HARDYDISK™ ANTIMICROBIAL SENSITIVITY TEST (AST)

INTENDED USE

HardyDisk™ AST Disks are used for semi-quantitative *in vitro* susceptibility testing by the agar diffusion test procedure (Kirby-Bauer) of rapidly growing and certain fastidious bacterial pathogens. Standardized methods for agar diffusion testing have been described for *Enterobacteriaceae*, *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Listeria monocytogenes*, *Enterococcus* spp., and by modified procedures, *Haemophilus* spp., *Neisseria gonorrhoeae, N. meningitidis* and *Streptococcus* spp. including *Streptococcus pneumoniae*.[5,6]

SUMMARY

Agar diffusion methods employing dried filter paper disks impregnated with specific concentrations of antimicrobial agents were developed in the 1940s. In order to eliminate or minimize variability in this testing, Bauer et al. developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium.[1,2]

Various regulatory agencies and standards-writing organizations subsequently published standardized reference procedures based on the Kirby-Bauer method. Among the earliest and most widely accepted of these standardized procedures were those published by the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO).[3-5] The procedure was adopted as a consensus standard by the Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS) and is periodically updated.[6,7] Mueller Hinton Agar is currently recommended for disk diffusion testing of non-fastidious organisms such as *Enterobacteriaceae, Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Listeria monocytogenes*, *Enterococcus* spp. and other streptococci.[1,2] Using modified procedures, Haemophilus Test Medium (HTM) is now recommended for disk diffusion testing of *Haemophilus* species. Similarly, GC Base with Supplements is recommended for *Neisseria gonorrhoeae* and Mueller Hinton with 5% Sheep Blood is recommended for *Streptococcus* spp., and *N. meningitidis*.[5,6] The latest CLSI documents should be consulted for current recommendations.[6-8]

FORMULA

HardyDisk™ AST Disks are prepared by impregnating high-quality, 6mm diameter, white filter paper disks with accurately determined amounts of antimicrobics or other chemotherapeutic agents. The disks are clearly marked on both sides with letters and numbers designating the agent and the drug content (See Table 1).

HardyDisk™ AST Disks are supplied in plastic cartridges containing 50 disks each. The cartridges are for use in single disk dispensers or multi-place dispensers such as BBL™ Sensi-Disc™ dispensers and BBL™ Self-Tamping dispensers.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20 to +8 degrees C. away from direct light. **Do not store at colder than -20 degrees C.** Disks should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Some disks (e.g. beta-lactams) should be kept frozen at -20 degrees C. A one week supply could be stored at 2-8 degrees C.

It is recommended that the disks be stored in a sealed container (Cat. no. 553K) with a desiccant (DesiView™, Cat. no. DV10). Return unused disks to the refrigerator/freezer as soon as possible after use.

The expiration date applies to the product in its intact packaging when stored as directed.

Refer to the keyword "Storage", in the Hardy Diagnostics' software program HUGO™, for more general information on storing culture media.

Products must be brought to room temperature before use.

Refer to the keyword "Storage", in the Hardy Diagnostics' software program HUGO™, for more information on storing culture media.

PRECAUTIONS

For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. This product is to be used only by adequately trained and qualified laboratory personnel. Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics' software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics' software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Direct specimen testing is not recommended. It is recommended that isolated organisms, established isolation techniques and tests for purity be performed before inoculating media for disk diffusion testing. Direct inoculation will produce erroneous results.

Preparation of inoculum with test and control cultures[6]

1. Perform a Gram stain using only pure cultures.

2. Select three to five similar colonies and transfer with inoculation needle or loop into 4-5mL of a suitable broth such as Tryptic Soy Broth or Mueller Hinton Broth for fastidious microorganisms.

3. Incubate the broth cultures at 35 degrees C. for two to six hours to develop a turbidity that exceeds or is equivalent to a 0.5 McFarland Standard (Cat. no. ML05). Alternatively, make a direct broth or saline suspension of colonies selected from an overnight culture (a non-selective medium such as Blood Agar, or Chocolate Agar for *Haemophilus* spp. and *N. gonorrhoeae* should be used). This procedure is preferred for *Streptococcus* spp., *Haemophilus* spp., *N. gonorrhoeae*, *N. meningitidis* and methicillin/oxacillin-resistant staphylococci.

4. Dilute to obtain turbidity equivalent to a 0.5 McFarland Standard (Cat. no. ML05). For diluent, use sterile broth or saline. Alternatively, standardize the inoculum photometrically to facilitate adjustment of rapidly growing microorganisms.

Note: Overnight broth cultures should not be used as inoculum.

Inoculation[6]
1. Within 15 minutes, dip a sterile swab into the properly adjusted inoculum, rotate it several times and press firmly against the upper inside wall of the tube to express excess fluid.

2. Streak the entire agar plate surface three times, turning the plate 60 degrees between streaks to obtain even inoculation. Mueller Hinton (MH) Agar is recommended for non-fastidious organisms; Mueller Hinton with 5% Sheep Blood for *Streptococcus* spp. and *N. meningitidis*; GC Base with Supplements for *N. gonorrhoeae*, and Haemophilus Test Medium (HTM) for *Haemophilus* spp.

3. The lid may be left ajar for three to five minutes, but no more than 15 minutes, to allow for any surface moisture to be absorbed before applying the drug-impregnated disks.

4. Select appropriate test disks.[6]

5. Using aseptic precautions, apply the disks by means of a dispenser. Deposit disks so that the centers are at least 24mm apart; up to 12 disks may be placed on a 150mm plate, 5 disks on a 100mm plate. In all cases, however, it is best to place disks that give predictably small zones (e.g. cephalosporins) in an effort to avoid overlapping zones. It is also important to pay attention to how close the disks are to the edge of the plate, regardless of how many disks are dispensed. If disks are placed too close to the edge of the plate, the zones may not form fully round diameters with some drugs. Because some of the drug diffuses almost instantaneously, a disk should not be relocated once it has come into contact with the agar surface. Instead, place a new disk in another location on the agar. If the D-test for inducible clindamycin resistance is being performed, refer to the current version of the M02 or M100 for guidance on disk placement. With *Haemophilus* spp., *N. gonorrhoeae*, and *S. pneumoniae*, use no more than nine disks per 150mm plate or four disks per 100mm plate. For *N. meningitidis*, use no more than five disks per 150mm plate or two disks per 100mm plate. If disks have been placed on the agar with other than a self-tamping dispenser, press the disks down with a sterile needle or forceps to make contact with the surface.

Note: It is important that the HardyDisk™ AST Cartridges are properly loaded into the multi-place dispensers to ensure proper dispensing. When using BBL™ Sensi-Disc™ dispensers, move the lever into the "Unlocked" position, insert the cartridge until an audible snap is heard, then move the lever into the locked position. Failure to load cartridges properly into dispensers may result in equipment malfunction and damaged cartridge(s).

6. Within 15 minutes, place the plates agar side up in a 35 +/- 2 degrees C. incubator (testing at temperatures above 35 degrees C. may not detect MRS [methicillin-resistant *Staphylococcus*]). *Haemophilus* spp., *N. gonorrhoeae, N. meningitidis* and *Streptococcus* spp. should be incubated in an atmosphere enriched with 5% CO_2.

