Malaysia (2), Philippines, Sri Lanka,
Southeast Asia	2013	9	17	100.0	Taiwan, Thailand (5), Viet Nam
	2014	12	22	95.5	
	2015	14	49	91.8	
	2016	14	54	85.2	

Table 12 (continued). Region-based categorization of laboratories performing *Shigella* serotyping in 2016

EQAS iteration	No. of participating laboratories	% correct test results	% minor deviations $(S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$	% major deviations $(S \rightarrow R)^{\wedge}$	% very major deviations $(R \rightarrow S)^{\wedge}$	% critical deviations $(S \rightarrow R \& R \rightarrow S)^{\wedge}$	% total deviations $(S \rightarrow R \& R \rightarrow S \&$ $S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$
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
2014	116	92	4	1	3	4	8
2015	116	93	4	1	1	3	7
2016	112	96	1	1	1	3	4

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

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

EQAS	No. of	Lab								Antimi	crobial						
iteration	labs	performance	AMP	CAZ	CHL	СІР	СТХ	GEN	MER	NAL	SMX	STR	SXT	ТЕТ	ТМР	CRO	OVERALL average
		No. of tests	52	44	51	48	48	50	-	52	7	27	52	52	4	42	529
2008	15	% 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
		No. of tests	423	358	388	426	372	396	-	388	211	293	388	386	218	301	4548
2009	111	% 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
		No. of tests	424	344	402	434	377	403	-	382	194	275	363	410	218	291	4517
2010	114	% 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
		No. of tests	403	322	353	396	343	359	-	369	179	246	371	376	178	289	4.184
2011	107	% 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
		No. of tests	462	376	427	464	400	430	-	442	196	291	396	426	215	337	4862
2012	120	% 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
		No. of tests	-	351	379	420	384	392	-	393	164	-	346	392	193	309	3723
2013	99	% 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
		No. of tests	441	390	386	441	389	424	-	405	188	-	413	398	189	331	4395
2014	116	% critical deviations*	2.5	9.7	2.1	7.9	1.3	4.0	-	2.5	4.8	-	3.9	3.5	5.3	2.1	4.1
		% total deviations^	2.9	14.1	3.9	34.2	1.5	5.4	-	5.2	4.8	-	4.1	6.5	6.3	3.9	8.1
		No. of tests	441	405	400	448	397	434	296	388	202	-	399	410	222	331	4773
2015	116	% critical deviations*	2.0	5.7	4.0	0.9	4.5	1.8	0.0	2.3	0.5	-	1.3	3.7	0.5	3.9	2.4
		% total deviations^	2.7	8.4	10.3	26.6	5.0	3.0	0.3	6.4	1.0	-	1.3	6.6	0.5	4.5	5.9
		No. of tests	418	391	380	310	377	409	340	361	195	-	374	390	224	339	4508
2016	112	% critical deviations*	2.6	7.2	2.6	1.0	2.7	2.9	0.3	1.9	4.1	-	1.9	2.3	3.1	2.4	2.7
		% total deviations^	2.9	7.4	7.1	7.1	2.9	4.2	0.3	3.0	4.1	-	2.1	3.3	3.1	2.4	3.9

Table 14. EQAS laboratories' performance of Shigella strains antimicrobial susceptibility testing categorized by antimicrobial

∞For antimicrobial abbreviations: see List of Abbreviations page 1

* $R \rightarrow S \& S \rightarrow R$ (R. resistant; S. susceptible) * $S \rightarrow R \& R \rightarrow S \& S \leftrightarrow I$ or $I \leftrightarrow R$ (I. intermediate)

- not determined

Strain						Ant	timicrobial	α					
	AMP	СТХ	CAZ	CRO	CHL	CIP	GEN	MER	NAL	SMX	TET	SXT	TMP
WHO 2016 SH-16.1	98 /0/4	2/0/90	2/0/ 93	1/0/ 81	1/1/ 91	1/1/ 99	3/1/ 95	0/0/82	0/1/86	44/0/5	92 /1/2	87 /0/3	53 /0/2
WHO 2016 SH-16.2	104 /0/2	2/0/93	4/0/ 95	2/0/84	87 /7/2	0/6/ 99	2/1/100	1/0/85	2/0/90	49 /0/0	94 /1/3	93 /0/2	55 /0/2
WHO 2016 SH-16.3	102 /0/3	93 /1/1	18/0/ 80	84 /0/2	90 /2/4	*	3/2/99	0/0/ 87	88 /1/3	48 /0/1	94 /2/3	93 /1/1	53 /0/3
WHO 2016 SH-16.4	102 /1/2	5/0/ 90	4/1/ 94	3/0/ 82	85 /7/3	2/12/90	4/1/ 98	0/0/85	2/2/86	46 /0/2	96 /1/1	93 /0/1	56 /0/0

Table 15. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2016 Shigella strains*

[∞]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.

* The results obtained from the combination of SH-16.3 and ciprofloxacin, i.e. the obtained interpretation has been disregarded. In the preparatory work for WHO SH-16.3, three independent tests towards ciprofloxacin showed an MIC-value at 1 mg/L and one test showed an MIC-value at 0.5 mg/L, therefore the expected result was set at 1 mg/L interpreted as 'resistant'. As the results were submitted and approved by the participants, it became clear that the MIC-values reported were lower than expected (consequently, the DD-zones were higher than expected). Following this observation, and 1) knowing that the differences in the obtained MIC-/DD-results could likely be due to expected method variability and 2) as the obtained MIC-/DD-results were found to vary closely around the interpretative criteria, the EQAS organizers have decided to disregard the results obtained from the combination of SH-16.3 and ciprofloxacin, i.e. the obtained interpretation will not be evaluated in neither the individual nor the overall report.

