Microbiology Data for Systemic Antibacterial Drugs — Development, Analysis, and Presentation

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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Clinical/Antimicrobial
Revision 2

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Contains Nonbinding Recommendations

Microbiology Data for Systemic Antibacterial Drugs — Development, Analysis, and Presentation Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. INTRODUCTION

The purpose of this guidance is to assist sponsors in the development, analysis, and presentation of microbiology data during antibacterial drug development.[2] Specifically, this guidance addresses the Food and Drug Administration's (FDA's) current thinking regarding the overall microbiology development program needed to support clinical development and approval of antibacterial drugs administered systemically as well as microbiology information collected after approval.[3]

This guidance replaces the guidance for industry *Microbiology Data for Systemic Antibacterial Drugs — Development, Analysis, and Presentation* issued in August 2016. Changes to this guidance compared to the August 2016 version include changes in the presentation of microbiology data as required by section 3044 of the 21st Century Cures Act, which added section 511A to the Federal Food, Drug, and Cosmetic Act (FD&C Act). Section 511A created new processes for susceptibility test interpretive criteria recognition, required FDA to establish a web page for susceptibility test interpretive criteria, and required changes to the labeling for antibacterial and antifungal drugs on susceptibility test interpretive criteria, among other changes. FDA's recommendations on implementing section 511A are described in section III.C.7., Location of Microbiology Information, section III.C.8., Postmarketing Microbiology Information, and Appendix D.

[1] This guidance has been prepared by the Division of Anti-Infective Products in the Center for Drug Evaluation and Research at the Food and Drug Administration. You may submit comments on this guidance at any time. Submit comments to Docket No. FDA-2009-D-0408 (available at https://www.regulations.gov/document?D=FDA-2009-D-0408-0017).

[2] For the purposes of this guidance, all references to *drugs* include both human drugs and therapeutic biological products unless otherwise specified.

[3] This guidance addresses the types of microbiology information that should be provided to support an investigational new drug application (IND), a new drug application (NDA), a biologics license application (BLA), and a supplemental NDA or BLA. The term *sponsor* is used in this guidance and refers to sponsors submitting an IND as well as applicants submitting an NDA or BLA.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. MICROBIOLOGY DEVELOPMENT PROGRAM

Microbiology data provide important information to guide clinical development of an investigational new drug. Microbiology data guide clinicians on the use of an antibacterial drug for its intended indications.

A. Early Development Nonclinical Considerations

1. Antibacterial Spectrum of Activity

Sponsors should evaluate the activity of an antibacterial drug, including its active components and major circulating metabolites, against a test panel of relevant bacteria early in clinical development. Sponsors should provide data on a sufficient range of clinically relevant bacteria to allow an assessment of the potential clinical efficacy of the antibacterial drug for the intended indication. Appendix A provides the suggested number of genera and species that should be tested and the recommended characteristics and diversity of the test isolates.

When conducting studies of the spectrum of activity, sponsors should test in parallel FDA-approved antibacterial drugs, especially those with the same mechanism of action as the investigational drug. In the case of a drug that acts by a new mechanism of action, we recommend that sponsors include FDA-approved antibacterial drugs with a spectrum of activity similar to the investigational drug. In the event there is no FDA-approved antibacterial drug with a similar spectrum of activity, we recommend that sponsors discuss with the FDA the approved drugs to include in these studies.

Sponsors should evaluate the minimum inhibitory concentrations (MICs) in the relevant target bacteria and provide the rationale for an estimate of the epidemiologic cutoff (EC) of the investigational drug against target bacteria.

Appendix B provides an example of the recommended elements of the study reports on antibacterial spectrum of activity.

2. Mechanism of Action

Sponsors should evaluate the mechanism of action of an investigational drug (e.g., inhibition of cell wall synthesis, lysis of cell membrane, protein synthesis). Sponsors should provide information about the drug's chemical structure and a description of any structural or biological similarities to known antibacterial drugs. Data to substantiate both physiological and

morphological effects on the microbial cells can provide a basis for understanding the development of resistance through alterations in the drug's target sites. Sponsors also should provide studies evaluating microbial killing (e.g., microbial kill curves).

3. Intracellular Antimicrobial Concentration Assessment

The ability of an antibacterial drug to achieve significant intracellular concentrations may have clinical importance when the target bacterium can reside within the cell (e.g., *Listeria*, *Chlamydophila*, *Legionella*). In situations where the antibacterial drug is intended to treat infections caused by bacteria that reside within the cell, sponsors should provide data on the drug's ability to penetrate into host cells and demonstrate the drug's activity against target bacteria inside the cell (e.g., assessment of viable intracellular microorganisms following exposure of the cells to various concentrations of an antibacterial drug).

