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# The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2017





**DTU Food**National Food Institute

# THE EXTERNAL QUALITY ASSURANCE SYSTEM OF THE WHO GLOBAL FOODBORNE INFECTIONS NETWORK YEAR 2017

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

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#### List of Abbreviations

AGISAR, WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance

AMR, Antimicrobial Resistance

AST, Antimicrobial Susceptibility Testing

ATCC, American Type Culture Collection

CAZ, Ceftazidime

CDC, Centers for Disease Control and Prevention

COL, Colistin

CRO, Ceftriaxone

CTX, Cefotaxime

DTU Food, Technical University of Denmark, National Food Institute

**EQAS**, External Quality Assurance System

ESBL, Extended Spectrum Beta-Lactamase

**GMI**, Global Microbial Identifier

IP, Institute Pasteur

MERO, Meropenem

MIC, Minimum Inhibitory Concentration

SMX, Sulfamethoxazole

**SXT**, Sulfamethoxazole-trimethoprim (co-trimoxazole)

WHO, World Health Organization

WHO GFN, WHO Global Foodborne Infections Network

#### 1. Introduction

Since 2000, 16 WHO External Quality Assurance System (EQAS) reports have been issued with this report being the 17<sup>th</sup>. 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) in bacterial pathogens by conducting laboratory-based surveillance of *Salmonella* and other important foodborne pathogens. Thus, the WHO EQAS 2017 aligns 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 the WHO EQAS has expanded to include additional foodborne pathogens than *Salmonella* such as *Shigella* and *Campylobacter*. *Salmonella*, *Campylobacter* and *Shigella* are among the most important foodborne pathogens worldwide and accounts 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 bacterial isolates are associated with infections characterized by increased morbidity and mortality.

In the 2017 iteration of the WHO EQAS, a major change was applied as it focuses only on *Salmonella* serotyping and antimicrobial susceptibility testing (AST). This adjustment was made to balance the costs and focus efforts at continuing the development of the genomic proficiency test adopted by WHO and offered through the Global Microbial Identifier (GMI) (http://www.globalmicrobialidentifier.org/workgroups/about-the-gmi-proficiency-tests).

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, and Institute Pasteur (IP) in Paris, France.

Individual laboratory data are confidential and known only 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 at 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 AST of *Salmonella* with a maximum error rate of 10% (either less than 5% very major / major errors and less than 5% minor errors, or less than 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 resistant strain as resistant (*i.e.* S  $\rightarrow$  R). Very major deviation is the classification of a resistant strain as susceptible (*i.e.* R  $\rightarrow$  S). In this report, the deviations of AST results are divided into two categories, *i.e.* critical deviations which include major and very major deviations, and total deviations which include also the minor deviations. In EQAS 2014, the regions were re-defined for all countries worldwide in relation to the analysis of data from the WHO GFN EQAS. This resulted in 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 2017.

#### 2. Summary

The summary report is divided into sections; the serotyping component, AMR as well as reporting resistance to Extended Spectrum Beta-Lactamases (ESBL) producing *Salmonella*. All results reported in the summary can be found in Appendix 1.

#### **Participation**

A total of 191 laboratories responded to the pre-notification and were enrolled in the WHO EQAS. When the deadline for submitting results was reached, 181 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, Bangladesh, Barbados, Belgium, Belize, Bolivia, Brazil (2), Brunei Darussalam, Bulgaria, Cambodia, Cameroon, Canada (11), Chile, China (15), Colombia (4), Costa Rica (2), Croatia, Cuba, Curaçao, Cyprus, Czech Republic (2), Denmark, Ecuador, Egypt, Gambia, Germany (2), Ghana, Greece (2), Guatemala (2), Honduras, India (4), Iran, Islamic rep. of (3), Ireland, Israel, Italy (14), Jamaica, Japan (2), Kenya (2), Korea, Rep of (2), Kosovo, Lao PDR, Luxembourg (2), Madagascar, Malaysia (5), Malta, Mauritius, Mexico (3), Morocco, Nepal (6), New Zealand, Nigeria (4), Panama (2), Paraguay, Peru, Philippines (2), Poland (3), Portugal, Serbia (2), Singapore (2), Slovakia, Slovenia, South Africa, Spain, Sri Lanka (2), Suriname, Sweden, Taiwan, Tanzania, United Republic of, Thailand (16), Trinidad and Tobago, Turkey, Ukraine, United Kingdom, United States of America (5), Uruguay, Venezuela (2), Viet Nam (2), Zambia, and Zimbabwe.

The level of participation in the WHO EQAS 2017 was the same as at the WHO EQAS 2016.

#### Salmonella EQAS components

The acceptance threshold for the EQAS *Salmonella* serotyping component was met by 77% (n = 111) of the 145 participating laboratories (Table 1). In addition, 88% (n = 127) of the laboratories tested all eight strains with a total of 90% (n = 1.014) of all tests being correct, representing results almost at the same level as in 2016 which was one of the best performances observed since the initiation of the EQAS (Table 2). The ability to correctly serotype the internal control strain increased in 2017 to the same level as in 2014, 98%, which is the best performance, recorded and only observed previously in 2011 and 2014. The increase in performance observed compared to

2016 was most likely due to a lower number of participating laboratories serotyping this specific strain. In 2017, the participation in testing the internal control strain decreased from 159 to 142, a level previously recorded over the years (Table 3). On a region-based categorization of participating laboratories, Africa and the Central Asia & Middle East both correctly serotyped between 63% and 66% of the test strains whereas the Caribbean, Southeast Asia, and Latin America, correctly serotyped between 81% and 89% of the test strains. The performance of correct serotyping in Europe, China, North America was between 94 and 99% but reached 100% correct serotyping of all eight strains in only Oceania. In 2017, Russia was again the only region not participating (Table 4). In all regions except for the Central Asia & Middle East region either a marked or consistent improvement was observed and in line with the other data presented.

In 2017, the main problem regarding the *Salmonella* serotyping appeared relatively to be associated with strain, WHO 2017 S-17.8 (Kentucky) whereas the deviations for the rest of the strains seems to be acceptable at a level of approximately 10% (n=5) and for the remaining two strains at 6% and 2%.

As indicated, WHO 2017 S-17.8 (Kentucky, I 8,20:i:z6), revealed a considerable level of deviation at 17.0% (Table 5). Of the 23 deviations, 14 were attributed to Tumodi (I 1,4,12:i:z6) which only differs from the somatic O antigen compared to Kentucky. It is surprising that the problem of the serotyping procedure seems very often to be associated with the somatic O antigen of relatively common antigens. The level of deviation is surprising since the serovars included the 2017 should not pose major difficulties. The somatic O antigens of all the test strains belong to the major serogroups such as O:4, O:3,10, O:7, O:8, and O:9, and the flagella antigens belong to well-known polyvalent antisera complex G and HMD.

Concerning the Salmonella AST component for the EQAS 2017, the performance slightly decreased compared to the EQAS of 2016, with deviations of 3% minor, 2% major, and 3% very major deviations. Thus, the percentages of critical deviation was 5% (Table 6). Deviations categorized by the tested antimicrobials revealed that ceftazidime (CAZ), ciprofloxacin (CIP), colistin (COL), ceftriaxone (CRO), cefotaxime (CTX), meropenem (MERO), sulfamethoxazole (SMX) and co-trimoxazole (SXT) caused most of the difficulties observed with the following level of total deviations: 22%, 18%, 6%, 7%, 8%, 6%, 7%, and 7%, respectively (Table 7). The deviations to CIP was mostly attributed to minor deviations and most likely due to the often observed hazy double zone when performing disk diffusion where the outer zone often incorrectly is measured. In this year's iteration, participating laboratories appears to have been too strict measuring the zone diameter categorizing the susceptible strains intermediate. Similarly, the deviations observed to SMX and SXT are due to the bacteriostatic effect complicating reading when conducting both disk diffusion and minimum inhibitory concentration (MIC) determination where 20% of the lawn of growth for disk diffusion equal to 80% reduction of growth for MIC determination determines the read-value. This year, a resistant isolate caused most problems. For the disk diffusion results, it was not surprising to see deviations in relation to COL as disk diffusion is not recommended as a method for AST to colistin. This resulted in 10 participants incorrectly reporting one isolate susceptible despite it being resistant. For the four antimicrobials used to confirm ESBL and carbapenemase production, CAZ, CRO, CTX and MERO, all were responsible

for critical deviations with 17% of all tests incorrect for CAZ, which is a great concern (Table 7, Table 8). Assessing the data for the four antimicrobials, no clear patterns was observed, resistance reported as susceptible and visa versa (Table 8).