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 $(R \rightarrow S \& S \rightarrow R)^{\wedge}$	% total deviations $(S \rightarrow R \& R \rightarrow S \&$ $S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$	Countries participating in the 2016 iteration	
	2009	17	93.3	2.4	3.5	0.8	4.3	6.8		
	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	Cameroun, Congo, Democratic Republic of the,	
A C	2012	17	82.6	4.2	2.5	10.7	13.2	17.4	Ivory Coast, Kenya (3), Madagascar, Mauritius,	
Africa	2013	14	87.6	7.2	2.5	2.7	5.2	12.4	Morocco, Nigeria, Senegal, South Africa, The	
	2014	18	85.3	6.1	2.3	6.4	8.7	14.7	Gambia (2), Zambia, Zimbabwe	
	2015	20	91.7	4.9	1.5	1.9	3.4	8.3		
	2016	16	90.3	3.5	1.1	5.1	6.2	9.7		
	2009	5	94.8	0.9	3.0	1.3	4.4	5.2		
	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		
Central Asia	2012	6	92.3	4.0	2.0	1.3	3.4	7.4	Bahrain, India (4), Iran, Islamic rep. Of (3), Iraq,	
& Middle	2013	6	86.9	8.5	3.9	0.8	4.6	13.1	Israel, Oman	
East	2014	16	85.6	6.7	1.7	6.0	7.7	14.4		
	2015	13	91.7	5.2	1.6	1.6	3.1	8.3		
	2016	11	91.3	1.5	5.1	2.1	7.2	8.7		
	2009	4	95.6	1.5	0.7	2.2	2.9	4.4		
	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		
a	2012	3	84.6	1.9	7.7	5.8	13.5	15.4		
Caribbean	2013	2	87.5	9.4	0.0	3.1	3.1	12.5	Barbados, Jamaica	
	2014	3	76.5	5.1	7.1	11.2	18.4	23.5		
	2015	4	90.7	6.4	2.9	0.0	2.9	9.3		
	2016	2	98.4	0.0	1.6	0.0	1.6	1.6		
	2009	22	98.1	1.1	0.7	0.1	0.8	1.9		
	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	Belgium, Bulgaria, Croatia, Cyprus, Czech	
Б	2012	24	96.6	1.7	0.4	1.4	1.7	3.4	Republic, Greece (2), Ireland, Italy (4),	
Europe	2013	23	93.6	4.8	1.2	0.3	1.5	6.4	Luxembourg, Malta, Norway, Poland, Portugal, Serbia (2), Spain, Turkey, Ukraine, United	
	2014	26	96.0	3.2	0.1	0.7	0.8	4.0	Kingdom	
	2015	25	95.2	3.7	0.4	0.8	1.1	4.8		
	2016	23	98.2	0.8	0.6	0.5	1.0	1.8		

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 $(R \rightarrow S \& S \rightarrow R)^{\wedge}$	% total deviations $(S \rightarrow R \& R \rightarrow S \&$ $S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$	Countries participating in the 2016 iteration			
	2009	6	100.0	0.0	0.0	0.0	0.0	0.0				
	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				
North	2012	6	89.5	0.0	2.1	8.4	10.5	10.5	Canada (2) United States of America			
America	2013	4	95.2	3.2	0.0	1.6	1.6	4.8	Canada (3), United States of America			
	2014	3	95.4	2.8	0.0	1.9	1.9	4.6				
	2015	4	96.2	3.8	0.0	0.0	0.0	3.8				
	2016	4	98.7	0.7	0.7	0.0	0.7	1.3				
	2009	-	-	-	-	-	-	-				
	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				
0	2012	1	90.0	7.5	0.0	2.5	2.5	10.0				
Oceania	2013	1	95.5	4.5	0.0	0.0	0.0	4.5	Australia, New Zealand			
	2014	2	96.2	3.8	0.0	0.0	0.0	3.8				
	2015	2	95.7	2.9	1.4	0.0	1.4	4.3				
	2016	2	98.6	0.0	1.4	0.0	1.4	1.4				
	2009	6	95.5	1.6	1.6	1.3	2.9	4.6				
	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				
D 1	2012	5	96.8	1.4	0.5	1.4	1.8	3.2				
Russia	2013	2	95.2	4.8	0.0	0.0	0.0	4.8	- none -			
	2014	3	98.4	0.8	0.0	0.8	0.8	1.6				
	2015	3	100.0	0.0	0.0	0.0	0.0	0.0				
	2016	-	-	-	-	-	-	-				
	2009	20	98.3	1.1	0.4	0.3	0.7	1.7				
	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	Argentina, Bolivia, Brazil (2), Chile (2),			
Latin	2012	24	91.7	1.3	0.6	6.5	7.1	8.3	Colombia, Costa Rica, Ecuador (2), El Salvador,			
America	2013	23	94.1	3.9	1.2	0.8	2.0	5.9	Guatemala (2), Honduras, Mexico, Panama,			
	2014	23	94.4	3.3	0.5	1.9	2.3	5.6	Paraguay, Peru, Suriname, Uruguay, Venezuela			
	2015	17	93.0	3.5	1.3	2.2	3.5	7.0				
	2016	21	98.2	0.4	0.2	1.2	1.4	1.8				

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

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

Region	Year	No. of labs	% correct test result	% minor deviations	% major deviations	% very major deviations	deviations	% total deviations	Countries participating in the 2016 iteration	
				$(S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$	(S→R)^	$(R \rightarrow S)^{\wedge}$	$(R \rightarrow S \& S \rightarrow R)^{\wedge}$	$(S \rightarrow R \& R \rightarrow S \& S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$		
	2009	18	94.1	3.9	0.3	1.7	2.0	5.9		
	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		
Southeast	2012	27	87.1	5.1	1.9	5.6	7.6	12.7	Cambodia, Japan, Korea, Rep of, LAO PDR,	
Asia	2013	19	86.2	7.5	2.9	3.1	6.0	13.5	Malaysia (2), Philippines, Sri Lanka (2), Taiwan Thailand (5), Viet Nam	
	2014	13	92.5	4.0	1.1	2.4	3.5	7.5		
	2015	15	93.1	4.8	0.8	1.3	2.0	6.9		
	2016	16	96.8	1.5	0.7	1.0	1.8	3.2		
	2009	12	96.3	2.2	1.0	0.5	1.5	3.7		
	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		
China	2013	5	92.7	3.4	0.4	3.4	3.9	7.3	China (16)	
	2014	8	94.6	2.2	0.3	3.0	3.2	5.4		
	2015	13	92.9	2.2	2.3	2.6	5.0	7.1		
	2016	16	97.1	0.8	1.5	0.6	2.1	2.9		

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

^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-producing *Salmonella* and *Shigella* strains.

Isolate no.	Expected interpretation	Confirmatory tests		
WHO 2016 S-16.1	No ESBL, AmpC or carbapenemase	-		
WHO 2016 S-16.2	Carbapenemase-phenotype	56/82 (68%)		
WHO 2016 S-16.3	No ESBL, AmpC or carbapenemase	-		
WHO 2016 S-16.4	No ESBL, AmpC or carbapenemase	-		
WHO 2016 S-16.5	No ESBL, AmpC or carbapenemase	-		
WHO 2016 S-16.6	AmpC-phenotype	61/80 (76%)		
WHO 2016 S-16.7	No ESBL, AmpC or carbapenemase	-		
WHO 2016 S-16.8	No ESBL, AmpC or carbapenemase	-		
WHO 2016 SH-16.1	No ESBL, AmpC or carbapenemase	-		
WHO 2016 SH-16.2	No ESBL, AmpC or carbapenemase	-		
WHO 2016 SH-16.3	ESBL-phenotype	72/75 (96%)		
WHO 2016 SH-16.4	No ESBL, AmpC or carbapenemase	-		