4. Resistance Studies

Characterization of the resistance mechanisms and their distribution within the proposed target bacteria may delineate the potential clinical usefulness of the drug. Mechanisms include alterations of the drug by production of enzymes (e.g., beta-lactamases, extended spectrum beta-lactamases), inability to reach the target, and changes in the affinity of the antibacterial drug for the target site. To determine if there may be a proportion of bacteria in the overall population that are resistant to the antibacterial drug (i.e., hetero-resistance), sponsors should conduct testing to evaluate for the presence of such bacteria. When possible, we recommend that sponsors provide the genotypic characteristic of resistance mechanisms.

Sponsors should compare the activity of an investigational antibacterial drug to the activity profile of approved and other existing antibacterial drugs with the same mechanism of action to assess the possibility of cross-resistance.

Under some circumstances, tentative inferences can be drawn about cross-resistance between antibacterial drugs within a specific population of isolates from regression analyses (i.e., MIC versus MIC, zone diameter versus zone diameter) of one drug compared to another drug. If cross-resistance exists between both the investigational and control drugs, a strong correlation between the MICs of both drugs would be expected to be observed, with a majority of the MICs clustered on a 45-degree diagonal. If resistance affects the activity of one drug over the other, the cluster is usually skewed in the direction of one drug and away from the expected diagonal.

Detailed information on the mechanism of action, resistance, or cross-resistance for an antibacterial drug with a novel mechanism of action may not be available for sponsors to include in the initial investigational new drug application. This information should be provided early in drug development and ideally before initiation of phase 2 clinical development.

Appendix B provides an example of the recommended elements of study reports evaluating resistance.

B. In Vitro Antimicrobial Susceptibility Test Methods During Drug Development

1. Early Clinical Development

Before conducting clinical trials, sponsors should describe the methods used for generating susceptibility data. Sponsors can reference a standard method[4] or evaluate susceptibility by other methods including modification of the method. Sponsors should provide a detailed description of the method including the justification for the modification of the method, the effect on susceptibility results, and the performance characteristics of the method (i.e., sensitivity, specificity, precision, linearity). Sponsors should discuss any modification of an established in vitro susceptibility test method with the FDA before implementation in the drug development program.

Modifications can include the addition of any substance (e.g., blood, body fluids, polysorbate). In some cases, isolates obtained during clinical trials may need to be tested for their susceptibility in the presence and absence of the substance and the results of both methods correlated with clinical and microbiological results. Sponsors also should conduct studies to address the influence of the growth medium (e.g., pH, divalent cations), inoculum density, incubation conditions (e.g., concentration of carbon dioxide), and additives (e.g., polysorbate), in both broth and agar medium on in vitro susceptibility test results.

If a sponsor proposes to use freeze-dried panels to assess the MIC of clinical isolates, the sponsor should conduct a comparative study to demonstrate comparability of MIC results for the frozen and freeze-dried panels. The sponsor should discuss this proposed study with the FDA to ensure that appropriate data are developed for the equivalency assessment. The sponsor should submit the data to the FDA before initiating phase 2 trials.

2. Provisional Antibacterial Susceptibility Test Interpretive Criteria

Provisional antibacterial susceptibility test (AST) interpretive criteria usually are based on the limited information available before the initiation of phase 3 clinical trials. In vitro microbiology data include distributions of MICs or zone diameters that are obtained by testing the antibacterial drug against a population of recent clinical isolates that represent the target bacteria for the indications being sought (see Appendix A for guidelines in the selection of target bacteria). Sponsors should provide testing data on a sufficient range of clinically relevant bacteria for the intended indications. Sponsors should identify the prominent genotypes, serotypes, biotypes, and isolates with known mechanisms of resistance and include these in the test panel. In addition, the mechanism of action of the investigational antibacterial drug and other drugs with the same mechanism of action should be considered when establishing susceptibility testing methods and provisional AST interpretive criteria.

[4] Standard methods for susceptibility testing are developed by organizations such as the Clinical and Laboratory Standards Institute; information can be found at https://clsi.org. Sponsors can describe the standard methods that they used by referencing recognized testing methodology.

When an investigational drug has a similar mechanism of action to an approved drug, the data justifying provisional AST interpretive criteria for the investigational drug should be presented as regressions of MIC versus MIC and zone diameter versus zone diameter. Sponsors should examine these data for clusters of isolates that are substantially different from those clusters near the expected regression line of MIC versus MIC or zone versus zone plots. For example, a cluster in a position away from the expected regression line suggests that one of the drugs is affected by a resistance mechanism that does not affect the other drug. Therefore, the two drugs are not interchangeable and provisional AST interpretive criteria of the investigational drug may not be similar to the AST interpretive criteria of an approved drug with a similar mechanism of action. When developing an antibacterial drug with a specific mechanism of action for the treatment of bacteria resistant to other antibacterial drugs with the same mechanism of action, these types of analyses can be extremely useful in demonstrating the activity of the investigational drug.