7. Examine the plates after 16 to 18 hours of incubation (20 to 24 hrs. for *Streptococcus* spp., *N. meningitidis* and *N. gonorrhoeae*). A full 24 hours of incubation is recommended for *Staphylococcus aureus* to detect methicillin-resistant staphylococci. Measure only zones showing complete inhibition as determined by gross visual inspection and record the diameters of zones to the nearest millimeter. For further details in measuring zones of inhibition, consult the listed reference.(6) If only isolated colonies grow, the inoculum is too light and the test should be repeated. Zone sizes around disks containing different drugs are not comparable for the purpose of comparing activity of drugs.

8. Control tests using prescribed cultures should be included each day susceptibility testing is performed or weekly if satisfactory performance can be documented according to the CLSI standard.(6) Typical zone sizes of *E. coli* ATCC® 25922, *S. aureus* ATCC® 25923, *P. aeruginosa* ATCC® 27853, *H. influenzae* ATCC® 49247, *H. influenzae* ATCC® 49766, *N. gonorrhoeae* ATCC® 49226, *S. pneumoniae* ATCC® 49619, *E. coli* ATCC® 35218 (beta-lactamase-producing strain) are given in the chart (or footnotes) and indicate the correct performance of the entire procedure. *E. faecalis* ATCC® 29212 (for quality control testing of gentamicin 120ug and streptomycin 300ug disks) and *E. faecalis* ATCC® 33186 are also recommended for evaluating new lots of Mueller-Hinton Agar for low thymine and thymidine content (refer to the current version of the M02). *H. influenzae* ATCC® 10211 is recommended as a useful additional quality control strain to verify the growth promotion properties of Haemophilus Test Medium (HTM) Agar.(6)

INTERPRETATION OF RESULTS

RESISTANT indicates that clinical efficacy is currently unreliable in treatment studies or specific microbial resistance mechanisms (e.g. beta-lactamases) may be likely.

INTERMEDIATE implies clinical applicability in body sites where the drug is physiologically concentrated or when a higher than normal dosage of the drug can be used. The MIC of the isolate may approach usually attainable blood and tissue levels but the response rate may be lower than for susceptible isolates.

SUSCEPTIBLE implies that an infection due to the organism may be treated with the concentration of antimicrobial agent used, unless otherwise contraindicated.

NONSUSCEPTIBLE is a category used for organisms that have only a susceptible interpretive category, but not intermediate or resistant interpretive categories. A susceptible-only interpretive category may be applied to new antimicrobial agents for which no resistant isolates have been encountered at the time the initial interpretive criteria were determined. Isolates that test with a MIC above or a zone measurement below the susceptible interpretive breakpoint are designated as nonsusceptible. A designation of nonsusceptible does not necessarily mean that a resistance mechanism exists in the isolate. The MIC (or zone measurement) of the isolate in the nonsusceptible range may be within the previously recognized wild-type distribution of susceptibility results; however, there is limited experience with these isolates in clinical trials.

LIMITATIONS

Disk performance and results depend not only on disk potency, but on use of proper inoculum and control cultures, functional plated media, proper storage conditions and other factors.

The test applies primarily to rapidly growing aerobic pathogens. Fastidious bacteria, other than *Haemophilus* spp., *N. gonorrhoeae, N. meningitidis* and *Streptococcus* spp., should be tested by a dilution method.[8]

Antimicrobial agents other than those listed in Table 1 may be in current use. Susceptibility tests employing these agents should be interpreted on the basis of presence or absence of a definite zone of inhibition and should be considered only as qualitative until such time as interpretive zones have been established. All zone diameters should be recorded. The approved pharmaceutical package insert and the latest CLSI documents should be consulted for current recommendations and definitive information.[6-9]

Refer to the keyword "Limitations", in the Hardy Diagnostics' software program HUGO™, for more information regarding general limitations when using culture media.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, slides, staining supplies, culture and susceptibility test media, 0.5 McFarland Standard (Cat. no. ML05), calipers, microscope, incinerators, and incubators, etc., are not provided.

QUALITY CONTROL

See Table 1 for acceptable quality control zone diameters. Quality control acceptance is specific to the procedure, control organism and antimicrobial agent combination. **Please refer to the most current version of the CLSI M100-S document for additional recommendations for testing conditions, reporting suggestions, warnings, comments, interpretive criteria and QC information.[7]**

User Quality Control: Check for signs of contamination and deterioration. Control tests using prescribed cultures should be included each day susceptibility testing is performed or weekly if satisfactory performance can be documented according to the CLSI standard.[6]

Quality Control Organism Maintenance: Avoid repeated subcultures of the organism. Retrieve new QC strains from stock. If using lyophilized strains, follow the maintenance recommendations provided by the manufacturer. Store *E. coli* ATCC® 35218 and *K. pneumoniae* ATCC® 700603 QC stock cultures at -60 degrees C. or below and prepare working stock cultures weekly.

Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics' software program HUGO™, for a description of the inoculation method.

Disk Diffusion Zone Diameter Chart (Table 1)