On a region-based categorization of participating laboratories, all regions performed poorly compared to 2016. A greater number of deviations was observed in developing regions, which partly could explain the results as well as the difficulties reporting the results for the third generation cephalosporins. The Caribbean region obtained the highest percentages of total deviations, 24.3% whereas a number of regions obtained a total deviations around 10%, *i.e.* Africa (12.8%), China (6.6%), Southeast Asia (8.1%), Latin America (8.9%), Europe (7.2%), and Central Asia & Middle East (11.1%). None of the regions obtained a performance of 100% correct AST results, however, North America and Oceania performed better than the other regions with 97.1% and 96.1% correct AST-results. Russia did not participate in the 2017 EQAS (Table 9).

For the 150 laboratories performing the *Salmonella* AST component (MIC (n = 41)/Disk diffusion (n = 74)), only 77% (115 laboratories) reported data for AST of the control strain E. coli ATCC 25922. As in previous years, this is a very alerting number as it is expected that all participating laboratories follow quality assurance procedures (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 the control strain E. coli ATCC 25922 free of charge to all new participants of the AST component, why there should not be any excuses not to test this strain.

#### **ESBL EQAS component**

The participants of the AST component are offered to detect and confirm ESBL production in the *Salmonella* strains. If participating in this component of the EQAS, all strains showing reduced susceptibility to cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ) ceftriaxone (CRO) and/or meropenem (MERO) should be tested for ESBL, AmpC and carbapenemase production.

For the EQAS 2017, four AmpC-, ESBL-, carbapenemase-producers were included represented by WHO 2017 S-17.1 Infantis (ESBL), WHO 2017 S-17.2 Havana (AmpC), WHO 2017 S-17.4 Rissen (ESBL), and WHO 2017 S-17.8 Kentucky (carbapenemase producers) (Table 11). The two ESBL producing strains harboured the *bla*<sub>CTX-M14b</sub>, and *bla*<sub>CTX-M14</sub> genes whereas the gene accounting for the AmpC phenotype till now curiously is unknown. The carbapenemase producer was conferred by *bla*<sub>NDM-1</sub> and *bla*<sub>CMY-16</sub>. The confirmatory tests (CAZ/Cl:CAZ and CTX/Cl:CTX) showed 87% (WHO 2017 S-17.1) and 90% (WHO 2017 S-17.4) of deviations in reporting correct ESBL results (based on phenotypic characteristics). For the WHO 2017 S-17.2 (AmpC) and WHO 2017 S-17.4 (carba), deviations of the confirmatory test resulted in 66% and 34%. In general, the level of correctly identified ESBL, AmpC and carbapenemase producing *Salmonella* is a great concern and it is suggested that the participating laboratories take steps to ensure that it is improved.

\_\_\_\_\_

#### 3. List of Appendices

Appendix 1: Figures and Tables

Appendix 2: Prenotification

Appendix 3: Expected results

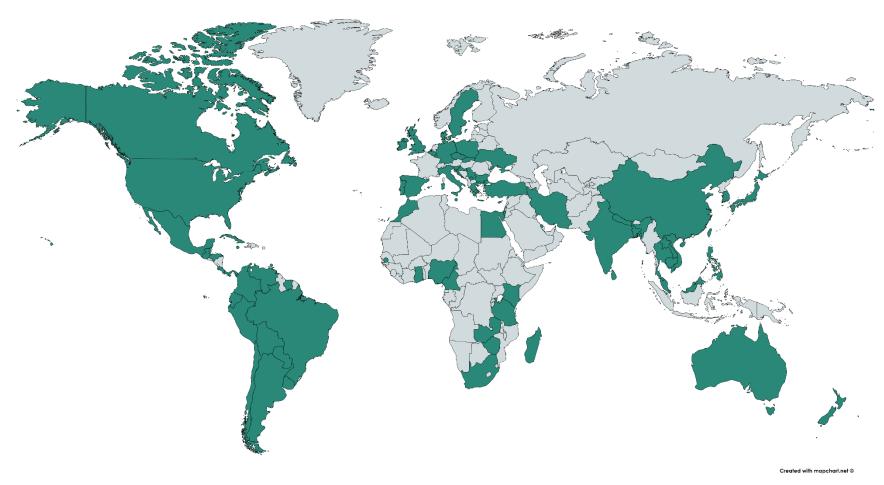
Appendix 4: WHO EQAS 2017 Protocol

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 2017



\*marked in green

#### List of Countries in the 10 Regions

**Africa** 

Algeria Gabon Reunion
Angola Gambia Rwanda
Benin Ghana Saint Helena

Botswana Guinea Sao Tome and Principe

Burkina Faso Guinea-Bissau Senegal Seychelles Burundi Kenya Cameroon Lesotho Sierra Leone Cameroun Liberia Somalia Cape Verde Libyan Arab Jamahiriya South Africa Central African Republic Madagascar South Sudan Chad Malawi Sudan Comoros Mali Swaziland

Congo (Brazzaville) Mauritania Tanzania, United Republic of

Congo, Democratic Republic of the Mauritius Togo Cote d'Ivoire (Ivory Coast) Mayotte Tunisia Uganda Djibouti Morroco Western Sahara Egypt Mozambique Equatorial Guinea Namibia Zambia Eritrea Zimbabwe Niger

Ethiopia Nigeria

Caribbean

Anguilla Dominica Saint Martin

Antigua and Barbuda Dominican Republic Saint Vincent and the Grenadines

Aruba Saint-Barthélemy Grenada Sint Maarten Bahamas Guadeloupe St. Kitts and Nevis Barbados Haiti Bonaire, Saint Eustatius and Saba Trinidad and Tobago Jamaica British Virgin Islands Turks and Caicos Islands Martinique Cayman Islands Monserrat Virgin Islands (US)

Cuba Puerto Rico Curação Saint Lucia

Central Asia & Middle East

Israel Pakistan Afganistan Armenia Jordan Palestine Azerbaijan Kazakhstan Oatar Saudi Arabia Bahrain Kuwait Bangladesh Syria Kyrgyzstan Tajikistan Bhutan Lebanon

GeorgiaMacaoTimor Leste (West)Hong KongMaldivesTurkmenistanIndiaMongoliaUnited Arab Emirates

Indonesia Myanmar (ex-Burma) Uzbekistan Iran, Islamic rep. Of Nepal Yemen

Iraq Oman

China

China

**Europe** 

Albania Guerney and Alderney Norway
Andorra Hungary Poland
Austria Iceland Portugal
Belarus Ireland Romania

BelgiumItalySan MarinoBosniaJerseySerbia

BulgariaKosovoSlovak RepublicCroatiaLatviaSlovakiaCyprusLiechtensteinSloveniaCzech RepublicLithuaniaSpain

Denmark Luxembourg Svalbard and Jan Mayen Islands

Estonia Macedonia Sweden
European Union Malta Switzerland
Faroe Islands Man, Island of Turkey
Finland Moldova Ukraine

France Monaco United Kingdom

Germany Montenegro Vatican City State (Holy See)

Gibraltar Netherlands

Greece

**Latin America** 

Argentina El Salvador Nicaragua Bolivia Falkland Islands (Malvinas) Panama Paraguay Brazil French Guiana Chile Guatemala Peru Suriname Colombia Guyana Costa Rica Honduras Uruguay Ecuador Venezuela Mexico

**North America** 

Bermuda Greenland United States of America

Canada Saint Pierre and Miquelon

**Oceania** 

Australia Papua New Guinea Guam

Kiribati Tonga New Caledonia New Zealand French Polynesia Samoa, American

Solomon, Islands Micronesia Vanuatu

Fiji Samoa
Marshall Islands Tuvalu

Russia Russia

**Southeast Asia** 

Brunei Darussalam Lao PDR Taiwan Cambodia Malaysia Thailand Japan Philippines Viet Nam