iteration	labs		Strain no.	No. of results submitted	% correct identification	Deviating results (*)
	97	C. jejuni	# 1	93	88%	C. coli (9) C. lari (3)
2003	97	C. coli	# 2	93	84%	C. jejuni (7) C. lari (4) C. upsaliensis (4)
	109	C. lari	# 1	97	79%	C. coli (11) C. jejuni (8)
2004	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)
2000	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) C. coli (10)
	154	C. lari	# 2	109	62%	C. con (10) C. jejuni (19) C. upsaliensis (13) C. upsaliensis (10)
2009	131	C. coli	# 1	87	77%	C. upsatiensis (10) C. jejuni (9) C. lari (1) C. upsaliensis (3)
	131	C. jejuni	# 2	87	95%	<i>C. lari</i> (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)
_	135	C. jejuni	# 1	112	96%	<i>C. coli</i> (4)
2012	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)
2014	101	C. coli	#2	101	85 %	C. jejuni (8) C. lari (6) C. upsaliensis (1)
2015	114	C jejuni	#1	112	93 %	C.coli (6) C.lari, C.upsaliensis
	114	C.coli	#2	110	89 %	C jejuni (8) C.lari (4)
2016 -	95	C jejuni	#1	94	94 %	C.coli (5) C.lari
	95	C.coli	#2	93	91 %	C jejuni (6) C.upsaliensis (2)

Table 18. EQAS	participating laborator	ies' performance of	<i>Campylobacter</i> s	trains identification

*number of participants reporting the specified deviating result

Region	Year	No. of labs	No. of strains identified	% strains correctly identified	Countries participating in the 2016 iteration		
	2009	9	15	53			
	2010	7	13	77			
	2011	10	19	32			
	2012	9	17	82	Egypt, Kenya (2), Mauritius, Senegal,		
Africa	2013	9	17	41	South Africa		
	2014	9	9	67			
	2015	12	24	88			
	2016	6	12	100			
	2009	14	27	85			
	2010	13	26	89			
	2011	2	4	50			
Central Asia &	2012	11	22	96			
Middle East	2013	1	8	50	Bahrain, Iran, Islamic rep. of, Oman		
	2014	7	7	57			
	2015	6	12	67			
	2016	3	6	100			
	2009	2	4	100			
	2010	3	6	67			
	2011	1	2	0			
~	2012	4	7	57			
Caribbean	2013	2	4	100	Barbados		
	2014	2	2	100			
	2015	3	6	67			
	2016	1	2	100			
	2009	29	55	89			
	2010	29	57	97			
	2011	25	48	85	Bulgaria, Croatia, Cyprus, Czech		
F	2012	29	56	95	Republic (2), Germany, Greece (2),		
Europe	2013	26	51	88	Italy (8), Luxembourg (2), Malta,		
	2014	26	26	89	Poland (2), Portugal, Serbia (2), Slovenia, Spain, Turkey (2)		
	2015	30	60	93	Slovenia, Spani, Turkey (2)		
	2016	28	56	96			
	2009	10	19	90			
	2010	11	22	86			
	2011	9	18	78			
North America	2012	13	26	96	Canada (7), United States of America		
North America	2013	10	18	100	(3)		
	2014	10	10	100			
	2015	13	26	100			
	2016	10	20	90			
	2009	2	4	100			
	2010	2	3	100			
	2011	2	4	100			
Ossaria	2012	2	4	100	Australia Niana 77 1 1		
Oceania	2013	2	4	100	Australia, New Zealand		
	2014	1	1	100			
	2015	2	4	100			
	2016	2	4	100			

Table 19. Region-based categorization of EQAS 2016 participating laboratories' performance of *Campylobacter* strains identification

		No. of	No. of	% strains	Countries participating in the		
Region	Year	labs	strains identified	correctly identified	2016 iteration		
	2009	2	4	100			
	2010	2	4	100			
	2011	2	4	50			
	2012	5	10	80			
Russia	2013	1	2	100	- none -		
	2014	3	3	100			
	2015	3	6	100			
	2016	0	0	-			
	2009	14	26	89			
	2010	19	37	78			
	2011	19	37	49			
Latin America	2012	22	40	95	Brazil (2), Colombia (2), Costa Ric		
	2013	20	36	83	Mexico, Panama, Paraguay		
	2014	22	22	86			
	2015	15	28	89			
	2016	8	13	85			
	2009	10	20	90			
	2010	14	27	93			
	2011	12	24	67	Brunei Darussalam, Cambodia, Japan,		
G (1 ())	2012	17	33	85	Korea, Rep of, LAO PDR, Malaysia		
Southeast Asia	2013	15	28	89	(2), Philippines, Sri Lanka, Taiwan,		
	2014	13	13	92	Thailand (7), Viet Nam (2)		
	2015	16	28	93			
	2016	19	38	79			
	2009	12	24	92			
	2010	10	20	85			
	2011	-	-	-			
China	2012	-	-	-	China (18)		
Ciiiia	2013	5	10	90	China (10)		
	2014	8	8	75			
	2015	14	28	93			
	2016	18	36	100			

Table 19 (continued). Region-based categorization of EQAS 2016 participating laboratories' performance of *Campylobacter* strains identification

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

EQAS iteration	No. of labs	% correct test results	% major deviations $(S \rightarrow R)^{\wedge}$	% very major deviations (R→S)^	% critical deviations $(R \rightarrow S \& S \rightarrow R)^{\wedge}$
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
2014	50	91.2	1.6	7.2	8.8
2015	56	89.5	5.2	5.2	10.5
2016	49	91.8	4.2	4.0	8.2

^S. susceptible; R. resistant

EQAS	No. of	Lab			An	timicro	bial		
iteration	labs	performance	CHL	CIP	ERY	GEN	NAL	STR	ТЕТ
2009	25	No. of tests	37	46	46	43	41	34	45
2009	23	% 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
2010	57	% 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
2011	30	% 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
2012	47	% 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
2013	47	% critical deviations*	5.6	6.7	8.0	0.0	8.9	23.5	8.1
2014	50	No. of tests	-	49	46	45	42	24	45
2014	30	% critical deviations*	-	8.2	0.0	0.0	11.9	16.7	11.1
2015	56	No. of tests	-	110	108	94	92	63	107
2015	30	% critical deviations*	-	5.5	6.5	11.7	8.7	6.3	22.4
2016	49	No. of tests	-	93	93	81	78	64	93
2016	49	% critical deviations*	-	9.7	5.4	4.9	9.0	17.2	5.4

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

^For antimicrobial abbreviations. See List of Abbreviations page 1

*R \rightarrow S & S \rightarrow R (R. resistant; S. susceptible)

Table 22. Antimicrobial susceptibility test results (number of R/S) for the EQAS 2016
Campylobacter strains*