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E coli* ATCC® 35218 [x,xii,xvii,xviii]	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Amikacin	AN-30	30ug				19--26	20--26	18--26	–	–	–	–	–
for *Staphylococcus* species			≤14	15--16	≥17								
for *Acinetobacter* species			≤14	15--16	≥17								
for *Enterobacteriaceae*			≤14	15--16	≥17								
for *Pseudomonas aeruginosa*			≤14	15--16	≥17								
Amoxicillin/Clavulanic Acid[vi]	AmC-30	20/10ug				18--24	28--36	–	17--22	15--23	–	–	–
for *Staphylococcus* species			≤19	–	≥20								
for *H. influenzae* and *parainfluenzae*			≤19	–	≥20								
for *Enterobacteriaceae*			≤13	14--17	≥18								
Ampicillin	AM-10	10ug				16--22	27--35	–	6	13--21	–	–	30--36
for *Enterobacteriaceae*			≤13	14--16	≥17								
for *Staphylococcus* species			≤28	–	≥29								
for *Enterococcus* species			≤16	–	≥17								
for *H. influenzae* and *parainfluenzae*			≤18	19--21	≥22								
for beta-hemolytic streptococci			–	–	≥24								
for *N. meningitidis*			–	–	–								
Ampicillin/Sulbactam	SAM-20	10/10ug				19--24	29--37	–	13--19	14--22	–	–	–
for *Enterobacteriaceae*			≤11	12--14	≥15								
for *Staphylococcus* species			≤11	12--14	≥15								
for *H. influenzae* and *parainfluenzae*			≤19	–	≥20								
for *Acinetobacter* species			≤11	12--14	≥15								
Azithromycin	AZM-15	15ug				–	21--26	–	–	13--21	–	–	19--25
for *H. influenzae* and *parainfluenzae*			–	–	≥12								
for *Staphylococcus* species			≤13	14--17	≥18								
for *Streptococcus* other than *S. pneumoniae*			≤13	14--17	≥18								
for *Streptococcus pneumoniae*			≤13	14--17	≥18								
for *N. meningitidis*			–	–	≥20								
Azlocillin	AZ-75	75ug				–	–	24--30	–	–	–	–	–
for *Pseudomonas aeruginosa*			≤17	–	≥18								
Aztreonam	ATM-30	30ug				28--36	–	23--29	–	30--38	–	–	–
for *H. influenzae* and *parainfluenzae*			–	–	≥26								
for *Enterobacteriaceae*			≤17	18--20	≥21								
for *Pseudomonas aeruginosa*			≤15	16--21	≥22								
Carbencillin	CB-10	100ug				23--29	–	18--24	–	–	–	–	–
for *Pseudomonas aeruginosa*			≤13	14--16	≥17								
for *Enterobacteriaceae*			≤19	20--22	≥23								
Cefaclor	CEC-30	30ug				23--27	27--31	–	–	–	25--31	–	24--32
for *H. influenzae* and *parainfluenzae*			≤16	17--19	≥20								
for *Staphylococcus* species			≤14	15--17	≥18								
for *Enterobacteriaceae*			≤14	15--17	≥18								
Cefamandole	MA-30	30ug				26--32	26--34	–	–	–	–	–	–
for *Enterobacteriaceae*			≤14	15--17	≥18								
for *Staphylococcus* species			≤14	15--17	≥18								
Cefazolin	CZ-30	30ug				21--27	29--35	–	–	–	–	–	–
for *Enterobacteriaceae*			≤ 19	20--22	≥23								
for *Staphylococcus* species			≤14	15--17	≥18								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 x,xii,xvii,xviii	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Cefdinir	CDR-5	5ug				24--28	25--32	–	–	–	24--31	40--49	26--31
for *Staphylococcus* species			≤16	17--19	≥20								
for *H. influenzae* and *parainfluenzae*			–	–	≥20								
for *Enterobacteriaceae*			≤16	17--19	≥20								
Cefditoren		5ug				22--28	20--28	-	-	25--34	-	-	27--35
Cefepime	FEP-30	30ug				31--37	23--29	24--30	–	25--31	–	37--46	28--35
for *Staphylococcus* species			≤14	15--17	≥18								
for *H. influenzae* and *parainfluenzae*			–	–	≥26								
for *N. gonorrhoeae*			–	–	≥31								
for beta-hemolytic streptococci			–	–	≥24								
for viridans group streptococci			≤21	22--23	≥24								
for *Enterobacteriaceae*			≤14	15--17	≥18								
for *Pseudomonas aeruginosa*			≤14	15--17	≥18								
for *Acinetobacter* species			≤14	15--17	≥18								
Cefetamet (investigational)		10ug				24--29	–	–	–	23--28	–	35--43	–
for *H. influenzae* and *parainfluenzae*			≤14	15--17	≥18								
for *N. gonorrhoeae*			–	–	≥29								
for *Enterobacteriaceae*			≤14	15--17	≥18								
Cefixime	CFM-5	5ug				23--27	–	–	–	25-33	–	37--45	16--23
for *H. influenzae* and *parainfluenzae*			–	–	≥21								
for *N. gonorrhoeae*			–	–	≥31								
for *Enterobacteriaceae*			≤15	16--18	≥19								
Cefmetazole	CMZ-30	30ug				26--32	25--34	–	–	16--21	–	31--36	–
for *Staphylococcus* species			≤12	13--15	≥16								
for *N. gonorrhoeae*			≤27	28--32	≥33								
for Enterobacteriaceae			≤12	13--15	≥16								
Cefonicid	CID-30	30ug				25--29	22--28	–	–	–	30--38	–	–
for *H. influenzae* and *parainfluenzae*			≤16	17--19	≥20								
for *Staphylococcus* species			≤14	15--17	≥18								
for *Enterobacteriaceae*			≤14	15--17	≥18								
Cefoperazone	CFP-75	75ug				28--34	24--33	23--29	–	–	–	–	–
for *Staphylococcus* species			≤15	16--20	≥21								
for *Enterobacteriaceae*			≤15	16--20	≥21								
for *Pseudomonas aeruginosa*			≤15	16--20	≥21								
Cefotaxime	CTX-30	30ug				29--35	25--31	18--22	–	31--39	–	38--48	31--39
for *H. influenzae* and *parainfluenzae*			–	–	≥26								
for *N. gonorrhoeae*			–	–	≥31								
for *N. meningitidis*			–	–	≥34								
for beta-hemolytic streptococci			–	–	≥24								
for viridans group streptococci			≤25	26--27	≥28								
for *Staphylococcus* species			≤14	15--22	≥23								
for *Enterobacteriaceae*			≤22	23--25	≥26								
for *Pseudomonas aeruginosa*			≤14	15--22	≥23								
for *Acinetobacter* species			≤14	15--22	≥23								
Cefotetan	CTT-30	30ug				28--34	17--23	–	–	–	–	30--36	–
for *N. gonorrhoeae*			≤19	20--25	≥26								
for *Staphylococcus* species			≤12	13--15	≥16								
for *Enterobacteriaceae*			≤12	13--15	≥16								
Cefoxitin	FOX-30	30ug				23--29	23--29	–	–	–	–	33--41	–
for *N. gonorrhoeae*			≤23	24--27	≥28								
for *Enterobacteriaceae*			≤14	15--17	≥18								
for *S. aureus* and *S. lugdunensis*			≤21	–	≥22								
for coag-neg *Staph*. (not *S. lugdunensis*)			≤24	–	≥25								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 x,xii,xvii,xviii	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Cefpodoxime	CPD-10	10ug				23--28	19--25	–	–	25--31	–	35--43	28--34
for *H. influenzae* and *parainfluenzae*			–	–	≥21								
for *N. gonorrhoeae*			–	–	≥29								
for *Staphylococcus* species			≤17	18--20	≥21								
for *Enterobacteriaceae*			≤17	18--20	≥21								
Cefprozil	CPR-30	30ug				21--27	27--33	–	–	–	20--27	–	25--32
for *Staphylococcus* species			≤14	15--17	≥18								
for *H. influenzae* and *parainfluenzae*			≤14	15--17	≥18								
for *Enterobacteriaceae*			≤14	15--17	≥18								
Ceftaroline	CPT-30	30ug				26--34	26--35	–	–	29--39	–	–	31--41
Ceftaroline- avibactam[xiv,xv]		**30/15ug**				**27--34**	**25--34**	**17--26**	**27--35**	**30--38**	–	–	–
Ceftazidime	CAZ-30	30ug				25--32	16--20	22--29	–	27--35	–	35--43	–
for *H. influenzae* and *parainfluenzae*			–	–	≥26								
for *N. gonorrhoeae*			–	–	≥31								
for *Staphylococcus* species			≤14	15--17	≥18								
for *Enterobacteriaceae*			≤17	18--20	≥21								
for *Pseudomonas aeruginosa*			≤14	15--17	≥18								
for *Acinetobacter* species			≤14	15--17	≥18								
for *Burkholderia cepacia*			≤17	18--20	≥21								
Ceftazidime-avibactam[xiv,xv]		**30/20ug**				**27--35**	**16--22**	**25--31**	**28--35**	**25--31**	–	–	–
Ceftibuten	CTB-30	30ug				27--35	–	–	–	29--36	–	–	–
for *H. influenzae* and *parainfluenzae*			–	–	≥28								
for *Enterobacteriaceae*[30] (investigational)			≤17	18--20	≥21								
Ceftizoxime	ZOX-30	30ug				30--36	27--35	12--17	–	29--39	–	42--51	28--34
for *H. influenzae* and *parainfluenzae*			–	–	≥26								
for *N. gonorrhoeae*			–	–	≥38								
for *Staphylococcus* species			≤14	15--19	≥20								
for Enterobacteriaceae			≤21	22--24	≥25								
for *Pseudomonas aeruginosa*			≤14	15--19	≥20								
Ceftobiprole[ix]	BRP-30	30ug				30--36	26--34	24--30	–	28--36	30--38	-	33--39
Ceftriaxone	CRO-30	30ug				29--35	22--28	17--23	–	31--39	–	39--51	30--35
for *H. influenzae* and *parainfluenzae*			–	–	≥26								
for *N. gonorrhoeae*			–	–	≥35								
for *N. meningitidis*			–	–	≥34								
for beta-hemolytic streptococci			–	–	≥24								