Korea, North Singapore Korea, Rep of Sri Lanka

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

Number						Part	icipatir	ng labo	ratorie	es				
of strains	EQ	AS	EQ	AS	EQ	AS	EQ	AS	EQ	AS	EQ	AS	EC	QAS
correctly serotyped		00	20		20			03		04	20			007
	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
<u>0</u>	37	3	11 96	11	1 99	100	0 127	100	4 127	3	3	100	0	0
In total	37	100	96	100	99					100	130	100	140	100
						Par	ticipati	ng iabo	эгацогі	es				
	EQ	AS	EQ	AS	EQ	AS	EQ	AS	EQ	AS	EQ	AS	EC	QAS
	2008 2009				20		20		20		20			014
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
8	50	33	76	50	91	61	82	67	68	47	52	41	70	47
7	36	24	29	19	16	11	17	14	29	20	29	23	32	21
6	11	7	7	5	12	8	10	8	14	10	15	12	17	11
5	14	9	13	8	9	6	2	2	9	6	8	6	6	4
4	12	8	5	3	6	5	4	3	5	3	7	6	5	3
3	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
						Par	ticipati	ng labo	oratori	es				
								rage						
	EQ		EQ		EQ		EQ							
	20	15	20	16	20	17	200 20	)0 - 17						
	No.	%	No.	%	No.	%	No.	%						
8	65	43	84	58	85	59	61	46						
7	25	17	22	15	26	18	24	19						
6	17	11	18	12	14	10	13	10						
5	22	15	5	3	7	5	9	7						
4	5	3	5	3	5	3	6	5						
3	2	1	5	3	3	2	6	5						
2	4	3	3	2	0	0	4	3						
1	7	5	4	3	4	3	5	4						
0	4	3	0	0	1	1	2	2						
In total	151	100	146	100	145	100	129	100						

Table 2. EQAS participating laboratories' performance of Salmonella serotyping

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			
2017	127	88	1014	90			
Average	101	76	817	86			

Table 3. EQAS participating laboratories' performance of internal quality control strain (WHO S-17.3, *Salmonella* Enteritidis) serotyping).

EQAS iteration	Labs ser S. Enteritid	
	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
2017	142	98
Average	123	94

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 2017
Africa	2001 2002 2003 2004 2006 2007 2008 2009 2010	6 9 11 9 16 11 10 15	37 62 70 51 95 73 71 94 83	73.0 87.1 71.4 62.7 71.6 80.8 49.3 75.5 67.5	Cameroun, Egypt, Kenya, Madagascar, Mauritius, Morocco, Nigeria (2), South
f	2011 2012 2013 2014 2015 2016 <b>2017</b>	10 10 8 11 12 8 <b>9</b>	57 65 51 63 68 58 <b>56</b>	79.2 60.0 74.5 76.2 61.8 62.1 <b>62.5</b>	Africa
Central Asia & Middle East	2002 2003 2004 2006 2007 2008 2009 2010	5 5 5 5 5 5 5	30 35 33 35 40 34 32 22	83.3 54.3 54.5 74.3 55.0 61.8 46.9 75.9	Bahrain, India, Israel, Nepal
Centra	2011 2012 2013 2014 2015 2016 <b>2017</b>	3 4 5 7 7 5 <b>4</b>	23 30 38 37 44 38 <b>32</b>	95.8 56.7 52.6 75.7 77.3 78.9 <b>65.6</b>	
bean	2001 2002 2003 2004 2006 2007 2008 2009	0 0 3 2 3 2 3 2 3	0 0 18 8 14 9 14	0 0 61.1 87.5 78.6 77.8 78.6 83.3	Barbados, Cuba, Curaçao,
Caribbean	2010 2011 2012 2013 2014 2015 2016 <b>2017</b>	2 1 2 1 3 5 2 <b>4</b>	13 7 16 5 15 24 16 <b>32</b>	92.9 87.5 62.5 100.0 60.0 58.3 60 <b>81.3</b>	Trinidad and Tobago
ec	2001 2002 2003 2004 2006 2007 2008	43 50 60 57 52 54 50	323 384 401 392 403 415 379	80.5 90.0 84.8 84.7 86.4 89.4 82.3	Belgium, Bulgaria, Croatia, Cyprus, Czech Republic (2), Denmark, Germany (2), Greece (3), Ireland, Italy (14),
Europe	2009 2010 2011 2012 2013 2014 2015 2016 <b>2017</b>	47 45 42 47 42 52 48 46 <b>42</b>	362 332 314 368 309 391 371 362 <b>330</b>	93.1 94.1 94.6 92.9 94.5 96.2 93.8 93.4	Luxembourg (2), Poland (3), Portugal, Serbia (2), Slovak Republic, Slovenia, Spain, Sweden, Turkey, Ukraine, United Kingdom

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 2017
					M EQ115 2017
	2001	4	32	87.5	
	2002 2003	2 6	16 41	100.0 95.1	
	2003	8	55	81.8	
	2006	10	80	96.3	
ca	2007	12	94	97.9	
eri	2008	11	84	95.2	
	2009	12	90	92.2	Canada (9), United States of
<b>h</b> A	2010	13	103	100.0	America (4)
North America	2011 2012	11 14	81 101	97.6 93.1	
Ž	2012	13	92	97.8	
	2014	13	84	100.0	
	2015	13	93	100.0	
	2016	13	100	99.0	
	2017	13	99	99.0	
	2001	4	30	100.0	
	2002	6	43	93.0	
	2003 2004	6 5	46 38	93.5 97.4	
	2004	5	37	94.6	
	2007	4	32	100.0	
<u>'ख</u>	2008	4	30	93.3	
Oceania	2009	4	32	96.9	Australia (3), New Zealand
) Se	2010	4	32	100.0	Australia (3), New Zealand
0	2011	4	32	100.0	
	2012 2013	4 4	32 31	100.0 100.0	
	2013	4	32	100.0	
	2015	4	31	100.0	
	2016	4	32	100.0	
	2017	4	31	100.0	
	2001	1	8	12.5	
	2002 2003	1 1	8 7	62.5 14.3	
	2003	4	26	69.2	
	2006	5	40	80.0	
	2007	8	51	80.4	
ಡ	2008	6	40	90.0	
ıssia	2009	7	49	91.8	- none -
Ru	2010 2011	8 7	54 48	87.1 87.3	none
	2011	6	48	87.5	
	2012	2	16	75.0	
	2014	4	30	93.3	
	2015	3	24	100.0	
	2016	-	-	-	
	2017	-	- 70	-	
	2001 2002	11 11	78 82	57.7 87.8	
	2002	13	83	75.9	
	2004	15	88	79.5	
	2006	13	84	84.5	Argentine Delivie Deseil (2)
ica	2007	15	107	88.8	Argentina, Bolivia, Brazil (2),
ner	2008	17	120	71.7	Chile, Colombia (4), Costa Rica
An	2009 2010	21 22	150 132	77.3 80.0	(2), Ecuador, Guatemala,
Latin America	2010	22 23	132 144	80.0 83.7	Honduras, Mexico (3), Panama
ati	2012	25	182	73.1	(2), Paraguay, Peru, Uruguay,
Ι	2013	22	154	83.1	Venezuela
	2014	24	166	84.9	
	2015	20	133	84.2	
	2016	23	165	87.9	
	2017	23	178	89.3	

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 2017
	2001	15	113	54.0	
	2002	12	90	92.2	
	2003	15	100	81.0	
	2004	17	130	81.5	
ಡ	2006	15	117	84.6	Brunei Darussalam, Cambodia,
Sis	2007	19	140	91.4	Japan (2), Korea, Rep of (2),
Southeast Asia	2008	18	125	81.6	
asi	2009	23	180	81.1	LAO PDR, Malaysia (4),
he	2010	24	172	90.5	Philippines (2), Singapore (2),
at	2011	23	180	98.4	Sri Lanka, Taiwan, Thailand
So	2012	28	207	77.8	(12), Viet Nam (2)
	2013	22	163	89.6	(/, (-/
	2014	22	166	94.6	
	2015	24	179	88.3	
	2016	28	211	87.7	
	2017	31	244	89.3	
	2001	4	32	96.9	
	2002	3	24	100.0	
	2003	8	60	75.0	
	2004	7	46	78.3	
	2006	6	48	85.4	
	2007	10	80	91.3	
ಡ	2008	15	108	94.4	
China	2009	16	126	95.2	China (15)
コ	2010	10	74	92.5	
	2012	10	78	80.8	
	2013	7	54	92.6	
	2014	9	71	93.0	
	2015	15	118	78.0	
	2016 17		136	95.6	
	2017	15	120	97.5	

Table 5. Salmonella serogroups (SG), serotypes (ST) and deviations (D), WHO EQAS 2017

