Quality control of antimicrobial susceptibility testing

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- Selection of quality control strains
- Storage and subculture of control strains
- Frequency
- Reading results
- Follow-up of QC results out of range
- Q&A
- Post-webinar assignment





 To monitor test performance of the AST method in order to have reliable results and therefore improved patient care





- Validating the entire AST method:
 - Media
 - Muller-Hinton agar
 - MIC broth
 - Antibiotic disks: potency, shelf life, storage
 - MIC plates
 - Incubation conditions
 - Contamination
 - Reading and interpretation



Selection of quality control strains

- Use the recommended routine quality control strains to monitor test performance
- Quality control strains with defined resistance mechanisms can be used to confirm the ability to detect resistance
- From culture collections or from commercial sources, i.e.:
 - ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, USA
 - NCTC, National Collection of Type Cultures, Health Protection Agency Centre for Infections, UK



Quality control strains

Table 1



European Committee on Antimicrobial Susceptibility Testing

Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST

Version 13.2, valid from 2023-06-29

Recommendations for p	prinicipal QC ¹	Recommendations for agents not covered by principal QC ¹						
Organism	QC strain	Agent	QC strain					
Enterobacterales ²	E. coli ATCC 25922	Colistin (MIC)	Add E. coli NCTC 13846					
Pseudomonas spp.	P. aeruginosa ATCC 27853	Piperacillin (zone diameter)	E. coli ATCC 25922					
		Ticarcillin (zone diameter)	E. coli ATCC 25922					
		Colistin (MIC)	Add E. coli NCTC 13846					
Stenotrophomonas maltophilia	E. coli ATCC 25922							
Acinetobacter spp.	P. aeruginosa ATCC 27853	Trimethoprim-sulfamethoxazole	E. coli ATCC 25922					
		(MIC and zone diameter)						
		Colistin (MIC)	Add E. coli NCTC 13846					
Staphylococcus spp.	S. aureus ATCC 29213	Roxithromycin (MIC)	H. influenzae ATCC 49766					
Enterococcus faecalis and	E. faecalis ATCC 29212	Ampicillin-sulbactam (MIC)	See table 2					
E. faecium		Amoxicillin (MIC)	E. coli ATCC 25922					
		Amoxicillin-clavulanic acid (MIC)	See table 2					
Streptococcus groups	S. pneumoniae ATCC 49619	Teicoplanin (MIC)	S. aureus ATCC 29213					
A, B, C and G		Minocycline (MIC)	S. aureus ATCC 29213					
		Trimethoprim (MIC)	S. aureus ATCC 29213					
			H. Influenzae ATCC 49766					
Streptococcus pneumoniae	S. pneumoniae ATCC 49619	Teicoplanin (MIC)	S. aureus ATCC 29213					
		Minocycline (MIC)	S. aureus ATCC 29213					
			A. Influenzae ATCC 49766					
Viridans group streptococci	S. pneumoniae ATCC 49619	Teicoplanin (MIC)	S. aureus ATCC 29213					
Haemophilus influenzae	H. influenzae ATCC 49766	Piperacillin-tazobactam (MIC)	See table 2					
		Ceftolozane-tazobactam (MIC)	See table 2					
Moraxella catarrhalis	H. influenzae ATCC 49766							
Anaerobic bacteria	B. fragilis ATCC 25285							
	C. perfringens ATCC 13124							
Listeria monocytogenes	S. pneumoniae ATCC 49619							
Pasteurella spp.	H. influenzae ATCC 49766	Benzylpenicillin (MIC)	S. pneumoniae ATCC 49619					
Campylobacter jejuni	C. jejuni ATCC 33560	Ciprofloxacin (MIC)	S. aureus ATCC 29213					
and C. coli		Erythromycin (MIC)	S. aureus ATCC 29213					
		Tetracycline (MIC)	S. aureus ATCC 29213					



Routine QC

EUCAST QC Tables v. 13.2, valid from 2023-06-29

Escherichia coli ATCC 25922

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

Test according to EUCAST methodology for non-fastidious organisms (MH broth and agar). See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.

Antimicrobial agent	(m	IIC g/L)	Disk content	Inhibition zone diameter (mm)			
•	Target ¹	Range ²	(Pa)	Target ¹	Range ²		
Amikacin	1-2	0.5-4	30	22-23	19-26		
Amoxicillin	4	2-8	-	-	-		
Amoxicillin-clavulanic acid ^{3,4}	4	2-8	20-10	21	18-24 ⁵		
Ampicillin	4	2-8	10	18-19	15-22 ⁵		
Ampicillin-sulbactam ^{4,6}	2	1-4	10-10	21-22	19-24 ⁵		
Azithromycin	-	-	15	17	14-20 ⁷		
Aztreonam	0.125	0.06-0.25	30	32	28-36		
Cefadroxil	-	-	30	17	14-20		
Cefalexin	8	4-16	30	18	15-21		
Cefazolin	2	1-4	30	24	21-27		
Cefepime	0.03-0.06	0.016-0.125	30	34	31-37		
Cefiderocol ⁸	0.125-0.25	0.06-0.5	30	27	24-30		
Cefixime	0.5	0.25-1	5	23	20-26		
Cefotaxime	0.06	0.03-0.125	5	28	25-31		
Cefoxitin	4	2-8	30	26	23-29		
Cefpodoxime	0.5	0.25-1	10	25-26	23-28		
Ceftaroline	0.06	0.03-0.125	5	27	24-30		
Ceftazidime	0.125-0.25	0.06-0.5	10	26	23-29		
Ceftazidime-avibactam ^{4,9}	0.125-0.25	0.06-0.5	10-4	27	24-30		



Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and B-Lactam Combination Agents^a

			QC Organisms and Characteristics											
		Escherichia coli ATCC ^{®b} 25922	Pseudomonas aeruginosa ATCC® 27853	Staphylococcus aureus ATCC® 25923	Escherichia coli ATCC® 35218 ^{c,d}	Klebsiella pneumoniae ATCC® 700603 ^{c,d,e}	Escherichia coli NCTC 13353 ^{c,d}	Klebsiella pneumoniae ATCC® BAA- 1705 ^{™C,d}	Klebsiella pneumoniae ATCC® BAA- 2814™	Acinetobacter baumannii NCTC 13304 ^{c,d}				
Antimicrobial	Disk	B- lactamase negative	Inducible AmpC	B-lactamase negative, <i>mec</i> A negative	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37 TEM-1	CTX-M-15 OXA-1	KPC-2 SHV	KPC-3 SHV-11 TEM-1	OXA-27				
Amovicillin-clavulanate	Content				Zone Dia		es, mm							
(2:1)	20/10 µg	18-24	-	28-36	17-22	-	-	-	-	-				
Ampicillin	10 µg	15-22	-	27-35	35 6		-	-	-	-				
Ampicillin-sulbactam (2:1)	10/10 µg	19-24	-	29-37	13-19	-	-	-	-	-				
Aztreonam	30 µg	28-36	23-29	-	31-38	10-16	-	-	-	-				
Aztreonam-avibactam	30/20 µg	32-38	24-30	-	31-38	26-32 ^f	-	-	-	-				
Cefepime	30 µg	31-37	25-31	23-29	31-37	23-29	6-15 ^g	-	-	6-16 ^g				
Cefepime- enmetazobactam ^f	30/20 µg	32-38	26-32	-	32-38	26-32	27-33	-	-	-				
Cefepime-taniborbactam	30/20 µg	31-37	25-31	-	31-37	24-31	24-30	22-27	-	-				
Cefepime-tazobactam	30/20 µg	32-37	27-31	24-30	-	25-30 ^f	27-31	-	-	-				
Cefepime-zidebactam	30/30 µg	33-40	29-35	-	-	28-34	29-35	-	-	19-25				
Cefotaxime	30 µg	29-35	18-22	25-31	-	17-25	-	-	-	-				
Cefpodoxime	10 µg	23-28	-	19-25	-	9-16	-	-	-	-				
Ceftaroline	30 µg	26-34	-	26-35	-	-	-	-	-	-				
Ceftaroline-avibactam	30/15 µg	27-34	17-26	25-34	27-35	21-27 ^f	-	-	-	-				
Ceftazidime	30 µg	25-32	22-29	16-20	-	10-18	-	-	-	-				
Ceftazidime-avibactam	30/20 µg	27-35	25-31	16-22	28-35	21-27 ^f	-	-	-	-				

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Storage and subculture of control strains

• EQAsia webinar

Short- and long-term storage of bacterial isolates 25 January 2024 13:30-15:15 ICT



13:30-13:35	Housekeeping, Welcome and Introduction					
	Short- and long-term storage of bacterial isolates – Rungtip C., CUVET					
13:35-14:45	Tips for storage of fastidious bacteria in human health - Sanghamitra R., NSW					
	Tips for storage of fastidious bacteria in animal health – Taradon L., CUVET					
14:45-15:00	Q&A/Post Webinar Test					
15:00-15:10	EQAsia reference and confirmatory testing - Tomislav K., DTU					
15:10-15:15	Wrap up and conclusion					



Storage and subculture of control strains

- Store strains at -80°C in duplicates:
 - 1 "working" vial
 - 1 "stock" vial
- QC strains can also be freeze-dried
- Subculture weekly from the "working" vial on appropriate nonselective media and check for purity
- Subculture from the purity plate each day for up to 7 days
- Fastidious microorganisms may be serially sub-cultured for 6 days
- When the "working" vial is depleted, subculture from the "stock" vial and prepare another "working" vial from the subculture





- Control tests should be set up and checked daily for all antibiotics which are part of routine panels and in routine use
- If performance is satisfactory (no more than 1 in 20 tests outside control limits) - testing frequency may be limited to once a week
- In addition, each new batch of Mueller-Hinton agar (for media prepared in-house) should be checked to ensure that zones are within range





- Each day that tests are set up, **examine the results** of the last 20 consecutive tests
- Examine results for trends and for zones falling consistently above or below the target
- If two or more of 20 tests are out of range investigation is required