for viridans group streptococci			≤24	25--26	≥27								
for *Staphylococcus* species			≤13	14--20	≥21								
for *Enterobacteriaceae*			≤19	20--22	≥23								
for *Pseudomonas aeruginosa*			≤13	14--20	≥21								
for *Acinetobacter* species			≤13	14--20	≥21								
Cefuroxime (oral)	CXM-30	30ug				20--26	27--35	–	–	–	28--36	33--41	–
for *Staphylococcus* species			≤14	15--22	≥23								
for *Enterobacteriaceae*			≤14	15--22	≥23								
for *H. influenzae* and *parainfluenzae*			≤16	17--19	≥20								
Cefuroxime (parenteral)	CXM-30	30ug				20--26	27--35	–	–	–	28--36	33--41	–
for *H. influenzae* and *parainfluenzae* (parenteral)			≤16	17--19	≥20								
for *N. gonorrhoeae* (parenteral)			≤25	26--30	≥31								
for *Staphylococcus* species (parenteral)			≤14	15--17	≥18								
for *Enterobacteriaceae* (parenteral)			≤14	15--17	≥18								
Cephalothin	CF-30	30ug				15--21	29--37	–	–	–	–	–	26--32
for *Staphylococcus* species			≤14	15--17	≥18								
for *Enterobacteriaceae*			≤14	15--17	≥18								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 x,xii,xvii,xviii	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Chloramphenicol	C-30	30ug				21--27	19--26	–	–	31--40	–	–	23--27
for *H. influenzae* and *parainfluenzae*			≤25	26--28	≥29								
for *S. pneumoniae*			≤20	–	≥21								
for *Staphylococcus* species			≤12	13--17	≥18								
for *Enterococcus* species			≤12	13--17	≥18								
for *Enterobacteriaceae*			≤12	13--17	≥18								
for *N. meningitides*			≤19	20--25	≥26								
for *Streptococcus* other than *S. pneumoniae*			≤17	18--20	≥21								
Cinoxacin	CIN-100	100ug				26--32	–	–	–	–	–	–	–
for *Enterobacteriaceae*			≤14	15--18	≥19								
Ciprofloxacin	CIP-5	5ug				30--40	22--30	25--33	–	34--42	–	48--58	–
for *H. influenzae* and *parainfluenzae*			–	–	≥21								
for *N. gonorrhoeae*			≤27	28--40	≥41								
for *N. meningitidis*			≤32	33--34	≥35								
for *Staphylococcus* species			≤15	16--20	≥21								
for *Enterococcus* species			≤15	16--20	≥21								
for *Enterobacteriaceae*			**≤20**	**21--30**	**≥31**								
for *Pseudomonas aeruginosa*			≤15	16--20	≥21								
for *Acinetobacter* species			≤15	16--20	≥21								
Clarithromycin	CLR-15	15ug				–	26--32	–	–	11--17	–	–	25--31
for *H. influenzae* and *parainfluenzae*			≤10	11--12	≥13								
for *Streptococcus* other than *S. pneumoniae*			≤16	17--20	≥21								
for *S. pneumoniae*			≤16	17--20	≥21								
for *Staphylococcus* species			≤13	14--17	≥18								
Clinafloxacin		5ug				31--40	28--37	27--35	–	34--43	–	–	27--34
Clindamycin[iii]	CC-2	2ug				–	24--30	–	–	–	–	–	19--25
for *Streptococcus* other than *S. pneumoniae*			≤15	16--18	≥19								
for *S. pneumoniae*			≤15	16--18	≥19								
for *Staphylococcus* species			≤14	15--20	≥21								
Colistin	CL-10	10ug		–	≥11	11--17	–	11--17	–	–	–	–	–
for *Pseudomonas aeruginosa*			≤10	–	≥11								
Dirithromycin	DTM-15	15ug				–	18--26	–	–	–	–	–	18--25
for *Staphylococcus* species			≤15	16--18	≥19								
for *S. pneumoniae*			≤13	14--17	≥18								
for *Streptococcus* other than *S. pneumoniae*			≤13	14--17	≥18								
Doripenem	DOR-10	10ug				27--35	33--42	28--35	–	21--31	–	–	30--38
for *Enterobacteriaceae*			≤19	20--22	≥23								
for *Pseudomonas aeruginosa*			**≤15**	**16--18**	**≥19**								
for *H. influenzae* and *parainfluenzae*			**≤1**	–	**≥16**								
Doxycycline	D-30	30ug				18--24	23--29	–	–	–	–	–	**25--34**
for *Staphylococcus* species			≤12	13--15	≥16								
for *Enterococcus* species			≤12	13--15	≥16								
for *Enterobacteriaceae*			≤10	11--13	≥14								
for *Acinetobacter* species			≤9	10--12	≥13								
Enoxacin	ENX-10	10ug				28--36	22--28	22--28	–	–	–	43--51	–
for *Staphylococcus* species			≤14	15--17	≥18								
for *N. gonorrhoeae*			≤31	32--35	≥36								
for *Enterobacteriaceae*			≤14	15--17	≥18								
Ertapenem	ETP-10	10ug				29--36	24--31	13--21	–	20--28	27--33	–	28--35
for *Staphylococcus* species			≤15	16--18	≥19								
for *H. influenzae* and *parainfluenzae*			–	–	≥19								
for *Enterobacteriaceae*			**≤18**	**19--21**	**≥22**								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 x,xii,xvii,xviii	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Erythromycin[iii]	E-15	15ug				–	22--30	–	–	–	–	–	25--30
for *S. pneumoniae*			≤15	16--20	≥21								
for *Staphylococcus* species			≤13	14--22	≥23								
for *Streptococcus* other than *S. pneumoniae*			≤15	16--20	≥21								
for *Enterococcus* species			≤13	14--22	≥23								
Faropenem		5ug				20--26	27--34	–	–	15--22	–	–	27--35
Fleroxacin	FLE-5	5ug				28--34	21--27	12--20	–	30--38	–	43--51	–
for *Staphylococcus* species			≤15	16--18	≥19								
for *H. influenzae* and *parainfluenzae*			–	–	≥19								
for *N. gonorrhoeae*			≤28	29--34	≥35								
for *Enterobacteriaceae*			≤15	16--18	≥19								
Fosfomycin	FOS-200	200ug				22--30	25--33	–	–				
for *Enterococcus* species			≤12	13--15	≥16								
for *Enterobacteriaceae*			≤12	13--15	≥16								
Fusidic acid	FA-10	**10ug**				–	**24--32**	–	–	–	–	–	**9--16**
Garenoxacin	GRN	5ug				28--35	30--36	19--25	-	33--41	-	-	26--33
Gatifloxacin	GAT-5	5ug				30--37	27--33	20--28	–	33--41	–	45--56	24--31
for *Staphylococcus* species			≤19	20--22	≥23								
for *Enterococcus* species			≤14	15--17	≥18								
for *H. influenzae* and *parainfluenzae*			–	–	≥18								
for *N. gonorrhoeae*			≤33	34--37	≥38								
for *S. pneumoniae*			≤17	18--20	≥21								
for *Streptococcus* other than *S. pneumoniae*			≤17	18--20	≥21								
for *Enterobacteriaceae*			≤14	15--17	≥18								
for *Pseudomonas aeruginosa*			≤14	15--17	≥18								
for *Acinetobacter* species			≤14	15--17	≥18								
Gemifloxacin	GEM-5	5ug				29--36	27--33	19--25	–	30--37	–	–	28--34
for *H. influenzae* and *parainfluenzae*			–	–	≥18								
for *S. pneumoniae*			≤19	20--22	≥23								
for *Enterobacteriaceae*			≤15	16--19	≥20								
Gentamicin[iii]	GM-10					19--26	19--27	16--21	–	–	–	–	–
for *Staphylococcus* species		10ug	≤12	13--14	≥15								
for *Enterobacteriaceae*			≤12	13--14	≥15								
for *Pseudomonas aeruginosa*			≤12	13--14	≥15								
for *Acinetobacter* species			≤12	13--14	≥15								
Grepafloxacin	GRX-5	5ug				28--36	26--31	20--27	–	32--39	–	44--52	21--28
for *Staphylococcus* species			≤14	15--17	≥18								
for *H. influenzae* and *parainfluenzae*			–	–	≥24								
for *N. gonorrhoeae*			≤27	28--36	≥37								
for *S. pneumoniae*			≤15	16--18	≥19								
for *Streptococcus* other than *S. pneumoniae*			≤15	16--18	≥19								
for *Enterobacteriaceae*			≤14	15--17	≥18								
Iclaprim	ICL	5ug				14--22	25--33	–	–	24--33	–	–	21--29
Imipenem	IPM-10	10ug				26--32	–	20--28	–	21--29	–	–	–
for *H. influenzae* and *parainfluenzae*			–	–	≥16								
for *Staphylococcus* species			≤13	14--15	≥16								
for *Enterobacteriaceae*			≤19	20--22	≥23								
for *Pseudomonas aeruginosa*			**≤15**	**16--18**	**≥19**								
for *Acinetobacter* species			≤13	14--15	≥16								
Kanamycin	K-30	30ug				17--25	19--26	–	–	–	–	–	–
for *Staphylococcus* species			≤13	14--17	≥18								
for *Enterobacteriaceae*			≤13	14--17	≥18								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 x,xii,xvii,xviii	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Levofloxacin	LVX-5	5ug				29--37	25--30	19--26	–	32--40	–	–	20--25
for *H. influenzae* and *parainfluenzae*			–	–	≥17								
for *S. pneumoniae*			≤13	14--16	≥17								
for *Streptococcus* other than *S. pneumoniae*			≤13	14--16	≥17								
for *Enterococcus* species			≤13	14--16	≥17								
for *Enterobacteriaceae*			≤13	14--16	≥17								
for *Staphylococcus* species			≤15	16--18	≥19								
for *Pseudomonas aeruginosa*			≤13	14--16	≥17								
for *Acinetobacter* species			≤13	14--16	≥17								
for *Stenotrophomonas maltophilia*			≤13	14--16	≥17								
Linezolid	LZD-30	30ug				–	25--32	–	–	–	–	–	25--34