Sponsors should analyze the data in terms of frequency distributions (e.g., histograms) of AST results. Frequency distribution analyses can help define which populations of isolates harbor specific resistance mechanisms that sponsors should identify. Frequency distributions can be analyzed for both dilution and diffusion susceptibility testing methods. Frequency distributions call for evaluation for each target bacteria, especially if there is no clear demarcation between the resistant and susceptible populations. EC values should be estimated for each targeted species or bacterial group.

Additional considerations to the provisional AST interpretive criteria include the methodological variability between diffusion and dilution susceptibility testing methods. Sponsors can suggest adjustments to the provisional AST interpretive criteria by evaluating scattergrams of dilution testing results compared with diffusion testing results of the same isolates tested with both methods. This evaluation can be performed using the error rate bounding method that compares diffusion testing to dilution testing. The computational algorithm generates AST interpretive criteria that minimize the number of isolates with diffusion testing results that fall outside these criteria.

Finally, evaluation of the frequency distribution analyses relative to the pharmacokinetic/pharmacodynamic (PK/PD) characteristics of the investigational antibacterial drug can further refine the provisional AST interpretive criteria.

3. Establishing In Vitro Antibacterial Susceptibility Test Interpretive Criteria

Sponsors should perform an analysis of the correlation between the clinical cure and microbiologic eradication rates in the clinical trials with the provisional AST interpretive criteria results to determine their clinical relevance. When appropriate, sponsors should assess the clinical response and microbiologic eradication rates as overall rates and as individual rates against bacteria with resistance to other antibacterial drugs as well as specific virulence factors. The available human PK/PD information, including target attainment analyses, assists in the selection of AST interpretive criteria. These analyses help to form the basis for the final selection of the AST interpretive criteria.

The purpose of establishing AST interpretive criteria is to guide the selection of appropriate antibacterial therapy. The accuracy and clinical relevance of such tests depend on adherence to standardized methods and appropriate consideration of the test results.

Sponsors also should consider the time it takes to develop a susceptibility test. FDA encourages sponsors to coordinate susceptibility test development with drug development so that susceptibility testing is available to inform use of the drug after it is marketed.

Appendix C provides the recommended electronic database format for the data from clinical trials as it pertains to AST interpretive criteria.

4. Quality Control Parameters

Sponsors should establish quality control (QC) parameters for AST before determining the activity of the antibacterial drug to ensure the generation of precise, accurate, and reproducible results. Routine QC procedures involve performance testing of designated QC strains that are genetically stable and have well-characterized susceptibility characteristics. Generally, the establishment of QC parameters should involve the use of 3 different lots of test medium, frozen panels in the case of MICs, 2 different lots of disks in the case of disk diffusion, and 10 replicates of each QC strain over 3 days in at least 7 different laboratories. This testing is done to generate enough data points to determine appropriate QC parameters.

Sponsors should obtain reference bacteria recognized from a reputable source such as the American Type Culture Collection (ATCC). In the event that sponsors do not use these recommended QC bacteria, they should justify the use of other well-characterized bacteria. If a QC bacterium is chosen that is different from these recommended QC bacteria, it should be deposited in a recognized culture collection (e.g., ATCC).

The use of established methods and concomitant use of QC strains lends confidence to the in vitro susceptibility data generated from the testing of bacteria. Therefore, sponsors should provide QC data with all susceptibility test results done on bacteria at each facility that is conducting susceptibility testing for clinical trials. Alternatively, if a central laboratory performs in vitro susceptibility tests, we recommend that sponsors provide the QC data generated by the central laboratory. In addition, we recommend that sponsors analyze the QC data generated during the conduct of clinical trials to determine whether adjustments to the QC ranges are necessary.

C. Other Considerations

1. First and Second Lists of Target Bacteria in Labeling

The *Microbiology* subsection of labeling contains two lists of bacteria. Sponsors should format this section as described in Appendix D.

The first list is based on bacteria evaluated during clinical trials that are included in the INDICATIONS AND USAGE section of labeling (21 CFR 201.57(c)(2)(i)(C)).

The second list is based on the relevance of the bacteria to the indication and its susceptibility to concentrations of the antibacterial drug that can be achieved using the proposed dosage. Appendix E provides a summary of the information needed to support the inclusion of bacteria in the second list. The inclusion of bacteria in the second list is not based on results from adequate and well-controlled clinical trials. Sponsors should provide information in support of the second list for each species proposed for inclusion by indication.