Strain ID	Correct s	serotype	No. of labs reporting SG	% D <sub>SG</sub>	No. of labs reporting ST	% <b>D</b> <sub>ST</sub>	Deviating results (*)
WHO 2017 S-17.1	Infantis	I 6,7:r:1,5	159	4.4	143	11.2	Choleraesuis (2), Goma, Irumu, Papuana, Paratyphi A, Paratyphi C, Surat, Thompson, Typhi, Virchow (6)
WHO 2017 S-17.2	Havana	I 13,23:f,g:-	149	10.7	137	10.9	Adelaide, Derby, II .1.,13,23:g,m,[s],t:[e,n,x], Kiel, Linton, Lomita, Paratyphi A, Paratyphi C, Raus (3), Rideau (2), Rissen, Rissen var. 14+
WHO 2017 S-17.3	Enteritidis	I 9,12:g,m;-	156	1.3	142	2.1	Berta, Typhi (2)
WHO 2017 S-17.4	Rissen	I 6,7:f,g:-	152	1.3	139	10.1	Alamo, Eingedi (2), Galiema, Menston, Montevideo, Othmarschen (4), Plumaugat, Typhi
WHO 2017 S-17.5	Weltevreden	I 3,10:r;z6	155	2.6	142	9.2	Assinie, Dumfries, Elisabethville (3), Fareham, Paratyphi B (2), Simi, Stockholm, Ughelli (2)
WHO 2017 S-17.6	Schwarzengrund	I 4,12:d:1,7	156	1.3	141	10.6	Ahmadi, Ayinde, Brezany, Kisangani, Kubacha, Kaapstad, Paratyphi A, Paratyphi B, Sarajane, Stanley (2), Travis, Typhimurium (2), Uppsala
WHO 2017 S-17.7	Typhimurium	I 4,5,12:i;1,2	157	1.3	143	6.3	Avonmouth, I 1,4,5,12:i:, Lagos (3), Paratyphi A, Saintpaul
WHO 2017 S-17.8	Kentucky	I 8,20:i:z6	145	15.9	135	17.0	Azteca, Bardo, Bargny, Enteritidis, Falkensee, Haardt, Paratyphi A (2), Sekondi, Tumodi (14)

<sup>\*</sup>number of participants reporting the specified deviating result

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

EQAS iteration	No. of EQAS participating laboratories	% correct test results	% minor deviations $(S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$	% 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
2017	150	91	3	2	3	5	8
Average*	139	93	4	2	1	4	8

<sup>\*</sup>Data do not include one strain which may have lost resistance due to transport or storage stress

<sup>^</sup>S, susceptible; I, intermediate; R, resistant

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

EQAS	No.										A	ntimicr	obial $^{\infty}$								
iteration	of labs	Performance	AMC	AMP	CAZ	CHL	CIP	COL	CRO	CTX	GEN	KAN	NAL	SMX	MER	STR	SXT	TET	TMP	XNL	OVERALL average
		No. of tests	-	343	-	343	334	-	-	-	343	312	328	248	-	312	-	335	295	-	798
2000	44	% critical deviations*	-	6	-	4	1	-	1	1	4	4	1	3	-	4	-	6	1	-	6
		% total deviations^	-	8	-	7	6	1	ı	-	5	16	4	5	-	12	-	13	1	-	14
		No. of tests	-	822	-	814	813	ı	1	-	821	623	726	431	-	679	757	804	416	-	1778
2001	108	% critical deviations*	-	4	-	2	1	-	-	-	2	2	2	6	-	7	2	7	1	-	6
		% total deviations^	-	7	-	3	4	1	-	-	4	7	8	9	-	27	5	18	2	-	15
		No. of tests	-	918	-	903	911	ı	1	-	905	680	885	495	-	718	724	861	499	-	1961
2002	119	% critical deviations*	-	2	-	2	0	ı	1	-	2	2	2	4	-	4	7	3	3	-	5
		% total deviations^	-	3	-	3	2	-	1	-	16	10	4	4	-	34	10	7	3	-	15
		No. of tests	-	1019	-	996	995	ı	1	-	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	-	-	956	1078	-	1035	649	-	896	996	1054	607	225	2256
2006	143	% critical deviations*	9	2	7	3	2	-	-	7	3	-	2	6	-	5	3	9	1	2	8
		% total deviations^	22	3	11	15	6	-	-	15	7	-	6	7	-	22	5	20	2	9	21
		No. of tests	908	1114	830	1105	1101	-	-	914	1111	-	1092	678	-	875	971	1047	583	258	2290
2007	143	% critical deviations*	6	5	1	0	1	-	-	1	3	-	2	5	-	4	3	4	1	0	5
		% total deviations^	17	7	1	6	1	-	-	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 (continued). EQAS participants' performance of Salmonella strains antimicrobial susceptibility testing categorized by antimicrobial.

EQAS	No.											Antimic	$\mathbf{robial}^{\infty}$								
iteration	of labs	Performance	AMC	AMP	CAZ	CHL	CIP	COL	CRO	CTX	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	ı	3	8	6	-	2	6	2	-	12
		No. of tests	-	1166	1016	881	1167	473	831	968	1113	-	921	487	921	-	1055	1014	553	-	1354
2017	150	% critical deviations*	-	4	17	4	1	6	6	6	4	-	2	5	6	-	6	2	5	-	3
		% total deviations^	-	5	22	5	18	6	7	8	5	-	2	7	6	-	7	4	5	-	9
		No. of tests	944	1076	930	1003	1069	473	793	996	1044	588	978	557	790	769	982	1011	562	242	800
Average•	139	% critical deviations*	7	3	5	2	4	6	3	3	3	3	2	6	4	6	3	4	2	1	4
		% total deviations^	17	5	6	4	10	6	4	7	5	10	5	7	4	31	4	10	2	8	8

 $<sup>^{\</sup>infty}$ For antimicrobial abbreviations: see List of Abbreviations page 1

<sup>\*</sup> $R \rightarrow S \& S \rightarrow R (R, resistant; S, susceptible)$ 

 $<sup>^</sup>S \rightarrow R \& R \rightarrow S \& S \rightarrow I \text{ or } I \rightarrow R \text{ (I, intermediate)}$ 

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

<sup>-,</sup> not determined

Table 8. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2017 Salmonella strains\*

Strain							Ant	timicrobia	1^						
	AMP	CTX	FOX	CAZ	CRO	CHL	CIP	COL	GEN	MER	NAL	SMX	TET	TMP	SXT
WHO S-17.1	<b>132</b> /0/13	<b>101</b> /2/18	9/4/ <b>101</b>	<b>77</b> /15/34	<b>90</b> /0/15	5/2/120	15/ <b>73</b> /56	2/0/ <b>58</b>	6/1/ <b>131</b>	9/1/ <b>106</b>	<b>111</b> /1/1	<b>60</b> /0/2	<b>124</b> /2/1	<b>50</b> /0/19	<b>88</b> /1/38
WHO S-17.2	10/2/ <b>134</b>	16/8/ <b>96</b>	<b>31</b> /36/46	<b>53</b> /14/60	7/0/ <b>97</b>	1/0/ <b>124</b>	4/11/ <b>132</b>	1/0/ <b>58</b>	4/2/133	5/0/ <b>109</b>	3/2/111	14/7/ <b>40</b>	2/2/123	1/0/ <b>68</b>	5/1/ <b>126</b>
WHO S-17.3	9/6/ <b>130</b>	7/3/112	-	5/6/117	8/1/ <b>95</b>	*	1/17/ <b>127</b>	5/1/ <b>53</b>	<b>124</b> /5/7	4/0/ <b>109</b>	2/1/ <b>113</b>	<b>56</b> /1/5	11/9/ <b>106</b>	2/0/ <b>69</b>	5/0/ <b>127</b>
WHO S-17.4	<b>142</b> /1/3	<b>117</b> /2/2	7/1/ <b>107</b>	<b>55</b> /18/54	<b>97</b> /1/6	<b>115</b> /0/10	0/15/ <b>130</b>	1/1/ <b>57</b>	5/2/132	4/0/111	0/1/ <b>114</b>	<b>56</b> /0/4	<b>122</b> /2/3	<b>64</b> /0/5	<b>122</b> /0/11
WHO S-17.5	6/0/ <b>140</b>	3/2/116	-	5/0/123	5/1/ <b>97</b>	<b>122</b> /2/1	1/13/ <b>132</b>	2/1/ <b>55</b>	5/0/135	5/0/110	3/1/ <b>112</b>	<b>60</b> /0/0	<b>125</b> /1/1	<b>69</b> /0/0	<b>132</b> /1/0
WHO S-17.6	6/0/ <b>140</b>	2/3/116	-	4/1/ <b>122</b>	2/0/101	7/0/119	0/15/ <b>129</b>	4/0/ <b>54</b>	4/1/ <b>135</b>	4/0/110	3/1/ <b>110</b>	<b>60</b> /0/0	<b>124</b> /2/1	<b>69</b> /0/0	<b>128</b> /0/5
WHO S-17.7	<b>141</b> /0/5	10/2/ <b>109</b>	-	6/4/ <b>117</b>	7/0/ <b>96</b>	<b>119</b> /1/6	30/ <b>92</b> /26	<b>52</b> /0/10	4/1/ <b>135</b>	8/0/ <b>107</b>	<b>112</b> /1/3	<b>60</b> /0/0	<b>124</b> /2/1	<b>69</b> /0/0	<b>131</b> /0/1
WHO S-17.8	<b>146</b> /0/0	<b>120</b> /1/0	111/2/0	<b>125</b> /1/0	<b>101</b> /0/4	<b>121</b> /0/3	140/4/4	2/0/ <b>56</b>	10/1/ <b>130</b>	<b>101</b> /6/12	115/0/0	<b>62</b> /0/0	<b>122</b> /2/2	<b>68</b> /0/0	<b>131</b> /0/2

