

Wide variation in disk quality in 16 disks from nine manufacturers

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Changes from previous versions

Version	Comments
23 October 2015	<ul style="list-style-type: none">• First publication
16 June 2016	<ul style="list-style-type: none">• Information on the responsibility of EUCAST added.• Data reanalyzed due to changes in QC criteria for cefotaxime and meropenem.• Specific comment for meropenem removed.• Specific comment for ciprofloxacin updated.• Information on evaluation of new disk lots on the request of manufacturers added.• Results for new meropenem lots from Bio-Rad added.

The responsibility of EUCAST

- EUCAST randomly performs quality control of materials used in antimicrobial susceptibility testing (AST). If a problem is detected, EUCAST will primarily contact the manufacturer and if the issue is not solved, publish a warning on the EUCAST website.
- Manufacturers are responsible for the quality of their products. EUCAST cannot systematically check AST materials and the lack of a warning against a product should not be considered an approval by EUCAST.
- Information on availability of AST materials from different manufacturers is listed in the document "Compliance of manufacturers". This document is based on information given by the manufacturers, who take full responsibility for the accuracy of data.
http://www.eucast.org/ast_of_bacteria/compliance_of_manufacturers/

Selection of disks for evaluation

- Sixteen antimicrobial disks from nine manufacturers were evaluated against EUCAST targets and ranges for relevant quality control (QC) strains.
- The disks were chosen either because of their central role in the EUCAST disk testing system (e.g. screening disks for important resistant mechanisms), or because problems have been detected by the EUCAST Development Laboratory (EDL) or other laboratories.

Study design

First study (2014)

- Nine manufacturers were asked to supply disks for the 16 selected antimicrobial agents and nominal disk potencies. Not all manufacturers supplied all disks. The results from this study was presented as a poster at ECCMID 2015, with manufacturers anonymised.

Second study (2015)

- The manufacturers were informed individually of their results and asked to supply new disk lots for all agents with deviating results. Some manufacturers chose not to supply new disk lots, whereas others sent new disk lots for a few agents, or all agents, included in the first study. All disks received were included in the second study.

Evaluation on the request of manufacturers (2016-)

- For the 16 disks included in these two studies, EUCAST will perform evaluations of new disk lots on the request of the manufacturer.

Disk diffusion testing

- Disk diffusion was performed according to EUCAST methodology.
- Each disk was tested against all QC strains with targets and ranges to the agent in the EUCAST Quality Control Tables v. 6.1.
- For each agent, all disks supplied for the study were placed on a single 150-mm circular agar plate, except for meropenem 10 µg, for which two plates were used due to the large inhibition zones.
- Each disk-strain combination was tested in triplicate (three individually prepared inoculum suspensions) on the same day.
- All tests were performed on in-house prepared Mueller-Hinton agar plates (MH and MH-F as recommended) using MH agar from two manufacturers.

Antimicrobial disks and QC strains

Antimicrobial agent	Disk content (µg)	QC Strain					
		<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. aureus</i> ATCC 29213	<i>E. faecalis</i> ATCC 29212	<i>S. pneumoniae</i> ATCC 49619	<i>H. influenzae</i> ATCC 49766
Benzylpenicillin	1 unit			X		X	X
Amoxicillin-clavulanic acid	20-10	X					
Piperacillin-tazobactam	30-6	X	X				
Oxacillin	1					X	
Mecillinam	10	X					
Cefotaxime	5	X				X	X
Cefoxitin	30			X			
Ceftazidime	10	X	X				
Meropenem	10	X	X			X	
Ciprofloxacin	5	X		X	X		
Norfloxacin	10	X		X	X		
Pefloxacin	5	X					
Gentamicin	10	X		X			
Tobramycin	10	X		X			
Erythromycin	15			X		X	
Tetracycline	30			X		X	X