2. Antibacterial Interactions and Fixed-Combination Studies

Drug interaction studies may provide information (e.g., synergy, antagonism, indifference) on the effects one antibacterial drug may have on another. Potential for interaction usually can be determined by qualitative or quantitative in vitro studies when the activity cannot be accurately anticipated from general knowledge of the drug characteristics. The preferred methods for the characterization of antibacterial interaction are kill curves. Another method is the checkerboard titration analyzed by fractional inhibitory concentration.

For fixed-combination drug products, including drugs that contain an antibacterial drug and a component that counteracts a resistance mechanism (e.g., beta-lactam and beta-lactamase inhibitor combination), we recommend that sponsors provide the in vitro and/or in vivo data to support the contribution of each of the drugs (separately and in combination) to the activity.

3. Additional Nonclinical Studies of Antibacterial Drugs

Antibacterial drugs may have various effects on target bacteria and/or interactions with the host. These phenomena include, but are not limited to, postantibiotic effect, postantibiotic leukocyte effect, sub-MIC effects, effects on endotoxin, effects on virulence factors, and interactions with the host immune system. The sponsor should provide data from studies that are designed to investigate these effects of the investigational antibacterial drug.

Some antibacterial drugs may be inactive when protein bound, or there may be insufficient free active drug at trough concentrations. Therefore, we recommend that sponsors characterize the effects of human serum proteins and other human body fluids (e.g., lung surfactant), when appropriate, on the in vitro and in vivo activity of the drug. The effects of human serum proteins and human body fluids on activity of the drug should be evaluated over the range of clinically relevant concentrations of the antibacterial drug.

4. Animal Models of Infection

The goal of the animal models is to investigate the antibacterial activity of the investigational drug. Ideally, the animal model of infection should be similar to the infection of interest in humans, and the bacteria used in the animal model should be similar in character (e.g., antibacterial resistance, virulence factors) to bacteria that cause human disease.

In addition to measuring survival, sponsors should measure bacterial burden in blood and relevant affected organs. For example, an evaluation of antibacterial activity in relevant tissue sites (e.g.,

spleen, kidney, lung, thigh) can be important to characterize the drug's potential in the treatment of particular body site infections in humans. Animal models can provide preliminary information on PK/PD parameters as they pertain to microbiology data (e.g., area under the plasma concentration time curve over the MIC, maximal concentration over the MIC and time above the MIC).[5]

Sponsors can conduct comparative studies of the investigational antibacterial drug with other antibacterial drugs exhibiting the same mechanism of action or drugs with the same spectrum of activity. Results can be reported as 50 percent effective dose (ED50), 50 percent protective dose (PD50), or 50 percent curative dose (CD50). Sponsors should also provide the bacterial burden data at baseline and various time points.

5. Microbiology Information Collected in Clinical Trials

We recommend that a sponsor use a central laboratory for microbiologic testing (including confirmation of bacteria identification and AST) during clinical trials. We recommend that sponsors provide clinical trial protocols and laboratory procedure manuals (including details of specimen collection, isolate/specimen transport, isolate characterization and identification, isolate preservation, and susceptibility testing methods) to the FDA for review before trial initiation.

6. Electronic Submission of Microbiology Information

Sponsors should provide microbiology information in the electronic common technical document (eCTD) as described in the guidance for industry *Providing Regulatory Submissions in Electronic Format — Certain Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications*.[6] Generally, sponsors should provide the information on microbiology in two sections of the eCTD as follows:

- Module 2, Section 2.7, Clinical Summary, subsection 2.7.2.4, Special Studies. This section should contain the microbiology summary reports and the information used to justify the microbiology information included in the labeling.

- Module 5, Clinical Study Reports, subsection 5.3.5.4, Other Study Reports. This section should contain the nonclinical study and clinical trial reports used in the construction of the summary information provided in subsection 2.7.2.4. All the study reports used to construct the summary report presented in section 2.7.2.4 should be cross-linked to the summary report. All sections should be cross-referenced to each other.

[5] The FDA encourages sponsors to consult the FDA when considering a nonanimal testing method believed to be suitable, adequate, validated, and feasible. The FDA will consider if the alternative method could be assessed for equivalency to an animal test method.

[6] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance web page at https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

7. Location of Microbiology Information

The AST interpretive criteria recognized by the FDA are listed on the FDA Susceptibility Test Interpretive Criteria web page (https://www.fda.gov/STIC). FDA will update the Interpretive Criteria web page at least every six months and upon approval of a new drug application or supplement, as applicable.[7] Recognized performance standards, method standards, and quality control parameters for AST can be found on the FDA's Recognized Consensus Standards web page.[8]

Other microbiology information (e.g., mechanism of action, antimicrobial activity, information regarding emergence of resistance) remains in the drug product labeling under Section 12.4 Microbiology.