Studie	Antimicrobial^							
Strain	CIP	ERY	GEN	NAL	STR	TET		
WHO 2016 C-16.1	42 /0/4	43 /0/3	39 /0/2	36 /0/4	26 /0/6	45 /0/1		
WHO 2016 C-16.2	5/0/42	2/0/45	2/0/38	3/0/35	5/0/ 27	4/0/ 43		

^For antimicrobial abbreviations. see List of Abbreviations page 1

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

Table 23. Region-based categorization of EQAS 2016 participants' performance of antimicrobial susceptibility testing of *Campylobacter* strains

Region	Year	No. of labs	% correct test result	% major deviations $(S \rightarrow R)^{\wedge}$	% very major deviations	% critical deviations (R→S &	Countries participating in the 2016 iteration
					$(S \rightarrow R)^{\wedge}$	S→R)^	
	2009	2	75.0	10.7	14.3	25.0	
	2010	2	95.2	0.0	4.8	4.8	
	2011	7	85.0	3.3	11.7	15.0	
Africa	2012	4	94.3	0.0	5.7	5.7	- none -
	2013	5	90.9	5.5	3.6	9.1	
	2014	7	51.5	39.4	9.1	48.5	
	2015	6	71.9	12.5	15.6	28.1	
	2016	•	-	•	-	-	
	2009	0	-	-	-	-	
	2010	0	-	-	-	-	
	2011	1	75.0	0.0	25.0	25.0	
Central Asia	2012	2	93.8	6.3	0.0	6.3	Iran, Islamic rep. of
& Middle East	2013	3	93.3	3.3	3.3	6.7	
	2014	3	100.0	0.0	0.0	0.0	
	2015	3	97.1	2.9	0.0	2.9	
	2016	1	40.0	40.0	20.0	60.0	
	2009	2	95.2	4.8	0.0	4.8	
	2010	1	100.0	0.0	0.0	0.0	
	2011	0	-	-	-	-	
China	2012	2	88.5	7.7	3.8	11.5	China (16)
Cinna	2013	3	95.2	2.4	2.4	4.8	
	2014	6	100.0	0.0	0.0	0.0	
	2015	8	86.5	5.2	8.3	13.5	
	2016	16	88.5	5.2	6.3	11.5	
	2009	0	-	-	-	-	
	2010	0	-	-	-	-	
	2011	0	-	-	-	-	
Caribbean	2012	1	75.0	25.0	0.0	25.0	Cuba, Jamaica
Caribbean	2013	1	100.0	0.0	0.0	0.0	Cuba, Jamaica
	2014	2	100.0	0.0	0.0	0.0	
	2015	2	73.3	20.0	6.7	26.7	
	2016	2	73.3	20.0	6.7	26.7	
	2009	10	94.8	3.0	2.2	5.2	
	2010	13	100.0	0.0	0.0	0.0	Czech Republic,
	2011	11	100.0	0.0	0.0	0.0	Greece (2), Italy (3),
Europe	2012	16	97.3	1.6	1.1	2.7	Luxembourg (2), Malta,
Larope	2013	16	94.9	3.5	1.5	5.1	Poland, Serbia, Spain,
	2014	16	97.4	1.3	1.3	2.6	Turkey
	2015	15	97.5	2.5	0.0	2.5	
	2016	13	94.1	5.0	0.8	5.9	
	2009	2	100.0	0.0	0.0	0.0	
	2010	5	93.8	6.3	0.0	6.3	
	2011	5	100.0	0.0	0.0	0.0	
North	2012	5	100.0	0.0	0.0	0.0	Canada (3), United States
America	2013	3	100.0	0.0	0.0	0.0	of America (3)
	2014	4	100.0	0.0	0.0	0.0	
	2015	5	97.9	2.1	0.0	2.1	
	2016	6	100.0	0.0	0.0	0.0	

^S. susceptible; R. resistant

Table 23 (continued). Region-based categorization of EQAS 2016 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 $(R \rightarrow S \& S \rightarrow R)^{\wedge}$	Countries participating in the 2016 iteration
	2009	0	-	-	-	-	
	2010	0	-	-	-	-	
	2011	1	100.0	0.0	0.0	0.0	
o :	2012	0	-	-	-	-	N. Zalad
Oceania	2013	0	-	-	-	-	New Zealand
	2014	0	-	-	-	-	
	2015	1	100.0	0.0	0.0	0.0	
	2016	1	100.0	0.0	0.0	0.0	
	2009	0	-	-	-	-	
	2010	1	78.6	7.1	14.3	21.4	
	2011	1	100.0	0.0	0.0	0.0	
Dereste	2012	0	-	-	-	-	
Russia	2013	0	-	-	-	-	- none -
	2014	0	-	-	-	-	
	2015	0	-	-	-	-	
	2016	0	-	-	-	-	
	2009	5	93.2	6.8	0.0	6.8	
	2010	8	89.6	6.0	4.5	10.4	
	2011	7	96.8	0.0	3.2	3.2	
Tatin America	2012	7	95.2	3.2	1.6	4.8	Brazil, Costa Rica,
Latin America	2013	7	92.4	4.5	3.0	7.6	Paraguay
	2014	6	100.0	0.0	0.0	0.0	
	2015	8	93.1	4.2	2.8	6.9	
	2016	3	84.2	0.0	15.8	15.8	
	2009	4	84.4	4.4	11.1	15.6	
	2010	7	77.2	9.8	13.0	22.9	
	2011	5	85.1	9.0	6.0	14.0	
Southeast Asia	2012	10	85.8	13.3	0.9	14.2	Korea, Rep of, Philippines,
Southeast Asia	2013	9	84.8	10.7	4.5	15.2	Sri Lanka, Thailand (6)
	2014	6	87.5	12.5	0.0	12.5	
	2015	8	82.9	6.1	11.0	17.1	
	2016	9	96.9	0.0	3.1	3.1	