for *Staphylococcus* species			≤20	–	≥21								
for *Enterococcus* species			≤20	21--22	≥23								
for *S. pneumoniae*			–	–	≥21								
for *Streptococcus* other than *S. pneumoniae*			–	–	≥21								
Linopristin-flopristin	LFE-10	10 ug				–	25--31	–	–	25--31	–	–	22--28
Lomefloxacin	LOM-10	10ug				27--33	23--29	22--28	–	33--41	–	45--54	–
for *H. influenzae* and *parainfluenzae*			–	–	≥22								
for *N. gonorrhoeae*			≤26	27--37	≥38								
for *Staphylococcus* species			≤18	19--21	≥22								
for *Enterobacteriaceae*			≤18	19--21	≥22								
for *Pseudomonas aeruginosa*			≤18	19--21	≥22								
Loracarbef	LOR-30	30ug				23--29	23--31	–	–	–	26--32	–	22--28
for *Staphylococcus* species			≤14	15--17	≥18								
for *H. influenzae* and *parainfluenzae*			≤15	16--18	≥19								
for *Enterobacteriaceae*			≤14	15--17	≥18								
Mecillinam	MEL-10	10ug				24--30	–	–	–	–	–	–	–
for *Enterobacteriaceae*			≤11	12--14	≥15								
Meropenem	MEM-10	10ug				28--34	29--37	27--33	–	20--28	–	–	28--35
for *Staphylococcus* species			≤13	14--15	≥16								
for *H. influenzae* and *parainfluenzae*			–	–	≥20								
for *N. meningitides*			–	–	≥30								
for *Enterobacteriaceae*			≤19	20--22	≥23								
for *Pseudomonas aeruginosa*			**≤15**	**16--18**	**≥19**								
for *Acinetobacter* species			≤13	14--15	≥16								
for *Burkholderia cepacia*			≤15	16--19	≥20								
Methicillin	MET-5	5ug				–	17--22	–	–	–	–	–	–
for *Staphylococcus* species			≤9	10--13	≥14								
Mezlocillin	MZ-75	75ug				23--29	–	19--25	–	–	–	–	–
for *Pseudomonas aeruginosa*			≤15	–	≥16								
for *Enterobacteriaceae*			≤17	18--20	≥21								
for *Acinetobacter* species			≤17	18--20	≥21								
Minocycline	MI-30	30ug				19--25	25--30	–	–	–	–	–	–
for *Staphylococcus* species			≤14	15--18	≥19								
for *Enterococcus* species			≤14	15--18	≥19								
for *N. meningitides*			–	–	≥26								
for *Enterobacteriaceae*			≤12	13--15	≥16								
for *Pseudomonas aeruginosa*			≤14	15--18	≥19								
for *Acinetobacter* species			≤12	13--15	≥16								
for *Burkholderia cepacia*			≤14	15--18	≥19								
for *Stenotrophomonas maltophilia*			≤14	15--18	≥19								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 [x,xii,xvii,xviii]	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Moxalactam	MOX-30	30ug				28-35	18-24	17-25	–	–	–	–	–
for *Staphylococcus* species			≤14	15--22	≥23								
for *Enterobacteriaceae*			≤14	15--22	≥23								
for *Pseudomonas aeruginosa*			≤14	15--22	≥23								
Moxifloxacin	MXF-5	5ug				28--35	28--35	17--25	–	31--39	–	–	25--31
for Staphylococcus species			≤20	21--23	≥24								
for *H. influenzae* and *parainfluenzae*			–	–	≥18								
for *S. pneumoniae*			≤14	15--17	≥18								
Nafcillin	NF-1	1ug				–	16--22	–	–	–	–	–	–
for *Staphylococcus* species			≤10	11--12	≥13								
Nalidixic Acid	NA-30	30ug				22--28	–	–	–	–	–	–	–
for *Enterobacteriaceae*			≤13	14--18	≥19								
for *N. meningitides*			≤25	–	≥26								
Netilmicin	NET-30	30ug				22--30	22--31	17--23	–	–	–	–	–
for *Staphylococcus* species			≤12	13--14	≥15								
for *Enterobacteriaceae*			≤12	13--14	≥15								
for *Pseudomonas aeruginosa*			≤12	13--14	≥15								
Nitrofurantoin	Fm-300	300ug				20--25	18--22	–	–	–	–	–	23--29
for *Staphylococcus* species			≤14	15--16	≥17								
for *Enterococcus* species			≤14	15--16	≥17								
for *Enterobacteriaceae*			≤14	15--16	≥17								
Norfloxacin	NOR-10	10ug				28--35	17--28	22--29	–	–	–	–	15--21
for *Staphylococcus* species			≤12	13--16	≥17								
for *Enterococcus* species			≤12	13--16	≥17								
for *Enterobacteriaceae*			≤12	13--16	≥17								
for *Pseudomonas aeruginosa*			≤12	13--16	≥17								
Ofloxacin	OFX-5	5ug				29--33	24--28	17--21	–	31--40	–	43--51	16--21
for *H. influenzae* and *parainfluenzae*			–	–	≥16								
for *N. gonorrhoeae*			≤24	25--30	≥31								
for *S. pneumoniae*			≤12	13--15	≥16								
for *Streptococcus* other than *S. pneumoniae*			≤12	13--15	≥16								
for *Enterobacteriaceae*			≤12	13--15	≥16								
for *Staphylococcus* species			≤14	15--17	≥18								
for *Pseudomonas aeruginosa*			≤12	13--15	≥16								
Omadacycline		**30ug**				**22--28**	**22--30**	–	–	**21--29**	–	–	**24--32**
Oxacillin	OX-1	1ug				–	18--24	–	–	–	–	–	≤12[vii]
for *S. aureus*			≤10	11--12	≥13								
for coag-neg *Staph.* and *S. lugdunensis*			–	–	–								
for *S. pneumoniae* (penicillin G susceptibility)			–	–	≥20								
Penicillin	P-10	10U				–	26--37	–	–	–	–	26--34	24--30
for *Staphylococcus* species			≤28	–	≥29								
for *N. gonorrhoeae*			≤26	27--46	≥47								
for beta-hemolytic streptococci			–	–	≥24								
for *Enterococcus* species			≤14	–	≥15								
for *N. meginitidis*			–	–	–								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 x,xii,xvii,xviii	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Piperacillin	PIP-100	100ug				24--30	–	25--33	12--18	–	–	–	–
for *Pseudomonas aeruginosa*			**≤14**	**15--20**	**≥21**								
for *Enterobacteriaceae*			≤17	18--20	≥21								
for *Acinetobacter* species			≤17	18--20	≥21								
Piperacillin/Tazobactam	TZP-10	100/10ug				24--30	27--36	25--33	24--30	33--38	–	–	–
for *Enterobacteriaceae*			≤17	18--20	≥21								
for *Pseudomonas aeruginosa*			**≤14**	**15--20**	**≥21**								
for *Acinetobacter* species			≤17	18--20	≥21								
for *Staphylococcus* species			≤17	–	≥18								
for *H. influenzae* and *parainfluenzae*			–	–	≥21								
Plazomicin		**30ug**				**21--27**	**19--25**	**15--21**	–	–	–	–	–
Polymyxin B	PB-300	300 units				13--19	–	14--18	–	–	–	–	–
for *Pseudomonas aeruginosa*			≤11	–	≥12								
Quinupristin-dalfopristin	QD-15	15ug				–	21--28	–	–	15--21	–	–	19--24
for *Enterococcus* species			≤15	16--18	≥19								
for *S. pneumoniae*			≤15	16--18	≥19								
for *Streptococcus* other than *S. pneumoniae*			≤15	16--18	≥19								
for *Staphylococcus* species			≤15	16--18	≥19								
Razupenem	RZM	10ug				21--26	–[xiii]	–	–	24--30	–	–	29--36
Rifampin	RA-5	5ug				8-10	26--34	–	–	22--30	–	–	25--30
for *H. influenzae* and *parainfluenzae*			≤16	17--19	≥20								
for *N. meningitidis*			≤19	20--24	≥25								
for *Staphylococcus* species			≤16	17--19	≥20								
for *S. pneumoniae*			≤16	17--18	≥19								
for *Enterococcus* species			≤16	17--19	≥20								
Solithromycin		**15ug**				–	**22--30**	–	–	**16--23**	–	–	**25--33**
Sparfloxacin	SPX-5	5ug				30--38	27--33	21--29	–	32--40	–	43--51	21--27
for *Staphylococcus* species			≤15	16--18	≥19								
for *S. pneumoniae*			≤15	16--18	≥19								
Spectinomycin	SPT-100	100ug								–	–	23--29	–
for *N. gonorrhoeae*			≤14	15--17	≥18								
Streptomycin[iii]	S-10	10ug				12--20	14--22	–	–	–	–	–	–
for *Enterobacteriaceae*			≤11	12--14	≥15								
Sulfisoxazole[iv]	G-25	250ug (or 300ug)				15--23	24--34	–	–	–	–	–	–
for *Staphylococcus* species			≤12	13--16	≥17								
for *Enterobacteriaceae*			≤12	13--16	≥17								
Tedizolid		**20ug**				–	**22--29**	–	–	–	–	–	**24--30**
Teicoplanin (investigational)	TEC-30	30ug				–	15--21	–	–	–	–	–	–
for *Staphylococcus* species			≤10	11--13	≥14								
for *Enterococcus* species			≤10	11--13	≥14								
Telavancin		30ug				-	16--20	-	-	-	-	-	17--24
Telithromycin	TEL-15	15ug				–	24--30	–	–	17--23	–	–	27--33
for *Staphylococcus* species			≤18	19--21	≥22								
for *H. influenzae* and *parainfluenzae*			≤11	12--14	≥15								
Tetracycline	Te-30	30ug				18--25	24--30	–	–	14--22	–	30--42	27--31
for *H. influenzae* and *parainfluenzae*			≤25	26--28	≥29								
for *N. gonorrhoeae*			≤30	31--37	≥38								
for *Streptococcus* other than *S. pneumoniae*			≤18	19--22	≥23								
for *S. pneumoniae*			≤18	19--22	≥23								
for *Staphylococcus* species			≤14	15--18	≥19								
for *Enterococcus* species			≤14	15--18	≥19								
for *Enterobacteriaceae*			≤11	12--14	≥15								
for *Acinetobacter* species			≤11	12--14	≥15								