8. Postmarketing Microbiology Information

Over time after approval, additional information regarding the methods for in vitro AST and/or the QC parameters used to monitor the performance of the test as well as how the susceptibility test results should be interpreted may become available. Changes in AST interpretative criteria may translate into a lack of efficacy and/or safety concerns when out-of-date AST information leads to failure to appropriately treat the infectious disease. Consequently, it is important that the in vitro AST methods, the QC parameters, and the AST interpretive criteria be reviewed on a regular basis and updated to reflect the most current information. Application holders should review data regarding their drug for any necessary updates and submit the rationale for any changes in the in vitro AST methods, QC parameters, spectrum of activity, or AST interpretive criteria, as well as new information regarding emergence of resistance, in an applicant's annual report under the new drug application. FDA will review these annual report submissions and determine whether changes or updates to the currently recognized AST interpretive criteria are appropriate. FDA will then update the Interpretive Criteria web page to reflect these changes, as needed. If the applicant determines that other microbiology information that is contained in the drug labeling should be updated, the applicant can submit a labeling supplement.

For applicants submitting an annual report with supporting data to update or change the method of determining the AST, we recommend including a description of the old and new methods with the changes noted, as well as validation data and QC parameters of the new method. Any change

[7] Section 511A(b) of the FD&C Act required the creation of the FDA Interpretive Criteria web page (https://www.fda.gov/STIC) for information about susceptibility test interpretive criteria for antimicrobial drugs. The FDA Interpretive Criteria web page recognizes appropriate interpretive criteria standards set by standard development organizations and provides exceptions, additions, or entire interpretive criteria when necessary (including when no relevant interpretive criteria are included in an FDA-recognized standard). In accordance with section 511A(d), antimicrobial drug labeling must include a reference to the FDA Interpretive Criteria web page. More information about AST interpretive criteria labeling can be found in the guidance for industry *Systemic Antibacterial and Antifungal Drugs: Susceptibility Test Interpretive Criteria Labeling for NDAs and ANDAs.*

[8] The FDA Recognized Consensus Standards web page is available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm. Occasionally, consensus standards for methods and quality control parameters may not be established at the time a new drug is approved. If appropriate, FDA will list acceptable methods and quality control information for the drug on the Interpretive Criteria web page along with the AST interpretive criteria identified for such drug.

to a test method (e.g., microbroth dilution) should be accompanied by data to show that the results correlate with other methods (e.g., agar dilution, disk diffusion testing) by which susceptibility testing can be done. When an applicant provides information to change the QC parameters, the applicant also should include the results of validation studies.

We recommend applicants include the following information when providing changes to the existing AST interpretive criteria:

- Rationale for change
- The MIC and zone diameter distributions against the genera and species of interest; data should be from isolates collected in the preceding 3 years of the submission
- Susceptibility to the antibacterial drug to determine microbiologically supported cutoffs (e.g., histograms)
- Categorical agreement between MIC and zone diameter breakpoints in graphical form
- Error-rate bounded method of Metzler and DeHaan (Metzler and DeHaan 1974) to determine discrepancies between the two methods; the Metzler and DeHaan method usually needs to be modified because two MIC values are normally described to define an *intermediate* category (Bruden et al. 1992)
- Relevant human PK data
- Clinical data from adequate and well-controlled trials and literature reports

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APPENDIX A:
GUIDELINES FOR BACTERIAL ISOLATE SELECTION FOR STUDIES OF IN VITRO ANTIBACTERIAL ACTIVITY

For the purposes of determining the antibacterial activity of a new molecular entity and for establishing correlation of test methods (i.e., minimum inhibitory concentration (MIC) and disk diffusion), we recommend that the sponsor provide antibacterial susceptibility test (AST) data for bacterial isolates intended for inclusion in labeling. The sponsor should consult with the division to determine an adequate number of tested isolates in those situations, as well as for special types of studies where significant information may be obtained using few isolates (e.g., bactericidal studies, time-kill studies).

Table A lists the suggested number of bacterial isolates of each species or bacterial group that should be tested in nonclinical studies, when those species will be included in labeling for the antibacterial drug (i.e., in either the first or second list of target bacteria).

We recommend that sponsors identify the prominent genotypes, serotypes, biotypes, and isolates with known mechanisms of resistance and include these in the test panel. When appropriate, the spectrum of activity against hetero-resistant bacteria should be determined (e.g., vancomycin hetero-resistant *Staphylococcus aureus*). The organisms tested should be recent clinical isolates collected within the last 3 years of the new drug application with susceptibility profiles that are representative of antibacterial drugs used to treat infections caused by the target bacteria for the indication being sought. Sponsors should provide summary data by subset of organisms demonstrating resistance (e.g., methicillin-resistant *S. aureus*, extended spectrum beta-lactamases).

