

# **TDM Roundtable Recommendations: Levetiracetam Assay Validation Guidance**

## **TDM Roundtable Participating Organizations:**

American Association for Clinical Chemistry  
American Society for Clinical Laboratory Science  
American Society for Clinical Pathology  
College of American Pathologists  
Food and Drug Administration

*The concept of the Therapeutic Drug Management (TDM) TDM Roundtable was developed by the AACCC TDM Renaissance Committee of the TDM-CT Division, in conjunction with the FDA, as means of fostering greater cooperation between the laboratory community and the agency to improve patient care. Special thanks to the TDM Roundtable workgroup that drafted the document.*

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TDM Roundtable. It is not an FDA document.**

# **Guidance for Industry and FDA Staff**

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## **Recommendations for Validation of Levetiracetam Test Systems**

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**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Devices and Radiological Health**

**Division of Chemistry and Toxicology Devices  
Office of In Vitro Diagnostic Devices Evaluation and Safety**

# **Preface**

## **Public Comment**

Written comments and suggestions may be submitted at any time for Agency consideration to the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852.

When submitting comments, please refer to the exact title of this guidance document. Comments may not be acted upon by the Agency until the document is next revised or updated.

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# Guidance for Industry and FDA Staff

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## Recommendations for Validation of Levetiracetam Test Systems

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### 1. Introduction

This guidance document is intended to serve as a set of recommendations to manufacturers to facilitate the development and validation of therapeutic drug management (TDM) assays for levetiracetam. Although the phrase “therapeutic drug monitoring (TDM)” has been used for many years and in many places to refer to quantitative measurement of therapeutic drugs in serum or plasma in order to assist a care provider to ensure that a patient is treated with optimal concentration of the drug in question, we have replaced “management” for “monitoring” in order to emphasize the purpose of the testing. “Management” implies that the laboratory measurement is an essential part of the treatment of the patient, whereas “monitoring” is focused on the analytical process, without reference to the clinical implications. The abbreviation “TDM” is retained throughout this document, but it is intended to refer to “management” and not to “monitoring.” As such it will establish scientifically sound expectations which are useful for documenting analytical performance of new testing devices or methods for levetiracetam.

Levetiracetam assays are quantitative measures of levetiracetam in plasma or serum, and serve to aid in the management of a patient’s drug therapy. As analytical techniques, they are expected to accurately measure the concentration of the target drug, with defined precision, sensitivity, and specificity. While the typical specimen is plasma or serum, it is possible to validate the assay to be used to test drug concentration in other biological samples e.g. whole blood, saliva, urine, or milk.

The remaining sections of this document describe the information generally recommended in an FDA application for a TDM assay, as well as specific information for levetiracetam. Before undertaking development of any new TDM assay, the manufacturer is strongly encouraged to contact the FDA, Office of In Vitro Diagnostics to discuss their validation strategy for FDA clearance or approval.

FDA's guidance documents, including this guidance, 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.

### **The Least Burdensome Approach**

The issues identified in this guidance document represent those that we believe should be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to follow the guidance and address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that there is a less burdensome way to address the issues, you should follow the procedures outlined in the “A Suggested Approach to Resolving Least Burdensome Issues” document. It is available on our Center web page at <http://www.fda.gov/cdrh/modact/leastburdensome.html>.

## **2. Background**

To date no commercially available immunoassay has been developed for levetiracetam and the majority of data regarding drug levels has been derived using either liquid (REFS) or gas chromatographic techniques (REFS). An optimal trough serum/plasma concentration range for levetiracetam is 10-60 mg/L (Levy et al., 2001, ssWelty et al., 2002, Johannessen et al., 2003).

Levetiracetam is predominantly eliminated via the kidney with approximately 64% of a given dose excreted unchanged in urine. The drug undergoes minimal hepatic metabolism, but hydrolysis of the acetamide function by a cytosolic amidase occurs to produce a carboxylic acid metabolite, 2-pyrrolidone-N-butyric acid. The acid metabolite is also excreted renally and accounts for approximately 27% of the administered dose. Oxidation of the 3 and 4 positions of the 2-oxopyrrolidine ring also occurs by hepatic metabolism to form minor metabolites that account for about 3% of the dose. Finally levetiracetam and 2-pyrrolidone-N-butyric acid could be oxidised at the 5 position of the 2-oxopyrrolidine ring and then hydrolysed with opening of the ring. Since hepatic metabolism is minimal levetiracetam is not subject to significant pharmacokinetic drug interactions with other drugs and there are no pharmacogenomic issues that affect levetiracetam concentration, however, there is pronounced inter-individual variability in levetiracetam pharmacokinetics.