Antimicrobial Agent	Code	Disk Potency	Zone Diameter Interpretive Stds (mm)[a]			QC Zone Diameter Limits (mm)							
			Resistant[b]	Intermediate[c]	Susceptible[d]	*E. coli* ATCC® 25922[h]	*S. aureus* ATCC® 25923	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218 x,xii,xvii,xviii	*H. influenzae* ATCC® 49247	*H. influenzae* ATCC® 49766	*N. gonorrhoeae* ATCC® 49226	*S. pneumoniae* ATCC® 49619[v]
Ticarcillin	TIC-75	75ug				24--30	–	21--27	6	–	–	–	–
for *Pseudomonas aeruginosa*			**≤15**	**16--23**	**≥24**								
for *Acinetobacter* species			≤14	15--19	≥20								
for *Enterobacteriaceae*			≤14	15--19	≥20								
Ticarcillin/Clavulanic Acid	TIM-85	75/10ug				24--30	29--37	20--28	21--25	–	–	–	–
for *Pseudomonas aeruginosa*			**≤15**	**16--23**	**≥24**								
for *Enterobacteriaceae*			≤14	15--19	≥20								
for *Acinetobacter* species			≤14	15--19	≥20								
for *Staphylococcus* species			≤22	–	≥23								
Tigecycline	TGC-15	15ug				20--27	20--25	9--13	–	23--31	-	30--40	23--29
for *Staphylococcus aureus*			–	–	≥19								
for *Streptococcus* other than *S. pneumoniae*			–	–	≥19								
for *Enterococcus faecalis*			–	–	≥19								
for *Enterobacteriaceae*			≤14	15--18	≥19								
Tobramycin	NN-10	10ug				18--26	19--29	19--25	–	–	–	–	–
for *Staphylococcus* species			≤12	13--14	≥15								
for *Enterobacteriaceae*			≤12	13--14	≥15								
for *Pseudomonas aeruginosa*			≤12	13--14	≥15								
for *Acinetobacter* species			≤12	13--14	≥15								
Trimethoprim[iv]	TMP-5	5ug				21--28	19--26	–	–	–	–	–	–
for *Staphylococcus* species			≤10	11--15	≥16								
for *Enterobacteriaceae*			≤10	11--15	≥16								
Trimethoprim/Sulfamethoxazole[iv]	SXT	1.25/23.75 ug				23--29	24--32	–	–	24--32	–	–	20--28
for *H. influenzae* and *parainfluenzae*			≤10	11--15	≥16								
for *S. pneumoniae*			≤15	16--18	≥19								
for *Staphylococcus* species			≤10	11--15	≥16								
for *Enterobacteriaceae*			≤10	11--15	≥16								
for *N. meningitidis*			≤25	26--29	≥30								
for *Burkholderia cepacia*			≤10	11--15	≥16								
for *Stenotrophomonas maltophilia*			≤10	11--15	≥16								
for *Acinetobacter* species			≤10	11--15	≥16								
Trospectomycin		30ug				10--16	15--20	-	-	22--29	-	28--35	-
Trovafloxacin	TVA-10	10ug				29--36	29--35	21--27	–	32--39	–	42--55	25--32
for *H. influenzae* and *parainfluenzae*			–	–	≥22								
for *N. gonorrhoeae*			–	–	≥34								
for *S. pneumoniae*			≤15	16--18	≥19								
for *Streptococcus* other than *S. pneumoniae*			≤15	16--18	≥19								
Ulifloxacin (prulifloxacin)[xi]	**ULI**	5ug				32--38	20--26	27--33	–				
Vancomycin	Va-30	30ug				–	17--21	–	–	–	–	–	20--27
for *S. pneumoniae*			–	–	≥17								
for *Streptococcus* other than *S. pneumoniae*			–	–	≥17								
for *Enterococcus* species			≤14	15--16	≥17								
for *Staphylococcus* species			–	–	–								