Manufacturers and disk lots

First study, 2014

Antimicrobial agent	Disk content (µg)	Bio-Rad	Liofilchem	BD	Abtek	SirScan	Oxoid	HiMedia	Bioanalyse	Mast
Benzylpenicillin	1 unit	3M5026	071013058	3261062	NC20	131224C	1339287	NA	140115	327562
Amoxicillin-clavulanic acid	20-10	3M5603	21414067	3304440	NJ07	130322A	1125891	0000198992	140221	325610
Piperacillin-tazobactam	30-6	3K5023	020714062	3305396	OA48	131128E	1459241	NA	140130	329419
Oxacillin	1	3L5109	022014011	3252444	ND23	130313N	1122963	0000194861	131230	328982
Mecillinam	10	3L5134	012214051	3283462	KF24	130813I	1345475	0000176945	140212	327579
Cefotaxime	5	3L5022	21714027	3326487	NA	130313E	1388864	NA	131104	324951
Cefoxitin	30	3L5197	11514076	3297024	NA	130924A	1345474	0000198987	140228	325015
Ceftazidime	10	3M5022	112013016	3283464	MG05	131128D	1403377	0000191676	140214	328947
Meropenem	10	3M5102	12114026	3241116	NG05	131218A	1487968	0000198998	140224	328923
Ciprofloxacin	5	3M5215	13014112	3154326	NJ44	130923A	1437292	0000196630	140129	326915
Norfloxacin	10	3M5077	112613058	3190105	OA32	131219B	1344060	0000196346	140203	327583
Pefloxacin	5	4B5318	12914067	2312175	NA	NA	1256563	0000196804	140219	314043
Gentamicin	10	3M5147	11614100	3154329	NJ61	NA	1409822	0000196441	140214	327253
Tobramycin	10	NA	102513037	3309450	NJ69	130923C	1402726	0000195929	140203	327564
Erythromycin	15	3L5267	20414067	3232212	NK17A	140130B	1424971	0000168251	140117	325620
Tetracycline	30	3M5330	10814027	3252416	NJ89	130814D	1337825	0000196801	140228	326933

NA = Not Available

Manufacturers and disk lots

Second study, 2015

Antimicrobial agent	Disk content (µg)	Bio-Rad	Liofilchem	BD	Abtek	SirScan	Oxoid	HiMedia	Bioanalyse	Mast
Benzylpenicillin	1 unit					150102B		NA	150227M	
Amoxicillin-clavulanic acid	20-10	5C5637			PA40			0000220437	150306A	
Piperacillin-tazobactam	30-6				OF23	140522C		NA	150123O	
Oxacillin	1		022315033			140409E		0000220057	150302N	
Mecillinam	10				OJ08			0000220060	150212N	
Cefotaxime	5				OF11			NA	150225C	
Cefoxitin	30	5C5205*	022015067		PA37			0000220434	150305B	
Ceftazidime	10				PA06			0000220436	150126J	
Meropenem	10		022315034		PA42	150219C	1634757	0000220444	150311C	
Ciprofloxacin	5				PB39			0000220054	150305A	
Norfloxacin	10				PB11			0000220053	150212C	
Pefloxacin	5		022015064		NA	140120C		0000220055	150217F	
Gentamicin	10				PB30A	140429B		0000220049	150313C	
Tobramycin	10	NA	022015068					0000220052	150306D	
Erythromycin	15		022015063		OH08			0000220048	150309B	
Tetracycline	30		022315035		PC02	140704A		0000220051	150303B	

Disk included in first study, but not supplied for second study

NA = Not Available

Data analysis

- Mean values from triplicate tests of each disk were evaluated against targets and ranges in EUCAST QC Tables v. 6.1.
 - EUCAST has evaluated and, if acceptable, adopted the CLSI quality control ranges for disk diffusion tests. In cases where EUCAST recommendations for strains, medium or disk contents differ from those of CLSI, EUCAST has developed separate criteria.
- The results were categorised as:
 - Mean value within ± 1 mm of the target value
 - Mean value >1 mm but within ± 2 mm of the target value
 - Mean value >2 mm from target value but still within the QC range
 - Mean value out of the QC range
- For disks with mean values >1 mm from QC target, we also reported results as above (H) or below (L) the target value.
- Disks with occasional readings out of range were marked with an asterisk.

Results from First (1) and Second (2) Study

Antimicrobial disk	Bio-Rad		Liofilchem		BD		Abtek		SirScan		Oxoid		HiMedia		Bioanalyse		Mast	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Benzylpenicillin 1 unit					L				H	H			NA	NA	H	H		
Amoxicillin-clav. 30 µg	H	H*					L						H	H		L		
Piperacillin-tazo. 36 µg							L	L	H				NA	NA				
Oxacillin 1 µg			L		L				L				H	H	L			
Mecillinam 10 µg							L		H				H		H			
Cefotaxime 5 µg ¹							NA		L				NA	NA				
Cefoxitin 30 µg ²	H*	H*	H	H*			NA	L					L*	L*		L		
Ceftazidime 10 µg							L	L					L	H				
Meropenem 10 µg ¹	H		H*				L	L			H		H					
Ciprofloxacin 5 µg ²	L				L		L	L					H	H*		L	L	
Norfloxacin 10 µg							L		L				H*	H				
Pefloxacin 5 µg			L	L	L		NA	NA	NA				H					
Gentamicin 10 µg					H		L		NA				H	H				
Tobramycin 10 µg	NA	NA	H										H*	H*				
Erythromycin 15 µg			L		L		L		L				H	H	L*	L		
Tetracycline 30 µg			L	L*	L*		L		L*					L	L		L	

Mean value within ± 1 mm of the target value

Mean value >1 mm but within ± 2 mm of the target value

Mean value >2 mm from target value but still within the QC range

Mean value out of the QC range

Disk included in first study, but not supplied for second study

NA = Not Available

H = High, mean value >1 mm above target

L = Low, mean value >1 mm below target

* One or more readings out of QC range

¹ Data has been reanalyzed due to changes in QC criteria for *H. influenzae* ATCC 49766 with cefotaxime 5 µg (2015) and *E. coli* ATCC 25922 with meropenem 10 µg (2016).