^S. susceptible; R. resistant

	Method used	Incubation	Labs'			Antimi	crobial ³		
	Method used	conditions	performance ^{1.2}	CHL	CIP	ERY	GEN	NAL	TET
-		42°C / 24h	No. ¹	3	6	6	6	4	6
	Microdilution	42°C / 24h	⁰∕₀²	67	83	100	83	75	83
	Microdifution	36-37°C / 48h	No. ¹	5	8	8	8	7	8
		30-37 C / 481	⁰∕₀²	80	88	88	75	86	88
EQAS 2010		42°C / 24h	No. ¹	-	6	6	6	-	-
(N=20)	Acandilution	42°C / 24n	⁰∕₀²	-	100	83	83	-	-
	Agardilution	26 2700 / 491	No. ¹	-	0	0	0	-	-
		36-37°C / 48h	⁰∕₀²	-	0	0	0	-	-
		Overall	No. ¹	8	20	20	20	11	14
		Overall	⁰∕₀²	75	90	90	80	82	86
		42°C / 24h	No. ¹	4	9	9	8	7	9
	Microdilution	42 C / 2411	% ²	100	67	100	88	100	67
	Wherounution	36-37°C / 48h	No. ¹	6	8	6	8	7	7
		50-57 C7 401	% ²	83	88	100	75	86	86
EQAS 2011		42°C / 24h	No. ¹	-	8	8	8	-	-
(N=26)	Agardilution	42 C / 2411	% ²	-	88	63	100	-	-
	Agaidifution	36-37°C / 48h	No. ¹	-	1	1	1	-	-
		30-37 C7 401	% ²	-	0	0	100	-	-
		Overall	No. ¹	10	26	24	25	14	16
		Overan	% ²	90	77	83	88	93	75
		42°C / 24h	No. ¹	9	12	12	12	10	12
	Microdilution	42 C / 241	% ²	67	75	83	83	80	75
	Wheroundhom	36-37°C / 48h	No. ¹	7	9	8	8	8	8
		50-57 C7 401	% ²	100	89	100	63	88	88
EQAS 2012		42°C / 24h	No. ¹	-	9	7	9	-	-
(N=34)	Agardilution	42 C7 241	% ²	-	89	86	89	-	-
	rigardifution	36-37°C / 48h	No. ¹	-	4	4	4	-	-
			⁰∕₀²	-	50	100	100	-	-
		Overall	No. ¹	34	80	75	78	43	50
		Overall	⁰∕₀²	82	81	88	83	86	80

Table 24. EQAS participants' performance of antimicrobial susceptibility testing of *Campylobacter jejuni* ATCC 33560

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

Table 24 (continued). EQAS participants'	performance of antimicrobial susceptibility testing of
Campylobacter jejuni ATCC 33560	

	Mathalanad	Incubation	Labs'			Antimi	crobial ³		
	Method used	conditions	performance ^{1.2}	CHL	CIP	ERY	GEN	NAL	ТЕТ
		42°C / 24h	No.1 %	6 83	8 88	8 100	8 88	7 86	8 100
	Microdilution	36-37°C / 48h	No.1 9/0 ²	<u>8</u> 88	12 92	100 12 83	11 73	11 91	100 12 75
EQAS 2013		42°C / 24h	No. ¹	-	9 89	9 67	8 75	-	-
(N=47)	Agardilution	36-37°C / 48h	No. ¹	-	7 86	7 86	6 100	-	-
		Overall	No.1 9%0 ²	<u>-</u> 14 86	36 89	36 83	33 82	18 89	20 85
		42°C / 24h	No. ¹	-	10 90	10 100	10 80	10 100	10 90
	Microdilution	36-37°C / 48h	No. ¹	-	10 100	10 10 80	9 89	8 100	10 100
EQAS 2014	A	42°C / 24h	No.1 9/0 ²	-	7 100	7 71	7 100		-
(N=32)	Agardilution	36-37°C / 48h	No. ¹ %0 ²	-	5 80	5 80	5 100		
		Overall	No. ¹ %0 ²	-	32 94	32 84	31 90	18 100	20 95
	Microdilution	42°C / 24h	No. ¹ %0 ²	-	19 68	19 84	18 94	17 94	17 76
FOAG	MICroditution	36-37°C / 48h	No. ¹ %0 ²	-	8 100	8 100	7 86	5 100	8 100
EQAS 2015 (N=32)	Agardilution	42°C / 24h	No.1 %02	-	7 100	7 71	5 100	-	-
(11=32)	Agaramution	36-37°C / 48h	No. ¹ %0 ²	-	5 40	5 40	5 40	-	-
		Overall	No. ¹ %	-	39 77	39 79	35 86	22 95	25 84
		42°C / 24h	No. ¹	-	24	24	23	23	24
	Microdilution	36-37°C / 48h	No. ¹	-	88 5	88 5	96 5	83 5	83 5
EQAS			% ² No. ¹	-	100 9	100 9	100 9	- 100	- 100
2016 (N=42)	Agardilution	42°C / 24h	% ²	-	67	78	78	-	-
		36-37°C / 48h	No. ¹	-	4	4 75	3 100	-	-
		Overall	No.1	-	42	42	40	28	29
15.2			$\frac{9/0^2}{20/0}$ percentage of	-	86	86	93	86	86

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

EOAS	Stroin ID	No	Demonstrane (0/) of lobe performing connect identifies the
EQAS iteration	Strain ID	No. of participating labs	Percentage (%) of labs performing correct identification
2003	<i>E. coli</i> 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	<i>Salmonella</i> Paratyphi B var. Java	134	23% (Salmonella spp) 7% (Salmonella O:B) 24% (Salmonella Paratyphi B var. java. In total 54% <u>Deviations:</u> Citrobacter freundii (1), Edwardsiella sp (1), Escherichia fergusonii (1), Proteus mirabilis (1), Salmonella serovar X* (24), Salmonella serovar Paratyphi B (34) * incorrect serovar
2013	<i>E. coli</i> O157:H16 non- VTEC	129	82% correct, including: Escherichia coli non-VTEC / O157 non-VTEC / O157:H16 non-VTEC E. coli non-VTEC / O157 non-VTEC / 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
2014	Yersinia pseudotuberculosis	117	75% correct, including: YERSINIA SPECIES Yersinia pseudotuberculosis Yersinia pseudotuberculosis I / O1 / O:1b / API 20 E [1014100] Deviations: Acinetobacter baumannii, Burkolderia sp., Citrobacter freundi, corynebacterium species, Sphingomonas paucimobilis, HELICOBACTER, Pasteurella maisi, Pasteurella sp., Pseudomonas luteola, Rhizobium radiobacter (6), Salmonella typhi, Shigella flexneri, Sphingomonas paucimobilis (4), unknown, Vibrio metschnikovii, Yersinia enterocolitica (4), Yersinia similis, Yestina pestis
2015	Hafnia alvei	142	87.3% correct, including: Hafnia alvei (116), Hafnia alvei 1(8) Deviations: Aeromonas spp., Aeromonas veronii, Serratia marcescens, Enterobacter, Enterobacter cloacae, Eschericha coli (3), Eschericia fergusonii, Bacillus, Hafnia alvei ATCC 13337, Plesiomonas shigelloides, Shigella flexneri, Shigella sonnei, Shigella spp. (2), Vibrio parahaemolyticus, Yokenella regensburgei
2016	Listeria monocytogenes	137	 86.1% correct, including: Listeria monocytogenes (101), Listeria monocytogenes 1/2 a (8), Listeria monocytogenes 2a, Listeria monocytogenes IIa, Listeria monocytogenes 0:1, Listeria monocytogenes 01/2, Listeria monocytogenes Serotype 1, Listeria monocytogenes Type 1, Listeria spp (3). Deviations: Actinomyces pyogenes, Aeromonas, Chromobacterium violaceum, Corynebacterium spp., Enterobacter agglomerans, Ewingella americana, Listeria ivanovii, Listeria monocytogenes/innocua, Listeria grayi (2), non-fermenter spp., Pantoae spp 3, Salmonella Dublin (9,12;gp), Salmonella enterica ssp enterica, Sphingomonas paucimobilis (2), Staphylococcus xylosus, Vibrio parahaemolyticus, Yersinia enterocolitica.