We recommend that at least 75 percent of isolates analyzed in these studies be collected in the United States. Sponsors who include isolates from outside the United States should compare the characteristics of those isolates with the same species found in the United States (e.g., phenotype, genotype, serotype, susceptibility profile, and virulence factors).

Sponsors should perform susceptibility testing using a range of drug dilutions that will minimize reporting *greater than or equal to* or *less than or equal to* MIC values (e.g., *greater than or equal to 16 mcg/mL, less than or equal to 0.012 mcg/mL*). All AST data should be determined using standard reference methods unless modifications of the standard methods are needed. Sponsors should present a summary of susceptibility testing results as MIC histograms or other frequency distributions displaying any proposed in vitro AST interpretive criteria. All susceptibility test data should be accompanied by appropriate quality control data.

Table A: Suggested Number of Isolates for Determination of In Vitro Antibacterial Activity and for Correlation of MIC and Disk Diffusion Methods

Bacteria or Bacterium	**Number of Isolates**
Enterobacteriaceae	≥ 300
Pseudomonas aeruginosa	≥ 100
Acinetobacter spp.	≥ 100
Staphylococcus spp.[1, 2]	≥ 100
Enterococcus spp.[1, 2]	≥ 100
Haemophilus influenzae and *H. parainfluenzae*	≥ 100
Neisseria gonorrhoeae	≥ 100
Streptococcus pneumoniae	≥ 100 (prefer > 300)
Beta-hemolytic streptococci[1]	≥ 100 (prefer > 300)
Alpha-hemolytic streptococci (other than *S. pneumoniae*)[1]	≥ 100 (prefer > 300)
Other single species	≥ 100

[1] Bacterial isolates in these genera or bacterial groups should be identified to species level (or, in some cases, to more specific groups (e.g., *Streptococcus anginosus* group)). In these cases, the recommended number of isolates applies to the particular species or group.

[2] When a particular phenotype (e.g., methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*) will be included in labeling, the recommended number of isolates applies to that phenotype.

APPENDIX B:
STUDY REPORTS OF SPECTRUM OF ACTIVITY AND RESISTANCE

Study Reports of Spectrum of Activity

We recommend that study reports of spectrum of activity include the following elements:

- The name and location of each investigator conducting or contributing to the study.
- The standardized methods for bacterial identification and antibacterial susceptibility test (AST) used to determine the activity of the antibacterial drug; details of the method and the performance characteristics of the assay in the actual laboratory where such testing is done should be included if a nonstandard method is used.
- A description of all susceptibility testing quality control (QC) measures; all AST results should be accompanied by QC data.
- The number of isolates tested in each laboratory, the specimen source, and the geographical region from which the isolates were obtained.
- A description of the spectrum of activity by all regions and individual geographic regions.
- The phenotypic and/or genotypic characterization of isolates relative to their resistance to other antibacterial drugs; the methodology and the criteria used to characterize isolates as resistant should be described.
- The phenotypic and/or genotypic characterization of isolates relative to virulence characteristics.

Results from studies evaluating antibacterial activity should be presented in tabular form under appropriate sections as described below:

- Genera and species tested — species with unique mechanisms of resistance should be grouped separately; serotype, phenotype, and/or genotype should be included if known
- Drug name
- The minimum inhibitory concentration (MIC) range and the number of isolates tested
- The values for the MIC required to inhibit growth of 50 percent of bacteria (MIC_{50}) and the MIC required to inhibit growth of 90 percent of bacteria (MIC_{90})
- Minimum bactericidal concentration (MBC)
- MIC:MBC ratio for members of clinically relevant genera

Study Reports of Resistance

Results from studies evaluating resistance should be presented in tabular form under appropriate sections as described below:

- Genera and species tested — species with unique mechanisms of resistance should be grouped separately; if serotype, phenotype, and/or genotype are known then that information should be included
- Drug name
- MIC range — for each group of organisms and the number of isolates tested in each laboratory
- MIC_{50} and MIC_{90} values
- MBC

The complete study report should include stability and lot numbers of the drug used for microbiology testing. In addition, reproducibility of test results should be submitted. If the data are derived from a publication, a copy of the publication should be included in the submission.