² For specific comments on cefoxitin and ciprofloxacin, see next slide.

Specific comments

For **cefoxitin 30 µg**, the results are media dependent and inhibition zones on Oxoid Mueller-Hinton agar are in the upper part of the range. Results when excluding Oxoid agar are shown below.

For **ciprofloxacin 5 µg** all zones for *E. coli* ATCC 25922 are in the lower part of the range, and this range is currently under review by EUCAST and CLSI. Results when excluding results for *E. coli* ATCC 25922 are shown below.

Antimicrobial agent	Bio-Rad	Liofilchem	BD	Abtek	SirScan	Oxoid	HiMedia	Bioanalyse	Mast
Cefoxitin 30 µg first study	H*	H		NA			L*		
Cefoxitin 30 µg first study (BBL media only)	H		L	NA			L		L
Cefoxitin 30 µg second study	H*	H*		L			L*	L	
Cefoxitin 30 µg second study (BBL media only)	H			L			L	L	
Ciprofloxacin 5 µg first study	L		L	L			H		L
Ciprofloxacin 5 µg first study (<i>E. coli</i> excluded)	L	H	L	L			H		L
Ciprofloxacin 5 µg second study				L			H*	L	
Ciprofloxacin 5 µg second study (<i>E. coli</i> excluded)				L			H*		

Evaluation of new disk lots on the request of manufacturers

- EUCAST will perform further evaluations of new disk lots for one or several of the 16 antimicrobial disks on the request of the manufacturer.
- Testing will be performed according to the same procedure as in the two initial studies.
- This document will be updated with results from new evaluations.
- EUCAST does not guarantee that disk lots tested are commercially available. For information on availability, contact the manufacturer.

Evaluation of new disk lots on request of manufacturers

Antimicrobial disk	Manufacturer	Previous results		New disk lot	Results new lot	New disk lot tested
		1	2			
Meropenem 10 µg	Bio-Rad	H		5K00E2		May 2016

Mean value within ± 1 mm of the target value

Mean value >1 mm but within ± 2 mm of the target value

Mean value >2 mm from target value but still within the QC range

Mean value out of the QC range

Disk included in first study, but not supplied for second study

NA = Not Available

H = High, mean value >1 mm above target

L = Low, mean value >1 mm below target

* One or more readings out of QC range

Conclusions

- The results from this study show that there are significant and unacceptable differences in antimicrobial activity in disks from different manufacturers.
- It is crucial for **laboratories using the EUCAST disk diffusion method** to perform internal quality control and to evaluate the results. Repeat testing using EUCAST quality control strains should yield mean values close to the recommended target value (± 1 mm). Individual zone diameter values should be randomly distributed within the recommended ranges.
- **Disk manufacturers** must continuously review their disk manufacturing process. The activity of each disk shall match the recommended target values in the EUCAST QC tables, none of which disagree with those recommended by CLSI. It should be possible to hit the target value ± 1 mm for most agents and ± 2 mm for all agents.

EUCAST recommendations

- **Green disks** are close to the QC targets and well calibrated for use with the EUCAST disk diffusion method.
- **Yellow disks** deviate from the QC targets, but the results are still acceptable. Manufacturers are advised to take note and review their manufacturing process.
- **Orange disks** deviate significantly from QC targets. Laboratories are warned against the use of these disks and manufacturers are urged to immediately review their manufacturing process.
- **Red disks** are completely out of range and most probably contain either less than half or more than twice of the nominal amount of the active agent. Laboratories are warned against the use these disks and manufacturers should withdraw them from the market.

Publications and support

- Publications based on these results:
 - Editorial in Clinical Microbiology and Infection (CMI) March 2016, Vol. 22; Issue 3, p. 211-212
<http://www.clinicalmicrobiologyandinfection.com/article/S1198-743X%2815%2900990-8/pdf>
 - Poster ECCMID Copenhagen 2015 (P1239)
 - Poster ECCMID Amsterdam 2016 (P0824)
- For support and questions, please contact the EUCAST Development Laboratory (EDL):
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