



Instructions for Use

HARDYDISK™ ANTIMICROBIAL SENSITIVITY TEST (AST) IVD only

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*.

Use of HardyDisk™ AST Cefiderocol 30µg (FDC30) for *in vitro* agar diffusion susceptibility testing is indicated when there is the need to determine the susceptibility of bacteria to Cefiderocol.

HardyDisk™ AST Cefiderocol at concentration 30µg can be used to determine the zone diameter (mm) of Cefiderocol against the following bacteria for which Cefiderocol has been shown to be active both clinically and *in vitro*:

Escherichia coli

Enterobacter cloacae complex

Klebsiella pneumoniae

Proteus mirabilis

Pseudomonas aeruginosa

SUMMARY

Agar diffusion methods employing dried filter paper disks impregnated with specific concentrations of antimicrobial agents were developed in the 1940's. 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) **Consult the FDA Susceptibility Test Interpretive Criteria (STIC) website (<https://www.fda.gov/STIC>) for the most up-to-date FDA interpretive criteria/categories. Refer to the current revision of the CLSI M100 document for the most updated recommendations, footnotes and comments for testing conditions, reporting suggestions, warnings, interpretive criteria and QC information.**

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.

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 less 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. 1922) 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.

Products must be brought to room temperature before use.

Refer to the document "[Storage](#)" on the Hardy Diagnostics [Technical Document](#) website for more information.

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 document "[Precautions When Using Media](#)" on the Hardy Diagnostics [Technical Document](#) website for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics website for more information.

PROCEDURE

Direct specimen testing is not recommended. It is recommended that isolated organisms, established isolation techniques and tests for purity be performed before inoculating medium 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 2 to 6 hours to develop a turbidity that exceeds or is equivalent to the 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 the 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 3 times, turning the plate 60 degrees between streakings 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 3 to 5 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. Apply the disks by means of a dispenser, using aseptic precautions. 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 large zones next to 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, no matter how many disks are dispensed. If disks are placed too close to the edge of the plate, the zones may not be fully round 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 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 properly load cartridges 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% CO₂.
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 Quality Control organisms are given in Table 2 and indicate the correct performance of the entire procedure.

INTERPRETATION OF RESULTS

Consult the FDA Susceptibility Test Interpretive Criteria (STIC) website (<https://www.fda.gov/STIC>) for the most up-to-date FDA interpretive criteria/categories. Refer to the current revision of the CLSI M100 document for the most updated recommendations, footnotes and comments for testing conditions, reporting suggestions, warnings, interpretive criteria and QC information.⁽⁷⁾

Cefiderocol Interpretive Criteria is also listed in Table 1 below.

RESISTANT indicates that clinical efficacy has not been reliably shown in treatment studies.

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 (i.e. susceptible-only interpretive category). 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 clinical 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.

Antimicrobial agents other than Cefiderocol 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. **Consult the FDA Susceptibility Test Interpretive Criteria (STIC) website (<https://www.fda.gov/STIC>) for the most up-to-date FDA interpretive criteria/categories. Refer to the current revision of the CLSI M100 document for the most updated recommendations, footnotes and comments for testing conditions, reporting suggestions, warnings, interpretive criteria and QC information.**⁽⁷⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" on the Hardy Diagnostics [Technical Document](#) website for more information.

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 2 for acceptable quality control zone diameters. Quality control acceptance is specific to the procedure, control organism and antimicrobial agent combination. Consult the FDA Susceptibility Test Interpretive Criteria (STIC) website (<https://www.fda.gov/STIC>) for the most up-to-date FDA interpretive criteria/categories and the current revision of the CLSI M100 document for the most updated QC information and footnotes.⁽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.

Refer to the document "[Inoculation Procedures for Media QC](#)" on the Hardy Diagnostics [Technical Document](#) website for more information.