APPENDIX C: DATABASE FOR FINAL IN VITRO ANTIBACTERIAL SUSCEPTIBILITY TEST INTERPRETIVE CRITERIA

We recommend a single electronic database for the clinical trial with the subject level data presented in columns. Each column heading should identify the scope of information below it. For instance, a subject identification (ID) number can include a coding arrangement that differentiates the trial center as well as the individual subject. Sponsors should discuss the format of the microbiology datasets with the FDA at the time of the pre-new drug application or pre-biologics license application meeting. We recommend that a sponsor provide the following information in the database under appropriate columnar headings:

- Clinical trial center number
- Subject ID number
- Treatment group
- Sample source
- Species of bacterial isolate
- Indication
- Subject-by-subject clinical evaluations including separate rows for each subject, the subject's status of microbiological eradication, and the subject's overall clinical response (e.g., cure, failure)
- Antibacterial susceptibility test (AST) results by diffusion methods for the investigational drug and the comparator drug
- AST results by dilution methods for the investigational drug and the comparator drug

Sponsors should provide an interpretation of the data described below for the investigational drug and comparator drugs. Because of possible geographic differences in antibiograms and the clonal nature of bacteria, sponsors should present data in both combined and separate formats (e.g., United States and non-United States in separate tables). Where appropriate, we recommend that U.S. data be broken down into regions (e.g., Northeast, Southeast, Midwest, Northwest, Southwest) and that non-U.S. data be broken down by region (e.g., Asia, Europe, Africa) and within region by country (e.g., France, Germany).

We recommend that a sponsor include the following in the database:

1. Minimum inhibitory concentration (MIC) values and subject microbiological responses for each baseline bacterium within each proposed indication; all subsets of bacteria demonstrating unique mechanisms of resistance (e.g., methicillin-resistant

Staphylococcus aureus, beta-lactamase-positive *Haemophilus influenzae*) and virulence should be listed separately.

2. MIC values and subject clinical response for each baseline bacterium for each proposed indication; all subsets of bacteria demonstrating unique mechanisms of resistance and virulence should be listed separately.

3. Zone diameter values and subject microbiological responses for each bacterium for each proposed indication; all subsets of bacteria demonstrating unique mechanisms of resistance and virulence should be listed separately.

4. Zone diameter values and subject clinical responses for each bacterium for each proposed indication; all subsets of bacteria demonstrating unique mechanisms of resistance and virulence should be listed separately.

5. For each subset of bacteria requiring defined AST interpretive criteria, all individual isolates in the range of MICs from two dilutions below the susceptible and two dilutions above the resistant provisional AST interpretive criteria.

6. For each subset of bacteria requiring defined zone diameter AST interpretive criteria, all individual isolates in the range of zone diameters from 4 to 6 millimeters above the susceptible and 4 to 6 millimeters below the resistant provisional AST interpretive criteria.

7. By indication and bacteria relevant to the indication, all MICs for isolates associated with microbiological failures. The bacteria should be identified to the species level.

8. By indication and bacteria relevant to the indication, all zone diameters for isolates associated with microbiological failures. The bacteria should be identified to the species level.

9. For each bacterium (e.g., nonfastidious, fastidious, and anaerobic), the MIC value indicating the number and percent of isolates at that MIC associated with each microbiological response. MIC values should be grouped by bacterial type.

10. For each bacterium (e.g., nonfastidious, fastidious), the zone diameter indicating the number and percent of isolates at the zone diameter associated with each microbiological response. Zone diameter information should be grouped by bacterial type.

11. For each group of bacteria, a histogram showing the number of isolates at each MIC from clinical trials overlaying isolates from nonclinical studies. Sponsors should present bacteria with characterized phenotypic resistance and virulence markers as a subset.

12. For each group of bacteria, a histogram showing the number of isolates at each zone diameter from clinical trials overlaying isolates from nonclinical studies. Sponsors

should present bacteria with characterized phenotypic resistance and virulence markers as a subset.

APPENDIX D: EXAMPLE FORMAT FOR SECTIONS OF LABELING THAT PERTAIN TO MICROBIOLOGY

Recommended language for and organization of the microbiology information that is discussed in the CLINICAL PHARMACOLOGY section of labeling are provided below along with additional recommendations in italics.[1] For additional information concerning this section of labeling, see the guidance for industry *Clinical Pharmacology Labeling for Human Prescription Drug and Biological Products — Considerations, Content, and Format*.[2] Also included are recommended citations for the REFERENCES section of labeling. We recommend a consistent formatting approach for headings and subheadings in the CLINICAL PHARMACOLOGY section and the other sections of labeling to help organize the information (e.g., underlining for headings and italics for subheadings). We recommend the use of title case for headings and subheadings.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

X is an anti- (e.g., bacterial, as appropriate) drug *[see Microbiology (12.4)]*.[3]

Recommendation: The antimicrobial mechanism of action should be described in subsection 12.4 Microbiology.