Note: Information in **boldface** type is considered new or modified since the previous publication.

REPORTING RESULTS[7]

a. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black, nonreflecting background illuminated with reflected light. The zone margin should be considered the area showing no obvious visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

b. The “resistant” (R) category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistance mechanisms (e.g. beta lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

c. The "intermediate" (I) category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g. quinolones and beta-lactams in urine) or when a higher than normal dosage of drug can be used (e.g. beta-lactams). This category also includes a "buffer zone" which should prevent small, uncontrolled technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

d. The “susceptible” (S) category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.

e. The “nonsusceptible” (NS) category is used for isolates for which only susceptible interpretive criteria have been designated because of the absence or rare occurrence of resistant strains. Isolates that have MICs above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible.

f. For some organisms excluded from this document, the current CLSI guidelinet M45—*Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria* provides suggestions for standardized methods for susceptibility testing, including information about drug selection, interpretation, and QC testing. The organism groups covered in that document are *Abiotrophia* and *Granulicatella* spp. (formerly known as nutritionally deficient or nutritionally variant streptococci); the *Aeromonas hydrophila* complex; *Bacillus* spp. (not *B. anthracis*); *Campylobacter jejuni*/*coli*; *Corynebacterium* spp. (including *C. diptheriae*); *Erysipelothrix rhusiopathiae*; the HACEK group: *Aggregatibacter* spp. (formerly the *Aphrophilus* cluster of the genus *Haemophilus* [i.e. *H. aphrophilus*, *H. paraphrophilus*, *H. segnis*]), *Actinobacillus actinomycetemcomitans*, *Cardiobacterium* spp., *Eikenella corrodens*, and *Kingella* spp.; *Helicobacter pylori*; *Lactobacillus* spp.; *Leuconostoc* spp.; *Listeria monocytogenes*; *Moraxella catarrhalis*; *Pasteurella* spp.; *Pediococcus* spp.; potential agents of bioterrorism; and *Vibrio* spp., including *V. cholerae*. For organisms other than those outlined above, studies are not yet adequate to develop reproducible, definitive standards to interpret results. These organisms may require different media or different atmospheres of incubation, or they may show marked strain-to-strain variation in growth rate. For these microorganisms, consultation with an infectious disease specialist is recommended for guidance in determining the need for susceptibility testing and in the interpretation of results. Published reports in the medical literature and current consensus recommendations for therapy of uncommon microorganisms may obviate the need for testing. If necessary, a dilution method is usually the most appropriate testing method, and this may require submitting the organism to a reference laboratory. Physicians should be informed of the limitations of results and advised to interpret results with caution.

g. Policies regarding the generation of cumulative antibiograms should be developed in concert with the infectious disease service, infection control personnel and the pharmacy and therapeutics committee. Under most circumstances, the percentage of susceptible and intermediate results should not be combined into the same statistics. See the current CLSI document M39—*Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*.