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

G00-06-001/01.12.2014 Kgs. Lyngby, Denmark, April 2016

SIGN-UP FOR EQAS 2016

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). We are pleased to announce the launch of the 2016 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. Note that in relation to the antimicrobial susceptibility testing of *Campylobacter*, results obtained by broth micro dilution or agar dilution, only, are accepted;

- Identification of one unknown bacterial isolate.

WHO SHOULD PARTICIPATE IN EQAS 2016?

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 2016. Laboratories which signed up and received bacterial isolates in year 2015 but did not submit any result should provide a consistent explanation for this if they want to participate in 2016.

COST FOR PARTICIPATING IN EQAS

There is no participation fee. 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 2016

This link will open a sign-up webpage: <u>http://eqas.food.dtu.dk/who/signup</u>

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 2016 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 Pedersen: E-mail <u>suska@food.dtu.dk</u>; 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 in August/September 2016.

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) in August/September 2016.

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

DEADLINE FOR SUBMITTING RESULTS TO THE NATIONAL FOOD INSTITUTE

Results must be submitted to the National Food Institute (DTU Food) by **31st December 2016** 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 2016 is 27th May 2016

Appendix 3, page 1 of 1

			Presumptive	Amp	picillin	Cefo	taxime	Synergy	Cel	oxitin	Cefta	zidime	Synergy	Ceftr	iaxone	Chloram	nphenicol	Ciprof	loxacin	Genta	amicin	Merop	benem	Nalidix	ic acid	Sulfon	namides	Tetra	cycline	Trimet	thoprim	Trim	n/Sulfa
			phenotype	Α	MP	c	тх	CTX:/CTX:CI	F	OX	C.	AZ	CAZ:/CAZ:CI	С	RO	C	HL	c	CIP	GE	EN	M	ER	N	AL	SI	мх	т	ΈT	١T	MP	S	SXT
WHO 2016 S-16.1	Salmonella Bovismorbificans/ Salmonella Hindmarsh	l 6,8:r:1,5	-	>64	RESIST	<=0.25	SUSC				<=0.5	SUSC		0.064	SUSC	<=8.0	SUSC	0.03	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2016 S-16.2	Salmonella Infantis	l 6,7:r:1,5	carbapenemase	>64	RESIST	>64	RESIST	no synergy	>64	RESIST	>128	RESIST	no synergy	64	RESIST	>128	RESIST	0.03	SUSC	1	SUSC	0.25	RESIST	<=4	SUSC	>1024	RESIST	4	SUSC	>32	RESIST	>32	RESIST
WHO 2016 S-16.3	Salmonella Enteritidis	l 9,12:g,m;-	-	4	SUSC	0.5	SUSC				1	SUSC		0.25	SUSC	<=8.0	SUSC	0.06	SUSC	>32	RESIST	0.06	SUSC	<=4	SUSC	>1024	RESIST	4	SUSC	<=0.25	SUSC	0.125	SUSC
WHO 2016 S-16.4	Salmonella Uganda	l 3,10:l,z13;1,5	-	<=1	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.064	SUSC	<=8.0	SUSC	0.03	SUSC	<=0.5	SUSC	<=0.03	SUSC	8	SUSC	32	SUSC	<=2	SUSC	<=0.5	SUSC	0.125	SUSC
WHO 2016 S-16.5	Salmonella Stanley	I 4,5,12:d;1,2	-	2	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.032	SUSC	128	RESIST	0.03	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	>1024	RESIST	32	RESIST	>32	RESIST	>32	RESIST
WHO 2016 S-16.6	Salmonella Heidelberg	l 4,12:r:1,2	AmpC	>64	RESIST	8	RESIST	no synergy	32	RESIST	16	RESIST	no synergy	32	RESIST	>128	RESIST	0.03	SUSC	<=0.5	SUSC	<=0.03	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	<=0.25	SUSC	0.25	SUSC
WHO 2016 S-16.7	Salmonella Altendorf	I 4,12,27:c:1,7	-	2	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.064	SUSC	<=8.0	SUSC	0.03	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	64	SUSC	<=2	SUSC	<=0.25	SUSC	0.06	SUSC
WHO 2016 S-16.8	Salmonella Plymouth	l 9,46:d:z6	-	2	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.064	SUSC	<=8.0	SUSC	0.03	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	16	SUSC	<=2	SUSC	<=0.25	SUSC	0.06	SUSC
WHO 2016 SH-16.1	Shigella flexneri 1b		-	>64	RESIST	<=0.25	SUSC				<=0.5	SUSC		0.064	SUSC	<=8.0	SUSC	<=0.015	SUSC	1	SUSC	<=0.03	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2016 SH-16.2	Shigella boydii 4		-	>64	RESIST	<=0.25	SUSC				<=0.5	SUSC		0.032	SUSC	64	RESIST	<=0.015	SUSC	1	SUSC	<=0.03	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2016 SH-16.3	Shigella flexneri 2b		ESBL	>64	RESIST	32	RESIST	synergy	4	SUSC	0.5	SUSC	no synergy	32	RESIST	128	RESIST	1	RESIST	1	SUSC	<=0.03	SUSC	>128	RESIST	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2016 SH-16.4	Shigella flexneri 3a		-	>64	RESIST	<=0.25	SUSC				<=0.5	SUSC		0.032	SUSC	128	RESIST	0.03	SUSC	2	SUSC	<=0.03	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST

		Ciprof	loxacin	Erythr	omycin	Gentam	nicin	Nalidi	kic acid	Stre	eptomycin	Tetra	cycline
		c	CIP		RY	GEN		NAL		STR		Т	ET
WHO 2016 C-16.1	C. jejuni	32	RESIST	>64	RESIST	>32	RESIST	>64	RESIST	>64	RESIST	>64	RESIST
WHO 2016 C-16.2	C. coli	0.06	SUSC	1	SUSC	1	SUSC	<=4	SUSC	<=4	SUSC	0.5	SUSC

WHO B-16.1 Listeria monocytogenes



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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 pathogen

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HISTORY OF CHANGES; protocol version 2

Interpretative criteria for meropenem adjusted in Table 1 (changes from protocol version 1 indicated with bold and italics)

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.





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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 2016 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 (NCIMB 12210) reference strain for quality control (QC),
- identification and antimicrobial susceptibility testing of two thermophilic *Campylobacter* isolates,
- antimicrobial susceptibility testing of *Campylobacter jejuni* ATCC 33560 (NCTC 11351) reference strain for QC,
- 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 QC reference strains are included in the parcel only for new participants of the EQAS who did not receive them previously. The QC 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 QC 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 Collaborating Centre website (see <u>www.antimicrobialresistance.dk</u>).