### **3. Benefits/Risks to Health**

Since seizures occur at irregular intervals, pharmacological therapy is often empiric and prophylactic in nature. In addition, occurrence of adverse effects may be insidious, and knowledge of an upper concentration limit can be useful in avoiding treatment-emergent adverse effects. Specifically, TDM can be useful in establishing an individual patient's optimal serum/plasma concentration range, and benchmarking serum concentrations at which seizures are controlled, as well as those associated with AED-specific adverse effects. TDM can also assist with management whenever a patient's medication regimen is changed (e.g. addition or removal of potentially interacting concomitant medications), or when physiologic changes occur (e.g. co-morbid hepatic or renal disease, pregnancy and normal physiological changes such as puberty and aging). TDM results in more efficient and effective optimization of therapy and patient management. AEDs that display significant pharmacokinetic interpatient variability, or are subject to multiple drug/drug interactions are likely to benefit most from TDM.

For TDM to be useful it is important that serum sampling is conducted in a consistent manner. With patients receiving chronic therapy this would include, whenever possible, obtaining steady-state, trough (pre-dose) blood samples. These should be submitted to the laboratory with an assessment of recent drug intake (e.g., medication dose administered, time of last dose, patient age, weight and height).

### **4. Scope**

The issues discussed in this guidance document apply to assays intended to quantitatively determine levetiracetam concentrations in serum or plasma in a central laboratory setting.

### **5. Supporting Information**

Typically the metabolism and pharmacokinetics of the drug which is the subject of the proposed assay will have been established and published in the scientific literature before a TDM assay is developed. The pharmacokinetics information, for the various matrices for which the test is intended and biological variations thereon, should be included in the information submitted to FDA, and in the information (package insert) provided to the user of the assay. Typically there is also information in the literature regarding optimal ranges. This information should also be presented.

### **6. Device Description**

We recommend that you include the following in your device description:

- a description of the method that your device uses to detect levetiracetam
- a description of the assay components included with the kit
- information on the antigens/antibodies detected or measured
- a clear explanation of the specific controls and calibrators to be used in the assay
- a description of the primary purpose for the quality control material

In your description of assay components, you should include the antigen source and explain how it was characterized. If a recombinant antigen is used, you should supply specific information concerning the specific epitopes present on the antigen and specific information for antigen characterization. For monoclonal antibodies, you should give specific information concerning epitopes that will be detected, and provide appropriate antibody characterization.

## **7. Performance Characteristics**

### **a. General Study Recommendations**

Patient samples or sample pools, derived from the intended use population (i.e., patients being treated with the drug in question) should be included in the analytical protocols described below. Spiked samples may be used under some circumstances, but at a minimum, samples from patients taking the target drug, must be included in the precision and recovery studies, as well as method comparison studies. This is important because patient samples reflect the relevant proportions of free and bound drug, and other drugs commonly co-administered to the type of patients who require the target drug; therefore this is essential to demonstrate the robustness of the assay.

Spiked samples can be used to supplement the studies; however caution must be exercised against using spiked samples as the only matrix in the evaluations, because spiked samples may provide a less complete assessment of the performance characteristics.

The effect of freezing/thawing samples, variables in collection and storages, should also be thoroughly investigated.

All analytical protocols should be performed according to the procedures specified by the manufacturer in the testing program. The package insert will subsequently be developed from the studies and will reflect the level of performance that can be achieved when the assay is performed according to the package insert. Therefore, each pre-analytical and analytical step must be specified and included in each of the analytical studies; pre-analytical pretreatment steps, for example, should be included for individual replicates in a precision study and for individual dilutions in a linearity study. All of the manufacturer's recommended quality control and calibration procedures must be followed.

Appropriate specifics concerning protocols should be provided so that results can be interpreted properly and duplicated, if necessary. These specifics are also necessary to aid users in interpreting information in the labeling. For example, when referring to Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) evaluation protocols or guidelines, indicate which specific aspect of the protocols or guidelines were followed.

In studies using spiked samples, information should be provided to document the purity of drugs, or potential interferences, as well as the type of sample that the drug is spiked into.

Serum/plasma is the matrix recommended for most TDM assays, and equivalence must be demonstrated using the commonly employed anticoagulants and collection devices (including gel tubes). In cases where whole blood or other biological matrices are to be analyzed, this should be clearly stated and appropriate correlations (comparison to serum or plasma assays and comparisons among different anticoagulants) must be provided.

#### **b. Specific Performance Characteristics**

The following performance characteristics should be assessed in order to document performance and properly label the device in conformance with 21 CFR 809.10(b)(12).