<sup>^</sup>For antimicrobial abbreviations: see List of Abbreviations page 1

 $Marked \ in \ bold: \ expected \ interpretation. \ Grey \ cell: <90\% \ of \ laboratories \ did \ correct \ interpretation. \ R, \ resistant/I, \ intermediate/S, \ susceptible.$ 

<sup>\*</sup>The result for the *Salmonella* strain WHO S-17.3 for chloramphenicol was omitted from evaluation (during the process of analyzing the WHO EQAS 2017 data, it was clear to the organizers that the database evaluation of the result related to the *Salmonella* strain WHO S-17.3 for chloramphenicol caused a large number of deviations. The expected result related to the testing of WHO S-17.3/chloramphenicol was 16/intermediate, only, due to the large number of deviations, the organizers decided not to evaluate the submitted results related to this strain/antimicrobial combination.)

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

	_							monetta ASI	
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→R & R→S & S↔I or I↔R)^	Countries participating in the 2017 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, Egypt,
	2007	16	90.7	4.4	4.0	0.9	4.9	9.3	Ghana, Kenya (2),
	2008	19	83.8	6.5	5.5	4.2	9.7	16.2	Madagascar, Mauritius,
Africa	2009	22	90.1	4.5	3.6	1.8	5.4	9.9	Morocco, Nigeria (4),
Afr	2010	22	84.7	6.0	6.5	2.8	9.3	15.3	South Africa, Tanzania,
	2011	17	87.0	5.0	4.7	3.3	8.0	13.0	United Republic of, The
	2012	18	89.4	5.3	3.5	1.9	5.4	10.6	Gambia, Zambia,
	2013	16	92.0	3.2	4.0	0.9	4.9	8.0	Zimbabwe
	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	
	2017	17	87.2	4.5	4.0	4.3	8.3	12.8	
	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	
st	2004	10	87.5	6.7	5.5	0.3	5.8	12.5	
Ea	2006	7	79.2	10.5	9.8	0.5	10.3	20.8	
dle	2007	8	87.8	5.0	6.2	1.1	7.3	12.2	
Central Asia & Middle East	2008	12	86.1	6.5	4.0	3.4	7.4	13.9	D 1 1 1 T 1' (4)
<u> </u>	2009	6	93.7	4.3	0.9	1.1	2.0	6.3	Bangladesh, India (4),
ia &	2010	7	95.8	2.6	0.2	1.4	1.6	4.2	Iran, Islamic rep. of (3), Israel, Nepal (6)
Asi	2011	4	91.8	4.1	1.8	2.3	4.1	8.2	israel, repar (0)
ral	2012	8	92.8	4.4	1.6	0.7	2.3	6.6	
ent	2013	8	93.6	5.2	1.0	0.1	1.2	6.4	
ŭ	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	
	2017	15	88.9	5.0	2.6	3.5	6.1	11.1	
	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	
	2007	4	95.0	3.1	0.9	0.9	1.8	5.0	
an	2008	5	90.7	5.5	0.9	2.9	3.8	9.3	Darlandan Cula
Caribbean	2009	4	93.2	1.8	3.2	1.8	5.0	6.8	Barbados, Cuba, Curaçao, Jamaica,
l ii	2010	4	90.9	5.4	2.7	0.7	3.4	8.8	Trinidad and Tobago
ご	2011	2	96.5	1.4	0.0	2.1	2.1	3.5	Timada dila 1000g0
	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	
	2017	5	75.7	5.0	10.1	9.1	19.2	24.3	

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

			ptibility te						
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→R & R→S & S↔I or I↔R)^	Countries participating in the 2017 iteration
	8,5	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, Croatia,
	2006	54	88.7	7.0	3.8	0.6	4.4	11.3	Cyprus, Czech
	2007	49	94.2	3.7	1.6	0.4	2.0	5.7	Republic, Denmark,
	2008	51	91.2	4.4	2.5	1.9	4.4	8.8	Greece (3), Ireland, Italy
Europe	2009	40	95.1	2.6	1.3	0.9	2.2	4.8	(8), Kosova,
ļ ,	2010	39	92.4	4.1	1.2	2.3	3.5	7.6	Luxembourg (2), Malta,
国	2011	36	92.5	4.5	1.7	1.3	3.0	7.5	Poland (3), Portugal, Serbia (2), Slovak
	2012	40	95.5	2.8	1.2	0.4	1.7	4.5	Republic, Slovenia,
	2013	37	95.7	2.5	1.4	0.3	1.7	4.2	Spain, Turkey, 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	
	2017	33	92.8	2.4	2.1	2.7	4.8	7.2	
	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	
ल	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	
me	2009	10	98.7	0.0	0.4	0.9	1.3	1.3	Canada (5), United States
h A	2010	11	94.8	2.6	0.2	2.4	2.6	5.2	of America (4)
ort	2011	9	92.1	2.6	1.5	3.8	5.3	7.9	
Z	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	
	2017	9	97.1	1.2	0.6	1.2	1.7	2.9	
	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	
ಡ	2008	4	93.9	3.8	0.0	2.3	2.3	6.1	
ani	2009	4	95.9	3.2	0.3	0.6	0.9	4.1	Australia, New Zealand
Oceania	2010	4	92.5	4.6	0.6	2.3	2.9	7.5	Tubirunu, New Zealand
	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	
	2017	2	96.1	2.6	0.0	1.3	1.3	3.9	

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

Region	bility test 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 $(R \rightarrow S)^{\wedge}$	% critical deviations $(S \rightarrow R \& R \rightarrow S)^{\wedge}$	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2017 iteration
	2001		01.0	15.0	2.0	0.0	2.0	,	
	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	
æ	2008	6	92.2 93.8	4.7	1.4	1.7	3.1	7.8 6.2	
Russia	2009	6 8		3.3	3.3	0.8	4.1	5.7	- none -
Ru	2010 2011	7	94.3 90.0	4.8	3.2	2.0	5.2	10.0	
	2011	6	90.0	2.0	0.0	0.6	0.6	2.6	
	2012	2	98.2	1.8	0.0	0.0	0.0	1.8	
	2013	4	98.2	0.3	0.0	0.6	1.5	1.8	
	2014	4	98.2	1.0	0.9	0.0	0.3	1.3	
	2013	-							
			-	-	-	-	-	-	
	2017	11	00.8	-	1.4	1.0	2.4	- 0.2	
	2001	13	90.8	6.9	1.4	1.0	2.4	9.2 6.3	
	2002	12	93.7 90.8	4.6	0.7 2.0	3.0	5.0	9.2	
	2003	17	90.8	4.7	0.8	0.1	0.9	5.6	
	2004	16	88.7	6.3	4.5	0.6	5.1	11.3	
	2007	17	94.9	1.8	1.9	1.4	3.3	5.0	Argentina, Belize, Bolivia,
ca	2007	20	93.0	3.4	1.5	2.1	3.6	7.0	Brazil (2), Chile, Colombia
Latin America	2009	20	95.6	2.1	1.1	1.2	2.3	4.4	(3), Costa Rica (2),
Am	2010	23	90.8	2.1	5.6	1.4	7.1	9.2	Ecuador, Guatemala (2), Honduras, Mexico (2),
ţi	2011	22	90.8	2.8	3.1	3.3	6.4	9.2	Panama (2), Paraguay,
Lat	2012	25	94.4	1.6	3.0	1.0	4.0	5.6	Peru, Suriname, Uruguay,
	2013	25	95.5	2.6	1.2	0.3	1.5	4.2	Venezuela
	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	
	2017	24	91.1	3.3	2.3	3.2	5.5	8.9	
	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	
а	2008	18	92.8	3.7	0.8	2.7	3.5	7.2	
China	2009	14	94.8	2.2	2.1	0.8	2.9	5.1	China (14)
C	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	
	2017	14	93.4	2.9	0.7	3.0	3.7	6.6	