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 2016

3.1 Shipping, receipt and storage of strains

In September 2016 around 200 laboratories located worldwide will receive a parcel containing eight *Salmonella* strains, four *Shigella* strains, two *Campylobacter* strains and one 'unknown' bacterial





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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. AmpC-, Extended-Spectrum Beta-Lactamase (ESBL)-, and carbapenemase-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 for QC and the *Campylobacter* strains are shipped lyophilised (LYFO DISK®). See section 3.1.1 below for additional info on handling and reconstitution of the lyophilised cultures.

On arrival, the bacterial cultures must be stored in a dark place at 2°C to 8°C until handling in the laboratory.

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 detect errors such as mis-labelling or contamination).

3.1.1 Instructions related to handling of LYFO DISK®

The microorganisms supplied as LYFO DISK® are packaged in re-sealable vials that contain a lyophilized pellet and a desiccant to prevent adverse accumulations of moisture.

The following instructions can be downloaded from the manufacturer's website (<u>http://microbiologics.com/Support-Center/KWIK-STIK-trade</u>):

- 1. Remove the unopened LYFO DISK® vial from 2°C to 8°C storage and allow the unopened vial to equilibrate to room temperature.
- 2. Aseptically remove the pellet with sterile forceps from the vial. Do not remove desiccant.
- 3. Place the pellet in 0.5 mL of sterile fluid (water, saline, TSB, or BHIB).
- 4. Crush the pellet with a sterile swab until the suspension is homogenous. Immediately heavily saturate the same swab with the hydrated material and transfer to agar medium.
- 5. Inoculate the primary culture plate(s) by gently rolling the swab over one-third of the plate.
- 6. Using a sterile loop, streak to facilitate colony isolation.
- 7. Using proper biohazard disposal, discard the remaining hydrated material.
- 8. Immediately incubate the inoculated media at temperature and conditions appropriate to the microorganism.





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Materials required but not provided:

- Microorganisms require sterile tubes and 0.5 ml of sterile liquid such as, Tryptic Soy Broth, Brain Heart Infusion Broth, saline, or deionized water to hydrate the lyophilized preparation.
- Sterile swabs or inoculating loops are needed to transfer the hydrated preparation to an agar plate.
- Non-selective, nutrient or enriched agar media and specific incubation times and conditions to optimize growth and recovery.

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 QC reference strain should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods <u>routinely used</u> in your laboratory.

For reconstitution of the *E. coli* QC reference strain (NCIMB 12210) which is supplied in the form of a LYFO DISK®, see instructions in section 3.1.1 above.

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



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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' ($I \leftrightarrow S$ or $I \leftrightarrow R$), '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).

Antimicrobials	Refere	nce value, MIC	(µg/mL)	Reference	value, Disk diff	usion (mm)
	Sensitive	Intermediate	Resistant	Resistant	Intermediate	Sensitive
Ampicillin, AMP	≤8	16	≥32	≤13	14-16	≥17
Cefotaxime, CTX*	≤1	-	>1	≤27	-	>27
Cefoxitin, FOX	≤8	16	≥32	≤14	15-17	≥18
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**	≤20mm (5µg)** or <23mm (1µg)***	21-30mm (5μg)** or - (1μg)***	$\geq 31 \text{mm} \ (5 \mu \text{g})^{**} \ \text{or} \ \geq 23 \text{mm} \ (1 \mu \text{g})^{***}$
Gentamicin, GEN	≤4	8	≥16	≤12	13-14	≥15
Meropenem, MER*	≤0.12	-	>0.12	<27	-	≥27
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

Table 1. Interpretive breakpoint for Salmonella and Shigella antimicrobial susceptibility testing

Reference values used in this EQAS are according to CLSI (M100-S25), with the following exceptions: * For the cephalosporins and meropenem, the application of the interpretative criteria is intended to indicate if the microorganism is a presumptive ESBL- or carbapenemase-producer. Reference values for the cephalosporins are according to CLSI M100-S25 Table 3A. These interpretative criteria are also applied for *Salmonella* and *Shigella* test strains for interpretation of AST results in this EQAS. *Reference values for meropenem are based on epidemiological cut off values from www.eucast.org.*

** 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. These interpretative criteria are also applied for *Shigella* test strains for interpretation of AST results in this EQAS.





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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 take into consideration mechanisms of resistance due to plasmid-mediated quinolone resistance genes (e.g. *qnr*-genes) and one-point-mutation in the gyrase gene.

Important notes: beta-lactam resistance

The following tests for detection of ESBL-, AmpC-, and carbapenamase-producing phenotypes are optional in relation to the current WHO GFN EQAS.

If choosing to participate in this component of the EQAS, all strains displaying reduced susceptibility to cefotaxime (CTX), ceftazidime (CAZ), and/or ceftriaxone (CRO) should be tested for ESBL-, AmpC, or carbapenemase-production by confirmatory tests. Reduced susceptibility to any of the above-mentioned antimicrobials indicates that the bacterial strain is an ESBL-, AmpC, or carbapenemase-producing phenotype.

Confirmatory test for ESBL production requires the 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) by microbroth dilution methods or E-test; $a \ge 3$ 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) by disk diffusion; $a \ge 5$ mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its ZON Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production.

Detection of AmpC-type beta-lactamases can be performed by testing the bacterial culture for susceptibility to cefoxitin (FOX). Resistance to FOX indicates the presence of an AmpC-type beta-lactamase.

Confirmatory test for carbapenemase production requires the testing of meropenem (MER). Reduced susceptibility to MER indicates that the bacterial strain is a carbapenemase-producer.

The classification of the phenotypic results should be based on the most recent EFSA (European Food Safety Agency) recommendations (EURL-AR Workshop 2016, <u>http://www.crl-ar.eu/data/images/ws_april-2016/f11_efsa_criteria.pdf</u>). The following summary of these recommendations indicate how the phenotypes should be categorized:





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ESBL-phenotype:

- CTX or CAZ > 1 mg/L AND
- MER \leq 0.12 mg/L AND
- FOX $\leq 8 \text{ mg/L AND}$
- Synergy for CTX : CTX/Cl and/or CAZ : CAZ/Cl

ESBL+AmpC-phenotype:

- CTX or CAZ > 1 mg/L AND
- MER ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- Synergy for CTX : CTX/Cl and/or CAZ : CAZ/Cl

AmpC-phenotype:

- CTX or CAZ > 1 mg/L AND
- MER \leq 0.12 mg/L AND
- FOX > 8 mg/L AND
- No synergy for CTX : CTX/Cl nor CAZ : CAZ/Cl

(note, presence of ESBLs is not excluded)

Carbapenemase-phenotype:

- MER > 0.12 mg/L

(note, presence of ESBLs or AmpCs is not excluded)

Other-phenotype:

- Not covered by any of the above categories AND
- CTX, CAZ, FOX, or MER > interpretative criteria as susceptible in Table 1 (i.e. exhibits reduced susceptibility)

No ESBL-, AmpC-, or carbapenemase:

CTX, CAZ, FOX, and MER ≤ interpretative criteria as susceptible in Table 1 (i.e. exhibits susceptibility)

The genotype obtained by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either of the categories, ESBL-, AmpC, and/or carbapenemase-producer, but is not requested as part of this WHO GFN EQAS.