##### **(1) Precision**

Within-run, and total precision should be characterized according to guidelines provided in “Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline” (1999) CLSI Document EP05-A<sup>2</sup>. That document includes guidelines for experimental design, computations, and format for statement of claims.

For levetiracetam the precision of the assay should be evaluated for at least three concentrations spanning most of the assay range. Typically these concentrations are chosen to represent (a) sub-optimal range or near low end of the reportable range (b) concentrations considered to be within the optimal range and (c) near high end of reportable range or toxic range.

Whenever possible, precision studies should be performed utilizing patient specimens. If patient specimens are not readily available at the time initial precision studies are performed then spiked serum/plasma samples may be used, but as soon as possible during assay development, precision utilizing patient specimens should be evaluated to confirm that other compounds present in the patients’ biological fluids do not affect the TDM assay precision. When interpreting the significance of precision values it is important to recognize that the smallest coefficient of variation is the goal. However, it is equally important to recognize that clinical decision points associated with the interpretation of TDM values are generally reflected by a 20% change. For levetiracetam the between batch precision goal is 10% or better.

The description of the protocol and results should include the items listed below:

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- sample types (e.g., pooled patient samples, spiked serum/plasma)
- point estimates of the concentration
- standard deviations of within-run and total precision
- sites at which precision protocol was run
- number of days, runs, and observations.
- calibration curve stability (if stored)

The factors that were held constant and which were varied during the evaluation (e.g., instrument calibration, reagent lots, and operators) should also be identified. Computational methods, if they differ from those described in CLSI EP05-A, should also be identified.

#### **(2) Recovery**

As a measure of accuracy, the percent recovery of levetiracetam should be characterized. Typically, these studies involve spiking known amounts of pure levetiracetam into samples that are either negative for this drug or from patients taking levetiracetam that contain known drug concentrations. Spiking into samples from patients taking levetiracetam should be included as part of the study. Final concentrations of the spiked samples should span a significant part of the reportable range and include potential medical decision levels.

Recovery should be determined at both sub optimal and toxic concentrations to verify consistent performance across the assay range.

Replicates of each concentration or sample should be evaluated and the number of replicates chosen to ensure that any clinically significant differences observed will be statistically significant. Description of the study protocol should include:

- sample types and concentrations
- statement of how target concentrations were determined
- materials used for spiking
- number of replicates
- definition or method of calculating recovery.

When reporting results, the range of recoveries for each concentration evaluated should be indicated since this approach is more informative than describing mean recoveries at each concentration level.

### **(3) Linearity**

For levetiracetam the linear range of the assay response should be 2-100 mg/L and should be characterized by evaluating samples whose levetiracetam concentrations are known relative to one another. When practical, the linearity of the assay should be characterized using dilutions of patient samples containing elevated levetiracetam concentrations. Spiked serum/plasma may be used when patient samples are not available, (for example at very high drug concentrations). If patient specimens are diluted, they should be diluted with the same biological fluid to maintain the physiological dynamics of the system.

A graphic display or table of the known concentration vs. the observed concentration should be included in the results. The sample concentrations should be evenly distributed across the reportable range of the assay. The appropriate number of replicates and concentration levels depends on the reportable range of the assay.. "Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline" (2003) CLSI Document EP06-A3 describes a protocol for sample preparation, value assignment, appropriate analyte range and concentrations to test, as well as statistical design and analysis methods, and a format for statement of claims.

Some immunoassays may exhibit a "high dose hook effect," in which there is a decrease in response of the assay at high concentrations. Whenever appropriate (e.g., for two-site or sandwich immunoassays), the linearity studies should be extended beyond the reportable range to the highest concentrations that may be encountered in clinical settings in order to evaluate whether the device exhibits a high dose hook effect.

The protocol description should include sample types and preparation, concentrations, number of replicates and statistical methods used. The description of results should include the acceptable maximal differences from linearity or the measured maximal differences (including confidence intervals) from linearity and the range of linearity, as described in CLSI EP06-A. Data from the high-dose hook evaluation, should be included

Information on how to treat samples with levetiracetam concentrations outside the reportable range should be provided. If users are recommended to dilute samples that are above the reportable range, a specific protocol for dilution, including a validation of that protocol, should be provided. It is also necessary to clarify how samples with concentrations outside the range of linearity are reported to the user.

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A validated protocol recommending how to dilute patient specimens without changing the assay's performance is an essential component of every TDM assay.