HardyDisk™ AST Cefiderocol 30µg (FDC30)

50 HardyDisks Store at -20 to 8°C. For IVD and Rx only	CEFIDEROCOL 30µg (FDC30) Susceptibility Disk Cat No: Z943 Lot No: 321456 <small>B/V0.2</small> Expires:2020-04-30
	 HARDY DIAGNOSTICS

Container Label Sample
HardyDisk™ AST Cefiderocol 30µg (FDC30)

	1 Cartridges of 50 HardyDisks™AST CEFIDEROCOL 30µg (FDC30) Susceptibility Disk
	REF Z9431 
	LOT 321456 (01) 00819398028103 (10) 321456 (17) 200430
	 2020-04-30
	    50
	Santa Maria, CA 93455 - (800) 266-2222 -20°C  8°C

Container Label Sample
HardyDisk™ AST Cefiderocol 30µg (FDC30)

	5 Cartridges of 50 HardyDisks™AST CEFIDEROCOL 30µg (FDC30) Susceptibility Disk
	REF Z9435 
	LOT 321456 (01) 00819398028110 (10) 321456 (17) 200430
	 2020-04-30
	    250
	Santa Maria, CA 93455 - (800) 266-2222 -20°C  8°C

HardyDisk™ AST Cefiderocol 30µg (FDC30)

Indications for Use/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*.

Use of HardyDisk™ AST Cefiderocol 30µg (FDC30) for *in vitro* agar diffusion susceptibility testing is indicated when there is the need to determine the susceptibility of bacteria to Cefiderocol.

HardyDisk™ AST Cefiderocol at concentration 30µg can be used to determine the zone diameter (mm) of Cefiderocol against the following bacteria for which Cefiderocol has been shown to be active both clinically and *in vitro*:

Escherichia coli

Enterobacter cloacae complex

Klebsiella pneumoniae

Proteus mirabilis

Pseudomonas aeruginosa

Cefiderocol Interpretive Criteria

Table 1. HardyDisk™ AST Cefiderocol 30µg (FDC30) – Zone Diameter Interpretive Standards (mm)

Indications For Use Organism(s)	Interpretive Criteria		
	S	I	R
<i>Enterobacteriaceae</i> ^a	≥ 18	14 – 17	≤ 13
<i>Pseudomonas aeruginosa</i>	≥ 25	19 – 24	≤ 18

^aIncludes *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* complex.

Statements on safety and efficacy of Cefiderocol

Per the FDA-Recognized Susceptibility Test Interpretive Criteria website, the safety and efficacy of antimicrobial drugs, for which antimicrobial susceptibility is tested by this AST device, may or may not have been established in adequate and well-controlled clinical trials for treating clinical infections due to microorganisms outside of those found in the indications and usage in the drug label. The clinical significance of susceptibility information in those instances is unknown. The approved labeling for specific antimicrobial drugs provides the uses for which the antimicrobial drug is approved.

Table 2. HardyDisk™ AST Cefiderocol 30µg (FDC30) – Quality Control

QC Organisms - Nonfastidious	QC Zone Diameter Limits (mm)
<i>Escherichia coli</i> ATCC 25922	25 – 31
<i>Pseudomonas aeruginosa</i> ATCC 27853	22 – 31

REPORTING RESULTS:

- 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” category is used for isolates for which only a 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 guideline 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 *Abitrophia* 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. diphtheriae*); *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.; *Lactobacillus* spp.; *Leuconostoc* spp.; *Listeria monocytogenes*; *Moraxella catarrhalis*; *Pasteurella* spp.; *Pediococcus* spp.; and *Vibrio* spp. 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. Each laboratory must develop its own policies for verification 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. Some comments may relate to dangerously misleading results that can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These are denoted with the word “**warning**.”
- k. **For screening and confirmatory tests for ESBLs: If laboratories have not yet implemented the new cephalosporin and aztreonam interpretive criteria, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam. If the laboratory has implemented the new cephalosporin and aztreonam interpretive criteria, then test interpretations for these agents do not need to be changed.**

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

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