<sup>^</sup>S. susceptible; I. intermediate; R. resistant

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

Region	EQAS iteration	No. of labs	% correct test result	% minor deviations $(S \leftrightarrow I \text{ or } I \leftrightarrow R)^{\wedge}$	% 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 or I $\leftrightarrow$ R) $^{\wedge}$	Countries participating in the 2017 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	
ಡ	2007	20	93.9	4.0	1.4	0.7	2.1	6.1	Cambodia Ianan (2)
Asia	2008	19	90.5	4.7	2.2	2.6	4.8	9.5	Cambodia, Japan (2), Korea, Rep of (2), LAO
ıst	2009	27	91.8	4.1	3.0	1.2	4.2	8.3	PDR, Malaysia (5),
Southeast	2010	25	92.8	3.8	1.5	1.9	3.4	7.2	Philippines (2), Singapore,
out	2011	26	90.5	3.5	2.4	3.5	5.9	9.5	Sri Lanka (2), Taiwan,
, v	2012	35	91.7	3.9	3.5	0.9	4.4	8.3	Thailand (13), Viet Nam
	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	
	2017	31	91.9	2.9	2.1	3.2	5.2	8.1	

<sup>^</sup>S. susceptible; I. intermediate; R. resistant

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

		Method	Perfor- mance <sup>4.5</sup>	AMP	CAZ	CHL	CIP	COL	CRO	СТХ	FIS (SMX) <sup>2</sup>	FOX	GEN	MER	NAL	STR	SXT	TET	ТМР
Acc	cepted	MIC (μg/ml)		2-8	0.06-0.5	2-8	0.004-0.016	0.25-2	0.03-0.12	0.03-0.12	8-32	2-8	0.25-1	0.008-0.06	1-4	4-16 <sup>3</sup>	≤0.5/9.5	0.5-2	0.5-2
	erval <sup>1</sup>	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	MIC & Disk	No.4	37	-	38	35	-	-	-	19	-	39	-	37	36	-	42	31
	(44) <b>2001</b>		% <sup>5</sup> No. <sup>4</sup>	27 97	-	37 97	20 97	-	-	-	53 53	-	23 99	-	35 74	22	90	42 96	30
	(107)	MIC & Disk	NO. 1	19	-	20	14	-	-	-	34	-	12	-	14	81 12	14	22	50 22
	2002	100 0 D. I	No. <sup>4</sup>	109	-	107	108	-	-	-	57	-	108	-	102	82	102	102	66
	(114)	MIC & Disk	% <sup>5</sup>	16	-	15	14	-	-	-	26	-	12	-	14	11	12	13	11
	2003	MIC & Disk	No.4	140	-	137	138	-	-	-	82	-	138	-	132	105	129	137	79
	(144)	MIC & DISK	% <sup>5</sup>	14	-	22	9	_	-	-	17	-	9	_	16	9	14	19	14
<i>∞</i>	2004	MIC & Disk	No.4	132	-	128	132	-	-	111	84	-	134	-	126	110	120	129	87
of participants)	(140)	Title & Dish	% <sup>5</sup>	10	-	13	8	-	-	18	16	-	10	-	9	6	11	13	9
pa	2006	MIC & Disk	No. <sup>4</sup> % <sup>5</sup>	133 14	96 15	126	127 8	-	-	115 21	74 29	-	131 14	-	122 20	106 11	122 19	125 12	74 17
[Ci]	(137) <b>2007</b>		No.4	124	92	18 123	121	-	-	104	64	-	124	-	120	97	107	117	67
i <del>.</del>	(126)	MIC & Disk	% <sup>5</sup>	111	92	14	121	-	-	16	22	-	6	-	7	6	13	7	10
pē	(120)	100 0 D. I	No. <sup>4</sup>	147	111	135	144	_	_	124	71	_	145	_	136	101	129	139	79
of		MIC & Disk	% <sup>5</sup>	12	9	10	8	-	-	14	14	-	8	-	8	12	13	7	13
	2008	MIC	No.4	33	23	24	33	_	-	23	18	-	31	-	23	19	22	28	16
(total no.	(147)	IVIIC	% <sup>5</sup>	0	5	0	6	-	-	9	11	-	0	-	0	11	9	0	13
ta		Disk	No.4	114	89	112	111	-	-	101	53	-	114	-	113	82	107	111	63
(tc		Dist	% <sup>5</sup>	16	10	12	8	-	-	15	15	-	11	-	10	12	14	9	13
n		MIC & Disk	No. <sup>4</sup> % <sup>5</sup>	128	100	121	124 7	-	88	107	63	-	123	-	117	98	113	122	70
io	2009		No.4	16 27	13 19	15 24	26	-	16 20	10 20	11 14	-	18 25	-	13 24	10 19	14 21	14 27	11 25
at	(129)	MIC (27)	NO. 1	11	19	8	8	-	20 15	15	21	-	12	-	8	19 5	19	11	13
iteration	(149)		No. <sup>4</sup>	101	81	97	98		68	87	49	_	98	-	93	79	92	95	55
		Disk (102)	% <sup>5</sup>	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
7		WHC & DISK	% <sup>5</sup>	11	9	9	6	-	10	14	11	-	11	-	5	5	12	5	15
E	2010	MIC (24)	No.4	25	15	21	25	-	15	17	12	-	24	-	19	17	17	24	11
	(116)	WHC (24)	% <sup>5</sup>	12	20	10	8	-	7	18	8	-	13	-	16	18	18	17	36
		Disk (91)	No.4	89	82	87	90	-	64	83	39	-	88	-	85	67	84	86	52
		. ,	% <sup>5</sup> No. <sup>4</sup>	9	6	102	4	-	9	11	10	-	103	-	103	72.	10	107	8
		MIC & Disk	No. <sup>-</sup> % <sup>5</sup>	111	89 4	102 11	109 7	-	76 7	96 9	50 8	-	103	-	8	4	99 16	107 7	51 14
	2011		No. <sup>4</sup>	23	15	18	22	-	16	15	13	-	22.	-	19	17	16	21	11
	(112)	MIC (23)	% <sup>5</sup>	4	7	0	9		6	0	8		9	-	0	6	6	5	0
	(112)	D: 1 (00)	No. <sup>4</sup>	88	74	84	87	-	60	81	37	-	81	-	84	55	83	86	40
		Disk (89)	% <sup>5</sup>	20	4	13	7	-	7	11	8	-	11	-	10	4	18	8	18