### **(4) Sensitivity**

The functional sensitivity (lower limit of quantification) of the assay is defined as the lowest levetiracetam concentration for which acceptable assay precision and accuracy are observed, and this should be characterized and reported. For levetiracetam the concentration at which the intra-assay coefficient of variation is not greater than 10% is adequate. The acceptance criteria for sensitivity of a TDM assay should take into account the expected serum/plasma concentrations at the lower limits of therapeutic dose and any possible patient non-compliance issues. The accuracy at the lower limit of quantification (LLOQ) should also be described, based on samples with known drug concentrations.

The description of the sensitivity evaluation should include sample type, definition of the measures of sensitivity and results. Clarify how levetiracetam measurements below the LLOQ are reported to the user. The sensitivity and CV may vary depending upon the sensitivity of the analytical techniques utilized.

### **(5) Specificity for parent compound**

Levetiracetam does not undergo extensive hepatic metabolism in humans and 64 % of a given dose is excreted unchanged, however, a cytosolic amidase hydrolyses approximately 24% of the levetiracetam dose to an inactive metabolite [(2s)-2-(2-oxo-pyrrolidin-1-y) butanoic acid] (Benedetti et al., 2004). In addition two minor metabolites that are probably produced by hepatic metabolism have been identified: hydroxy-2-oxopyrrolidine (2% of dose) and ring opened 2-oxopyrrolodine (1% of dose) (Patsalos, 2003, Coupez R, et al. 2003).

As a measure of assay specificity, cross-reactivity with all of the significant levetiracetam metabolites, if available, should be characterized.

Levetiracetam does not undergo extensive hepatic metabolism in humans, therefore, the issue of cross-reactivity with levetiracetam biotransformation products is not probable since 64 % of a given dose is excreted unchanged and 3% as two minor metabolites whose metabolic pathway has not been established. It is to be noted that blood esterases hydrolyze approximately 24% of the levetiracetam dose to L057, an inactive metabolite [(2s)-2-(2-oxopyrrolidin-1-y) butanoic acid] (Benedetti et al., 2004). Two minor metabolites: hydroxy-2-oxopyrrolidine (2% of dose) and opened 2-oxopyrrolodine (1% of dose) have been identified. (Patsalos, 2003, Coupez R, et al. 2003).

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As a measure of assay specificity, cross-reactivity with levetiracetam metabolites should be characterized. Theoretically neither L057 nor the minor metabolites would be anticipated to interfere with the parent compound assay. Cross reactivity for L057 should be evaluated.

When metabolites of high purity are available, drug free plasma/serum should be spiked with the metabolites to a final concentration consistent with the highest concentration expected in the intended use population. When such metabolites are not available in high purity, the metabolite concentrations that are present in patient specimens should be measured by an appropriate method, and their effect on the proposed assay estimated. Specimens from patients with elevated creatinine concentration should be included, when available, because such patients typically show higher than average metabolite concentrations. Drug metabolite glucuronides concentrations also increase in renal failure. These glucuronides may cross react with parent drug assays. In either case, replicates should be evaluated, and the exact protocol, along with details of the metabolite purity, should be described. It may be helpful to consult with FDA prior to undertaking this alternative type of study.

The description of your evaluation should include description of types of samples used for spiking, number of replicates, concentration of metabolite, computation or definition of cross-reactivity used and percent cross-reactivity for each metabolite.

It is to be noted; highly purified samples of most drug metabolites can often be obtained from the drug manufacturer. However, the drug manufacturer may not have samples of minor metabolites available. If you cannot obtain such samples from the manufacturer, it is suggested you consult with the FDA for further guidance.

#### **(6) Interference**

The effects of potential interferents on assay performance should be characterized. Potential sources of interference that you should test include, but are not limited to, the following:

Endogenous compounds, particularly those listed below; at the suggested concentrations. The object of these studies is to confirm that analyte concentrations of naturally occurring compounds that may occasionally be elevated do not interfere with the TDM assay.

- bilirubin (60 mg/dL)
- triglycerides (1500 mg/dL)

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- cholesterol (500 mg/dL)
- uric acid (20 mg/dL)
- rheumatoid factor (500 IU/ml)
- hematocrit (15-60%)
- albumin (12 g/dL)
- gamma globulin (12 g/dL)
- human anti-mouse antibodies, HAMA
- hemoglobin (20-2000 mg/dl, due to hemolysis)
- blood substitutes

Whenever possible plasma/serum specimens from patients in renal failure who are taking levetiracetam should be retrieved and the reported concentration compared to that of specimens spiked to the same reported concentration in the same drug free serum/plasma. If the results are significantly different between the two specimens, a cross reactivity problem should be suspected. Another approach is to obtain assay results performed on patient specimens, particularly from patients with compromised renal function, and to compare such results with the results of a highly specific assay, such as mass spectrometry. It may be helpful to consult with FDA prior to undertaking this alternative type of study.