3.4 Handling the *Campylobacter* strains

The *Campylobacter* test strains as well as the *C. jejuni* reference strain (NCTC 11351) are supplied in the form of LYFO DISK[®]. To revive the strains, see instructions in section 3.1.1 above







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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 ATCC33560 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 (European Committee on Antimicrobial Susceptibility Testing; <u>www.eucast.org</u>; 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.

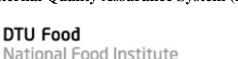
Table 2. Interpretive criteria for Campylobacter antimicrobial susceptibility testing

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

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



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

Identification of the unknown enteric pathogen 3.7

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

REPORTING OF RESULTS AND EVALUATION 4

We recommend that you write your results in the enclosed test forms and that you read 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 2016

Results must be submitted directly via the Internet based database. Should you not be able to access the Internet, you may return the completed test forms scanned by e-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 WHO GFN EQAS **Coordinator:**

Susanne Karlsmose Pedersen National Food Institute, Technical University of Denmark Søltofts Plads, Building 221, DK-2800 Kgs. Lyngby - DENMARK Tel: +45 3588 6601 E-mail: suska@food.dtu.dk





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6 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please carefully 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 can browse back and forth in the pages of the database. Always remember to save your input before leaving a page.

1) Enter the WHO Collaborating Centre website (from <u>http://www.antimicrobialresistance.dk</u>), then

- a. Click on 'EQAS'
- b. Click on the link for the interactive database (<u>http://eqas.food.dtu.dk/who</u>)
- c. Write your username and password in lower-case letters and click on 'Login'. You can find your username and password in the letter following your strains. Your username and password will remain unchanged in future trials. Do not hesitate to contact us if you experience problems with the login.
- 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' ALWAYS 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)



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- 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, select the appropriate result.
- 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

- 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 2016 start page', 'Review evaluated results' (a printer friendly version of the evaluation report is also available) or 'Go to WHO GFN homepage'.

End of entering your data - thank you very much!







SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1.1 Purpose

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

1.2 References

M100-S24, January 2014 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A9, January 2012 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard)

1.3 Definition of Terms

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

<u>Reference Stock Culture</u>: 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.

<u>Working Stock Cultures</u>: 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.

<u>Subcultures (Passages)</u>: 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

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- 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.

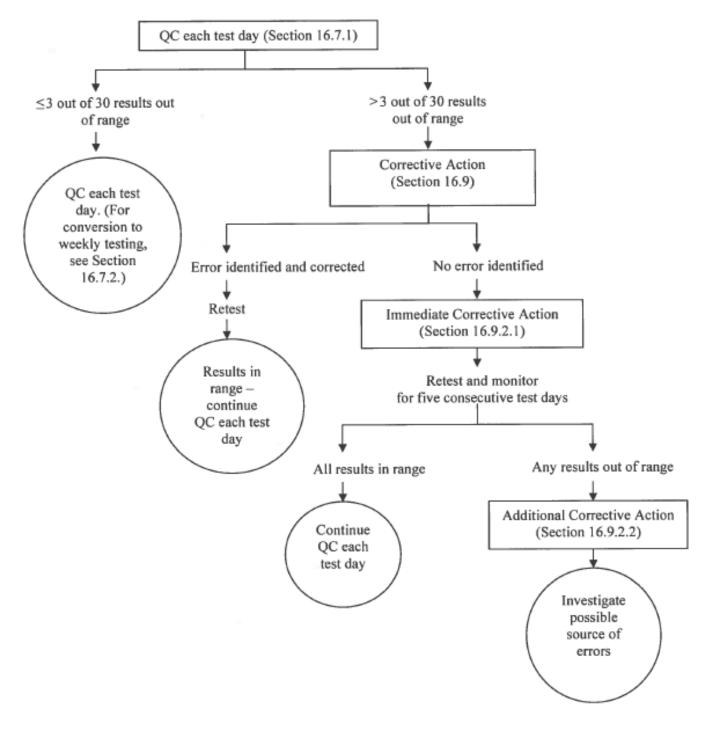
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DAILY MIC QC CHART

Appendix A. Quality Control Protocol Flow Charts





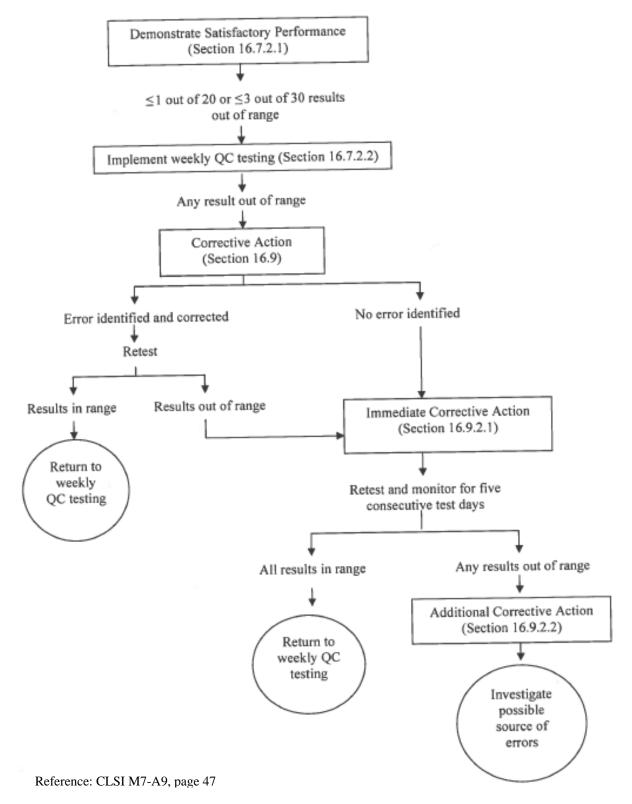
Reference: CLSI M7-A9, page 46

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Appendix A. (Continued)



QC Protocol: Weekly Testing





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INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Instructions adjusted from Czech Collection of Microorganisms (CCM) document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz.

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 (see Figure 1)
- 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

Notes:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue (see http://www.sci.muni.cz)
- 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!

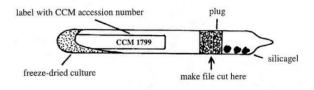


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz

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