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

		Method	Perfor- mance <sup>4.5</sup>	AMP	CAZ	CHL	CIP	COL	CRO	CTX	FIS (SMX) <sup>2</sup>	FOX	GEN	MER	NAL	STR	SXT	TET	ТМР
Acc	epted	MIC (μg/ml)		2-8	0.06-0.5	2-8	0.004-0.016	0.25-2	0.03-0.12	0.03-0.12	8-32	2-8	0.25-1	0.008-0.06	1-4	4-16 <sup>3</sup>	≤0.5/9.5	0.5-2	0.5-2
inte	rval <sup>1</sup>	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. <sup>4</sup>	134	111	121	131	-	90	115	53	-	127	-	121	89	112	129	66
		MIC & DISK	% <sup>5</sup>	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)		% <sup>5</sup>	3	4	0	3	-	0	4	5	-	3	-	3	8	0	0	9
		Disk (98)	No. <sup>4</sup>	97	85	90	96	-	67	87	34	-	92	-	90	63	89	94	44
		` ′	% <sup>5</sup>	16	14	9	7	-	15	11	15	-	11	-	11	8	16	14	27
		MIC & Disk	No. <sup>4</sup> % <sup>5</sup>	117 12	100	112 5	119 7	-	82 4	107 8	10	-	113	-	113 11	-	101 8	114 8	59 11
	2013		% No.4	31	25	28	32	_	19	27	17	-	32	-	28	-	22	32	22
	(122)	MIC (33)	% <sup>5</sup>	6	4	4	13	_	5	11	18	_	9	_	11	_	5	6	5
of participants)	(122)		No. <sup>4</sup>	86	75	84	87	_	63	80	27	_	81	_	85	-	79	82	37
раі		Disk (89)	% <sup>5</sup>	13	8	6	5		5	6	7	-	4	_	9	-	10	7	8
ici		100 0 D. I	No. <sup>4</sup>	111	99	101	108	-	75	97	49	-	111	-	103	-	102	104	50
art		MIC & Disk	% <sup>5</sup>	5	7	7	6	-	7	14	14	-	8	-	8	-	8	7	2
i pë	2014	MIC (28)	No.4	27	21	24	27	-	16	22	16	-	28	-	24	-	21	25	12
	(115)		% <sup>5</sup>	4	5	4	15	-	6	14	0	-	14	-	8	-	14	0	0
(total no.		Dick (87)	No. <sup>4</sup>	84	78	77	81	-	59	75	33	-	83	-	79	-	81	79	38
al 1		Disk (87)	% <sup>5</sup>	6	8	8	4	-	7	15	21	-	6	-	8	-	6	9	3
tot		MIC&Disk	No. <sup>4</sup>	113	101	101	112		78	99	54	75	112	74	100	-	104	106	57
		MICCEDISK	% <sup>5</sup>	8	5	7	7	-	9	6	11	9	9	12	7	-	13	8	9
[0]	2015	MIC (31)	No.4	30	26	25	30	-	16	25	15	20	30	19	24	-	24	27	16
ati	(117)		% <sup>5</sup>	3	8	4	13	-	0	12	7	10	7	11	4	-	8	7	13
er		Disk (85)	No. <sup>4</sup>	83	75	76	82	-	62	74	39	55	82	55	76	-	80	79	41
EQAS iteration		` ′	% <sup>5</sup>	10	4	8	5	-	11	4	13	9	10	13 88	8	-	14 91	97	59
AS		MIC&Disk	No. <sup>4</sup> % <sup>5</sup>	111	93 5	95 13	101 9	-	76 16	94 15	54 24	84 7	8	10	91	-	8	10	14
Õ	2016		70 No. <sup>4</sup>	27	24	24	27	-	17	24	13	22	29	25	20	-	20	25	16
田	(106)	MIC (30)	% <sup>5</sup>	4	4	0	7	_	12	4	23	0	3	4	0	_	0	8	13
	(100)		No. <sup>4</sup>	74	69	71	74	_	59	70	41	62	70	63	71	_	71	72	43
		Disk (76)	% <sup>5</sup>	14	6	17	9	-	17	19	24	10	10	13	11	-	10	11	14
			No. <sup>4</sup>	114	101	103	113	56	82	93	56	92	107	93	89	-	95	99	61
		MIC&Disk	% <sup>5</sup>	13	11	10	10	20	16	14	27	10	7	10	7	-	6	10	8
	2017		No. <sup>4</sup>	41	33	35	41	28	25	31	24	30	38	34	31	_	29	34	26
	(115)	MIC (41)	% <sup>5</sup>	5	6	0	7	4	12	6	17	3	5	6	3	_	0	6	0
	(113)		No. <sup>4</sup>	73	68	68	72	28	57	62	32	62	69	59	58	_	66	65	35
		Disk (74)	% <sup>5</sup>	18	13	15	11	36	18	18	34	13	7	12	9	-	9	12	14
00	4111.1.1	   abbreviations: see Li				13	11	30	10	10	34	13	/	12	9	_	9	1.2	14

<sup>&</sup>lt;sup>0</sup>For antimicrobial abbreviations: see List of Abbreviations page 1

<sup>&</sup>lt;sup>1</sup>CLSI standard. Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 22nd Informational supplement. CLSI document M100-S22. 2012 Wayne. PA. USA

<sup>&</sup>lt;sup>2</sup>FIS (sulfisoxazole) covers the group of SMX (sulfonamides); <sup>3</sup>Quality control range developed by the manufacturer of Sensititre®; <sup>4</sup>No.. number of laboratories performing the analysis; <sup>5</sup>%. percentage of laboratories reporting erroneous results; -, not determined

Table 11. Proportion of laboratories that obtained the expected result. Number (n/N) and percentages of laboratories which correctly detected and confirmed the ESBL-producing *Salmonella* strains.

Isolate no.	Expected interpretation	Confirmatory tests
WHO 2017 S-17.1	Presumptive ESBL-phenotype	80/92 (87%)
WHO 2017 S-17.2	Presumptive AmpC-phenotype	26/77 (34%)
WHO 2017 S-17.3	-	-
WHO 2017 S-17.4	Presumptive ESBL-phenotype	82/91 (90%)
WHO 2017 S-17.5	-	-
WHO 2017 S-17.6	-	-
WHO 2017 S-17.7	-	-
WHO 2017 S-17.8	Presumptive carbapenemase-phenotype	62/94 (66%)

G00-06-001/01.12.2014

Kgs. Lyngby, Denmark, May 2017

#### **SIGN-UP FOR EQAS 2017**

Greetings to the WHO Global Foodborne Infections Network (WHO GFN) Members:

WHO GFN strives to increase the quality of laboratory-based surveillance of *Salmonella* 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 2017 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.

#### WHO SHOULD PARTICIPATE IN EQAS 2017?

All national and regional reference laboratories which perform analysis on *Salmonella* 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 the EQAS.

The WHO GFN Regional Centers in cooperation with the EQAS Coordinator will evaluate the list of laboratories that sign up for EQAS 2017. Laboratories which signed up and received bacterial isolates in year 2016 but did not submit any result should provide a consistent explanation for this if they want to participate in 2017.

#### **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 2017**

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

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 25922 E. coli reference strain
- Components of EQAS 2017 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, please contact the EQAS Coordinator Susanne Karlsmose Pedersen: E-mail suska@food.dtu.dk.

#### TIMELINE FOR SHIPMENT OF ISOLATES AND AVAILABILITY OF PROTOCOLS

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 *October* 2017.

In order to minimize delays, **please send a valid import permit to the EQAS coordinator**. Please apply for a permit to receive the following: "UN3373, Biological Substance Category B": eight *Salmonella* strains, and (for new participants performing antimicrobial susceptibility testing on *Salmonella*) one *Escherichia coli* reference strain.

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

#### DEADLINE FOR SUBMITTING RESULTS TO THE NATIONAL FOOD INSTITUTE

Results must be submitted to the National Food Institute (DTU Food) by **28<sup>th</sup> February 2018** 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 the WHO GFN EQAS 2017 is August 4th, 2017

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#### Appendix 3, page 1 of 1

				Presumptive Ampicillin		Cefot	axime	Synergy	Cef	oxitin	Ceftazidime		Synergy	Ceftriaxone		Chloramphenicol			
			phenotype AMP		CTX		CTX:/CTX:CI	FOX		CAZ		CAZ:/CAZ:Cl		RO	CHL		С	IP.	
WHO 2017 S-17.1	Salmonella Infantis	I 6,7:r:1,5	ESBL	>64	RESIST	>64	RESIST	synergy	4	SUSC	16	RESIST	synergy	>32	RESIST	<=8	SUSC	0.25	INTER
WHO 2017 S-17.2	Salmonella Havana	I 13,23:f,g:-	AmpC	<=1	SUSC	1	SUSC	no synergy	32	RESIST	4	RESIST	no synergy	0.12	SUSC	<=8	SUSC	0.06	SUSC
WHO 2017 S-17.3	Salmonella Enteritidis	I 9,12:g,m;-	-	4	SUSC	0.5	SUSC				1	SUSC		0.25	SUSC	16	INTER	0.06	SUSC
WHO 2017 S-17.4	Salmonella Rissen	I 6,7:f,g:-	ESBL	>64	RESIST	32	RESIST	synergy	8	SUSC	2	RESIST	synergy	64	RESIST	>128	RESIST	0.03	SUSC
WHO 2017 S-17.5	Salmonella Weltevreden	I 3,10:r;z6	-	<=1	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.03	SUSC	128	RESIST	0.03	SUSC
WHO 2017 S-17.6	Salmonella Schwarzengrund	I 4,12:d:1,7	-	2	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.06	SUSC	<=8	SUSC	0.03	SUSC
WHO 2017 S-17.7	Salmonella Typhimurium	I 4,5,12:i:1,2	-	>64	RESIST	0.5	SUSC				<=0.5	SUSC		0.12	SUSC	64	RESIST	0.5	INTER
WHO 2017 S-17.8	Salmonella Kentucky	I 8,20:i:z6	Carbapenemase	>64	RESIST	>64	RESIST	no synergy	64	RESIST	>128	RESIST	no synergy	>256	RESIST	>128	RESIST	8	RESIST