Follow-up after QC results out of range

- If two non-consecutive control zone diameters of 20 tests are outside the acceptable range – then report susceptibility test results and investigate
- If two consecutive control zone diameters of 20 tests are outside the acceptable range – then investigate before reporting susceptibility test results. The tests may have to be repeated
- If multiple disks (>2) are out of range on one day then investigate before reporting susceptibility test results. The tests may have to be repeated
- If resistance in a resistant control strain is not recognized then put susceptibility test results on hold, investigate and retest



Disk diffusion – potential sources of error

	Improper storage							
	Not prepared according to instructions							
	Batch to batch variation or change of supplier of agar							
Media	Supplements (batch to batch variations, incorrect amount or expired)							
	рН							
	Agar depth/Agar volume							
	Expiration date							
	"15-15-15"-rule not adhered to (suspension used within 15 min, disks applied within15 min, incubation within 15 min)							
Conditions	Incubation (temperature, atmosphere and time)							
Conditions	Inoculum (too light, too heavy or unevenly spread)							
	Reading conditions							
	Reading zone edges							



Disk diffusion – potential sources of error

	Incorrect disk (wrong agent or wrong disk potency)						
Disks	Improper storage, use beyond expiry date						
	Disks not at room temperature						
	Too many disks on plate (interference between agents)						
	Incorrect QC strain chosen						
Quality Control	Contamination						
microorganism	Improper storage						
	Old culture used						



Follow-up and checks

- **zones of inhibition** were properly measured and registered;
- inoculum standard used is valid, properly stored and well vortexed before use;
- **all consumables**, used in the process, are within their expiry date and properly stored;
- the **temperature and the atmosphere** in the incubator comply with the requirements;



- **disks** are stored in the presence of a desiccator and at the appropriate temperature;
- **suspension** is properly prepared and had standard density;
- inoculum was prepared by using correctly incubated bacterial culture (not more than 24-hour) – special conditions may apply for some drugbug combinations



EQAsia Coursera course

DTU ₩

Antimicrobial resistance - theory and methods

🛪 Taught in English | 20 languages available | Some content may not be translated



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https://www.coursera.org/learn/antimicrobial-resistance









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- All participants in this webinar will receive an email with a template and brief instructions for the assignment
- Test *E. coli* ATCC 25922 quality control strain every day for 20 consecutive working days (part of routine procedures)
- Record results for all required antibiotics in the template Excel spreadsheet
- Also enter the actions performed if/when results were out of range.



	Enter the method used here								used here				
А	В	С	D	E	F	G	Н		J	К	L	м	N
2 Name of laborate	bry												
3 Country								/					
4													
5	Antimicrobial agent	MIC (mg/L) Target	MIC (mg/L) Range ▼	Disk content (mcg)	Inhibition zone diameter (mm) Target	Inhibition zone diameter (mm) Range	Method used	Day 1 Result	Day 1 Interpretation	Day 2 Result	Day 2 Interpretation	Day 3 Result ▼	Day 3 Interpretation
6	Amikacin	1-2	0.5-4	30	22-23	19-26	Disk diffusion	27	outside of range				
7	Amoxicillin-clavulanic acid	4	2-8	20-10	21	18-24							
8	Ampicillin	4	2-8	10	18-19	15-22							
9	Aztreonam	0-125-0.25	0.06-0.5	30	32	28-36			\mathbf{i}		ntor the inter	prototion	of the
10	Cefepime	0.03-0.06	0.016-0.125	30	34	31-37			\mathbf{X}		measure	ment here	or the
11	Cefotaxime	0.06	0.03-0.125	5	28	25-31					measure		
12	Cefoxitin	4	2-8	30	26	23-29			$\langle \rangle$				
3	Ceftazidime	0.125-0.25	0.06-0.5	10	26	23-29				Entor the	obtained va	luo of the	
4	Ceftriaxone	0.06	0.03-0.125	30	32	29-35					testing here	ue or the	
5	Ciprofloxacin	0.008	0.004-0.016	5	33	29-37					0		
6	Chloramphenicol	4	2-8	30	24	21-27							
7	Ertapenem	0.008	0.004-0.016	10	32-33	29-36							
8	Gentamicin	0.5	0.25-1	10	22-23	19-26							
9	Imipenem	0.125-0.25	0.06-0.5	10	29	26-32							
20	Levofloxacin	0.016-0.03	0.008-0.06	5	33	29-37							
21	Meropenem	0.016-0.03	0.008-0.06	10	31-32	28-35							
22	Nalidixic acid	2	1-4	30	25	22-28							
23	Nitrofurantoin	8	4-16	100	20	17-23							
24	Piperacillin-tazobactam	2-4	1-8	30-6	24	21-27							
25	Tigecycline	0.06-0.125	0.03-0.25	15	23-24	20-27							
26	Tobramycin	0.5	0.25-1	10	22	18-26							
27	Trimethoprim-sulfamethoxazole	≤0.5	-	1.25-23.75	26	23-29							
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- Assignments are due by April 15, 2024
- Only one assignment per laboratory
- Laboratories will receive individual feedback after the deadline
- All participants from each laboratory that submitted the assignment will receive a **certificate for completion** of this EQAsia training module







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