12.4 Microbiology

Mechanism of Action

Resistance

Interaction With Other Antimicrobials

[Heading Title] [Other relevant information to be determined on a case-to-case basis]

Recommendation: Additional relevant microbiological information that provides characterization of the antimicrobial drug should be placed under another appropriately named heading.

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[1] As provided for in the final rule "Requirements on Content and Format of Labeling for Human Prescription Drug and Biological Products" (71 FR 3922, January 24, 2006), the microbiology portion of labeling should be added as subsection 12.4 under the CLINICAL PHARMACOLOGY section.

[2] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance web page at https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

[3] Note that this is an exception to the preferred presentation of cross-referencing in the Full Prescribing Information, which is to use the section heading followed by the numerical identifier.

Antimicrobial Activity

[Name of drug] has been shown to be active against most isolates of the following microorganisms, both in vitro and in clinical infections *[see Indications and Usage (1)]*:

Recommendation: Each organism in this list must be associated with an indication in the INDICATIONS AND USAGE section (e.g., acute bacterial skin and skin structure infections caused by susceptible isolates of Staphylococcus aureus*) (21 CFR 201.57(c)(2)(i)(C)).*

[Microorganisms should be listed under the following categories in alphabetical order]

- Aerobic bacteria
  - Gram-positive bacteria
  - Gram-negative bacteria
- Anaerobic bacteria
  - Gram-positive bacteria
  - Gram-negative bacteria
- Other microorganisms (as applicable)

The following in vitro data are available, but their clinical significance is unknown.[4] At least 90 percent of the following bacteria exhibit an in vitro minimum inhibitory concentration (MIC) less than or equal to the susceptible breakpoint for [name of drug] against isolates of similar genus or organism group. However, the efficacy of [name of drug] in treating clinical infections caused by these bacteria has not been established in adequate and well-controlled clinical trials.

[Microorganisms should be listed under the following categories in alphabetical order]

- Aerobic bacteria
  - Gram-positive bacteria
  - Gram-negative bacteria
- Anaerobic bacteria
  - Gram-positive bacteria
  - Gram-negative bacteria
- Other microorganisms (as applicable)

Recommendation: For an organism to be included in the above list (i.e., the second list), the organism at a minimum should: (1) be relevant to an indication granted in the labeling; and (2) have an MIC_{90} below the concentration of the antimicrobial achievable in the plasma or at the infection site using the dosing regimen approved in the labeling as determined from in vitro testing of the targeted species or organism group. See Appendix A for the recommended number and characteristics of test isolates. See Appendix E for a summary of information for microorganisms to be included on the second list as well as additional information.

[4] This statement must be included for the second list of bacteria; see 21 CFR 201.57(c)(13)(ii)(A).

Susceptibility Testing

For specific information regarding susceptibility test interpretive criteria, and associated test methods and quality control standards recognized by FDA for this drug, please see https://www.fda.gov/STIC.

APPENDIX E: INFORMATION REGARDING THE SECOND BACTERIA LIST IN THE MICROBIOLOGY SUBSECTION OF LABELING

Factors to Consider When Developing the Lists

The following factors should be considered when developing the bacteria lists:

- Certain bacteria are disease-specific and therefore can be properly placed only in the first list. Examples of such pathogens are *Mycobacterium tuberculosis*, *Bacillus anthracis*, *Mycobacterium leprae*, *Yersinia pestis*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and *Brucella species*.

- There should be scientific evidence demonstrating that the bacteria are frequently associated with an indication for which approval is being sought.

- Sponsors should support the species included in the second list with antibacterial susceptibility test (AST) results of recent clinical isolates (minimum inhibitory concentrations (MICs)) correlated with the achievable concentrations of the antibacterial drug using the recommended dosing regimen.

- Bacteria included in the second list should have MIC_{90} values less than or equal to the clinically relevant AST interpretive criteria established for the particular genera, species, or group of bacteria related to a specific indication or indications.

Summary Information for the Second List

The summary should include the following information for the bacteria on the second list:

- A discussion of the relevance of the bacteria to a specific clinical indication

- The frequency in which the bacteria is shown to cause disease in the general population

- Relevant literature references and/or laboratory test data summary tabulations of susceptibility data (e.g., range, MIC_{50}, MIC_{90} for relevant antibacterial drugs) and annotated supporting literature for the listed bacteria

- In vitro susceptibility information for (e.g., MIC, MIC range, MIC_{50}, MIC_{90}) of the bacteria proposed for the second list (see Appendix C for the suggested number of isolates and characteristics of pathogens that should be included for testing), accompanied by appropriate quality control (QC) data

- A discussion of the methods used and their comparability to assess susceptibility as described in the supporting literature

- Comparisons of U.S. and foreign data analyzed separately and together
- Susceptibility data that are accompanied by the appropriate QC data