			Presumptive		istin		amicin	Merope			kic acid		onamides		cycline		hoprim		/Sulfa
			phenotype	C	OL	G	EN	MER	MER		NAL		SMX	TET		TMP		SXT	
WHO 2017 S-17.1	Salmonella Infantis	I 6,7:r:1,5	ESBL	<=1	SUSC	<=0.5	SUSC	0.06	SUSC	>128	RESIST	>1024	RESIST	>64	RESIST	>32	RESIST	>4	RESIST
WHO 2017 S-17.2	Salmonella Havana	I 13,23:f,g:-	AmpC	<=1	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	64	SUSC	4	SUSC	0.5	SUSC	0.12	SUSC
WHO 2017 S-17.3	Salmonella Enteritidis	I 9,12:g,m;-	-	2	SUSC	32	RESIST	0.06	SUSC	8	SUSC	>1024	RESIST	4	SUSC	<=0.25	SUSC	0.12	SUSC
WHO 2017 S-17.4	Salmonella Rissen	I 6,7:f,g:-	ESBL	<=1	SUSC	1	SUSC	0.06	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.5	Salmonella Weltevreden	I 3,10:r;z6	-	<=1	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.6	Salmonella Schwarzengrund	I 4,12:d:1,7	-	2	SUSC	<=0.5	SUSC	0.06	SUSC	8	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.7	Salmonella Typhimurium	I 4,5,12:i:1,2	-	8	RESIST	<=0.5	SUSC	0.06	SUSC	>128	RESIST	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.8	Salmonella Kentucky	I 8,20:i:z6	Carbapenemase	<=1	SUSC	1	SUSC	1	RESIST	>128	RESIST	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST





#### PROTOCOL for

serotyping and antimicrobial susceptibility testing of Salmonella test strains

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#### 1 INTRODUCTION

In 2000, the Global Foodborne Infections Network (formerly known as WHO Global Salm-Surv) launched an External Quality Assurance System (EQAS). The EQAS is organized by the National Food Institute, Technical University of Denmark (DTU Food), in collaboration with partners and Regional Sites in WHO GFN.

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

The WHO EQAS 2017 includes serotyping and antimicrobial susceptibility testing of eight *Salmonella* strains and antimicrobial susceptibility testing of the *Escherichia coli* ATCC 25922 (CCM 3954) reference strain for quality control (QC).

The above-mentioned QC reference strain is included in the parcel only for new participants of the EQAS who did not receive it previously. The QC reference strain supplied is an original







CERTIFIED culture provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The QC reference strain 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 <a href="https://www.antimicrobialresistance.dk">www.antimicrobialresistance.dk</a>).

#### 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 2017

#### 3.1 Shipping, receipt and storage of strains

In October 2017 around 200 laboratories located worldwide will receive a parcel containing eight *Salmonella* strains. An *E. coli* ATCC 25922 reference strain will be included for participants who signed up to perform antimicrobial susceptibility testing (AST) and did not receive it previously. All provided strains belong to UN3373, Biological substance category B. Extended Spectrum Beta Lactamase (ESBL)-, AmpC- or 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* strains are shipped as agar stab cultures whereas the reference strain is shipped lyophilised. On arrival, the agar stab culture must be stored in a dark place at 2°C to 8°C. If receiving a lyophilized reference culture, store in a dark, cool place. The agar stab cultures must be sub-cultured 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).

#### 3.2 Serotyping of Salmonella

The eight *Salmonella* strains should be serotyped by using the method routinely used in the laboratory. Also serogroup results will be evaluated, therefore, if you do not have all the necessary antisera for a serotyping, please go as far as you can in the identification and report the serogroup. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (Grimont and







Weill, 2007. 9<sup>th</sup> 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 strains and Escherichia coli ATCC 25922

The *Salmonella* strains as well as the *E. coli* ATCC 25922 reference strain should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods <u>routinely used</u> in your laboratory.

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

Testing of gentamicin susceptibility may be valuable for monitoring purposes. Therefore we kindly ask you to disregard, for the purpose of this proficiency trial, that the Clinical and Laboratory Standards Institute (CLSI) guidelines state that *Salmonella* should not be reported as susceptible to aminoglycosides.

The breakpoints used in this EQAS for interpreting MIC results are in accordance with CLSI values (Table 1). Consequently, interpretation of MIC results will lead to categorization of strains into three categories: resistant (R), intermediate (I) and susceptible (S). In the evaluation report you receive upon result submission, you can find that obtained interpretations in accordance with the expected interpretation will be defined as 'correct', whereas deviations from the expected interpretation will be defined as 'minor' (I  $\leftrightarrow$  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).





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**Table 1.** Interpretive breakpoint for Salmonella antimicrobial susceptibility testing

Antimicrobials	Referei	nce value, MIC	(μg/mL)	Reference value, Disk diffusion (mm)						
	Susceptible	Intermediate	Resistant	Resistant	Intermediate	Susceptible				
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)**	≥31mm (5µg) or ≥23mm (1µg)**				
Colistin, COL***	≤2	-	≥4	Not	Not	Not				
				applicable	applicable	applicable				
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				

Reference values used in this EQAS are according to CLSI (M100, 27<sup>th</sup> edition), with the following exceptions:



<sup>\*</sup> 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 Table 3A. These interpretative criteria are also applied for *Salmonella* 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.

<sup>\*\*</sup> 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.

<sup>\*\*\*</sup> Reference values for colistin are based on CLSI M100 Table 2A-2. In the current EQAS these values should be applied for the interpretation of *Salmonella* AST results into the category as susceptible or resistant.



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Concerning ciprofloxacin susceptibility tests, the applied breakpoints 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  $\beta$ -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  $\ge 8$ ) 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 zone when tested alone (CLSI M100 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 recommendations (available in The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015, EFSA Journal 2017;15(2):4694,212 pp (page 43).

The following summary of these recommendations indicate how the phenotypes should be categorized:







#### ESBL-phenotype:

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

#### ESBL+AmpC-phenotype:

- CTX or CAZ > 1 mg/L **AND**
- MER  $\leq$  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)

#### <u>Carbapenemase-phenotype</u>:

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.







#### 4 REPORTING OF RESULTS AND EVALUATION

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 28<sup>th</sup> February 2018.

If you do not have access to the Internet, or if you experience difficulties in entering your results, please contact the EQAS Coordinator directly, explaining the issues that occur.

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

We are looking forward to receiving your results.

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

Susanne Karlsmose Pedersen

National Food Institute, Technical University of Denmark

Kemitorvet, Building 204, DK-2800 Lyngby - DENMARK

Tel: +45 3588 6601

E-mail: suska@food.dtu.dk

Direct communication with the EQAS organisers must be in English.

#### 5 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.





## DTU Food National Food Institute

- 1) Enter the WHO Collaborating Centre website (from <a href="http://www.antimicrobialresistance.dk">http://www.antimicrobialresistance.dk</a>), then
  - a. Click on 'EQAS'
  - b. Click on the link for the interactive database (<a href="http://eqas.food.dtu.dk/who">http://eqas.food.dtu.dk/who</a>)
  - 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)
  - c. Enter the zone diameters in mm or MIC values in  $\mu$ g/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





## DTU Food National Food Institute

- 5) In the data entry page 'E. coli reference strain':
  - a. Enter the zone diameters in mm or MIC values in  $\mu$ g/ml. Remember to use the operator keys to show e.g. equal to (=), etc.
  - b. Click on 'Save and go to next page'
- 6) 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.
- 7) After browsing all pages in the report, you will find a new menu. You can choose 'EQAS 2017 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 PURPOSE AND REFERENCES

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

The following can be regarded as a summary of information that should be followed for subculturing and maintaining QC-strains when performing AST by broth dilution methods. For full information related to this subject, the following standards are relevant: M100 (Performance Standards for Antimicrobial Susceptibility Testing) and M7 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard).

#### 2 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.

#### 3 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 (after 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.

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

#### 4 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.

#### 5 FREQUENCY OF TESTING

#### Weekly vs. daily testing

Weekly testing is possible if the laboratory can demonstrate satisfactory performance with daily testing according to the descriptions in the CLSI guidelines.

- Documentation showing reference strain results from 20 or 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more one out of 20 or three 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

If five acceptable QC results are available, no additional days of QC-testing are needed.

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

Repeat the 30 days validation before resuming weekly testing.





## 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!

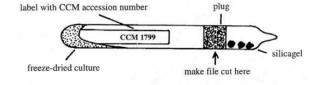


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz

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