h. Multiple test parameters are monitored by following the QC recommendations described in the current CLSI M100 standard. However, acceptable results derived from testing QC strains do not guarantee accurate results when testing patient isolates. It is important to review all results obtained from all drugs tested on patient isolates before reporting results. This should include, but are not be limited to, ensuring that 1) the antimicrobial susceptibility results are consistent with the proper identification of the isolate; 2) the results from individual agents within a specific drug class follows the established hierarchy of activity rules; and 3) the isolate is susceptible to those agents for which resistance has not been documented and for which only “susceptible” interpretive criteria exist in the M100 document. Unusual or inconsistent results should be **confirmed** by **re**checking **various parameters of testing**. Each laboratory must develop its own policies for **confirmation** of unusual or inconsistent antimicrobial susceptibility test results. This list should emphasize those results that are **most** likely to affect patient care.

i. Isolates that are initially susceptible may become intermediate or resistant after initiation of therapy. Therefore, subsequent isolates of the same species from a similar body site should be tested in order to detect resistance that may have developed after initiation of therapy. This can occur within as little as three to four days and has been noted most frequently in *Enterobacter*, *Citrobacter*, and *Serratia* spp. with third-generation cephalosporins; in *P. aeruginosa* with all antimicrobial agents; and in staphylococci with quinolones. For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. Laboratory guidelines on when to perform susceptibility testing on repeat isolates should be determined after consultation with medical staff.

j. **Refer to the most current version of the CLSI M100-S document for the appropriate screening and confirmatory tests for ESBLs. For laboratories that have not implemented the current interpretive criteria, ESBL testing should be performed as described in the current M100 document.**

k. **Screen and confirmatory tests should be performed and reported using the new instructions for a positive MHT described in the current CLSI M100 document. It is not necessary to test an isolate for a carbapenemase by the modified Hodge test when all of the carbapenems that are reported test either intermediate or resistant (i.e. these carbapenem susceptibility results should be reported as tested). However, if the isolate tests intermediate or resistant, the MHT may be performed for epidemiological purposes to determine if a carbapenemase is present.**

l. **Not all carbapenemase-producing isolates of *Enterobacteriaceae* are MHT positive; thus, MHT-positive results may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.**

FOOTNOTES AND COMMENTS

Notes for Quality Control Strains - used to Monitor Accuracy of Disk Diffusion Testing of Non-fastidious and Fastidious Microrganisms (using Mueller Hinton Medium without blood or other supplements)

i. ATCC is a registered trademark of the American Type Culture Collection.

ii. When disk approximation tests are performed with erythromycin and clindamycin, *S. aureus* ATCC® BAA-977 (containing inducible *ermA*-mediated resistance) and *S. aureus* ATCC® BAA-976 (containing *msrA*-mediated macrolide-only efflux) are recommended as supplemental QC strains (e.g. for training, competency assessment, or test evaluation). *S. aureus* ATCC® BAA-977 should demonstrate inducible clindamycin resistance (i.e. a positive D-zone test), whereas *S. aureus* ATCC® BAA-976 should not demonstrate inducible clindamycin resistance. *S. aureus* ATCC® 25923 should be used for routine QC (e.g. weekly or daily) of erythromycin and clindamycin disks using standard MHA.

iii. For control limits of gentamicin 120-ug and streptomycin 300-ug disks, use *E. faecalis* ATCC® 29212 (gentamicin: 16-23mm; streptomycin: 14-20mm).

iv. These agents can be affected by excess levels of thymidine and thymine. See the current version of the CLSI M02 document for guidance should a problem occur with QC.

v. Despite the lack of reliable disk diffusion interpretive criteria for *S. pneumoniae* with certain beta-lactams, *Streptococcus pneumoniae* ATCC® 49619 is the strain designated for QC of all disk diffusion tests with all *Streptococcus* spp.

vi. When testing *Haemophilus* on HTM, the acceptable limits for QC strain *E. coli* ATCC® 35218 are 17 to 22mm for amoxicillin-clavulanic acid when incubated in ambient air.

vii. Deterioration in oxacillin disk content is best assessed with QC organism *S. aureus* ATCC® 25923, with an acceptable zone diameter of 18 to 24mm.

viii. Some lots of Mueller Hinton agar are deficient in calcium and give small zones.

ix. Either *H. influenzae* ATCC® 49247 or 49766 may be used for routine QC testing.

x. Because this strain may lose its plasmid, careful organism maintenance is required; refer to the current CLSI M02 document for more information.

xi. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxaxin should be used for antimicrobial susceptibility testing.

xii. QC strain recommended when testing beta-lactam/beta-lactamase inhibitors.

xiii. Razupenem tested with S. aureus ATCC® 25923 can often produce the double or target zone phenomen. For accurate QC results, use S. aureus ATCC® 29213 (no double zones) with acceptable limit 33-39mm.

xiv. **QC limits for E. coli ATCC 35218 in HTM: ceftaroline-avibactam 26-34mm; ceftasidime-avibactam 27-34mm**

xv. **QC limits for K. pneumoniae ATCC 700603 with ceftaroline-acibactam and ceftazidime-avibactam is 21-27mm. This strain is considered supplemental QC only and is not required as routine user QC testing.**

xvi. The 200ug fosfomycin disk contains 50ug of glucose-6-phosphate.

xvii. This strain may lose its plasmid and develop susceptibility to beta-lactam antimicrobial agents after repeated transfers onto culture media. Minimize by removing new culture from storage at least monthly or whenever the strain begins to show increased zone diameters to ampicillin, poeracillin, or ticarcillin; refer to M02-A11 section 15.4.

xviii. This strain is considered supplemental QC only and is not required as routine user QC testing.

REFERENCES

1. Bauer, A.W., et al. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*; 45:493-496.

2. Ryan, K.J., et al. 1970. Disc sensitivity testing. *Hospital Practice*; 5:91-100.

3. Federal Register. Rules and regulations. Antibiotic susceptibility discs. *Fed. Regist.*; 37:20525-20529, 1972. *Erratum*; 38:2756, 1973.

4. Ericsson, H.M., et al. 1971. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathol. Microbiol. Scand.*, Section B, Suppl.; 217:1-90.

5. World Health Organization Expert Committee on Biological Standardization. 1977. Technical report series 610. W.H.O., Geneva.

6. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS). 2012. *Performance Standards for Antimicrobial Disk Susceptibility Tests*; Approved Standard – Tenth Edition. M02-A11. CLSI, Wayne, PA.

7. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS). 2012. *Performance Standards for Antimicrobial Susceptibility Testing, Nineteenth Informational Supplement.* M100-S22. CLSI, Wayne, PA.

8. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS). 2012. *Methods for dilution Antimicrobial Susceptibility Tests for bacteria that grow aerobically*; Approved Standard – Eighth Edition. M07-A9. CLSI, Wayne, PA.

9. Bushby, S.R.M. 1973. Trimethoprim-sulfamethoxazole. *In vitro* microbiological aspects. *J. Infect. Dis.*; p.10-30.

10. Hitchings. 1974. Trimethoprim-sulfamethoxazole: Microbiological, pharmacological and clinical considerations. University of Chicago Press, Chicago.

11. York, M.K., et al. 1996. Comparison of PCR detection of *mec*A with standard susceptibility testing methods to determine methicillin resistance in coagulase-negative staphylococci. *J. Clin. Microbiol.*; 34:249-253.

031312kdp

HARDY DIAGNOSTICS
1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@HardyDiagnostics.com

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · Florida

The Hardy Diagnostics' manufacturing facility and quality management system is certified to ISO 13485.