Commonly co-administered drugs including, but not limited to those listed below. Drugs commonly co administered to treat a specific disease should also be evaluated for potential TDM assay interferences; the list of specific drugs to be checked is dependent upon the TDM assay under development.

- all available antiepileptic drugs and relevant metabolites (see appendix)
- all available antipsychotic and antidepressant drugs
- common tranquilizers and hypnotics
- commonly prescribed antibiotics
- common over-the-counter drugs

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Anticoagulants or preservatives with which the sample is likely to come into contact, such as EDTA, fluoride/oxalate and heparin, various types of gels contained in serum separator blood collection tubes, and different collection and storage tube materials, such as plastic and glass. When testing these interferents, the concentrations of levetiracetam in the sample should be adjusted to medical decision levels. Typically, interference studies involve adding potential interferents to the sample containing the drug and determining any bias in recovery of levetiracetam, relative to a control sample (to which no interferents have been added). In addition to anticoagulants and endogenous substances it is essential that the various specialized biological fluid collection devices e.g. serum gel separators, filter paper, and ultra filtration membranes also be evaluated.

Recommended guidelines for interference testing are described in detail in “Interference Testing in Clinical Chemistry; Approved Guideline” (2002) CLSI Document EP07-A4. This document includes guidelines for setting decision criteria as well as for protocol designs, statistical methods, evaluating interference using patient specimens and establishing validating and verifying interference claims. The following considerations should be included when interferent testing is being planned:

- For endogenous substances, test at the highest concentration expected based on experience with the intended use population. Interference studies using samples naturally high in the endogenous compound being tested can be informative and this approach should be considered when such samples are available.
- For drugs, test to levels 3 times the highest acute peak concentration reported following therapeutic dosage.
- For specimen additives, test up to levels five times the recommended concentration.

If interference is observed at the concentration levels tested, lower levels should be tested in order to determine the lowest concentration that could cause interference. Replicate samples should be tested in these protocols. The description of the evaluation should include the following items (if description of the protocol refers to CLSI EP07-A, clarify which aspects of the guidelines were followed):

- names and concentrations of interferents tested
- sample type (e.g., spiked whole blood pools, samples naturally high in endogenous compounds)
- concentrations of levetiracetam in the sample

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- number of replicates tested
- definition or method of computing interference.

When reporting results, any observed trends in bias (i.e., negative or positive) across the concentration range of interferents tested must be identified. Include the standard error of the observed recoveries at each concentration or the range of observed recoveries at each concentration evaluated for a potential interferent. This approach is more informative than listing average recoveries alone.

For substances listed as non-interfering, state the criteria on which this is based, e.g., inaccuracies due to these substances are less than 10% at a given levetiracetam concentration. If any compounds are known from the literature or other sources to interfere with the test system, these should be included among the information in the labeling. It may not be necessary to perform additional interference testing with these known interferents.

#### **(7) Specimen collection and handling conditions**

The labeled recommendations for specimen storage and transport must be substantiated, by assessing whether the device can maintain acceptable performance (e.g., precision and accuracy) over the storage times and temperatures (including freeze/thaw cycles), recommended as acceptable by the manufacturer. An appropriate study includes analysis of sample aliquots stored under the conditions of time, temperature, or allowed number of freeze/thaw cycles recommended in the package insert. Storage conditions and freeze- thaw cycles are especially important for research studies where long specimen storage periods are required. Manufacturers' should update the package insert as new information on storage criteria becomes available.

#### **(8) Method comparison**

The new levetiracetam assay must be compared with a reference method, specific for the parent compound. Fully validated chromatographic methods that specifically measure parent drug should be used as the comparator in such a study (Vermej et al. 1994, Isoherranen et al., 2000, Johannessen et al., 2003, Wilson et al. , 2004 ). If the discordance exceeds 10% relative to the reference procedure, the reasons for the discordance should be addressed. It behoves the TDM assay developer to compare their new assay to any analytical technique that may be routinely utilized in clinical chemistry laboratories for drug analysis particularly if simpler LC, GC, immunoassay or other techniques are published or available. Such initial comparisons allow the manufacturer to establish the performance of the TDM assay under various analytical conditions.

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Guidelines provided in the document, “Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline” (1995) CLSI document EP09-A5 concerning experimental guidelines and statement of claims should be followed. Epileptic patient samples with levetiracetam concentrations distributed across the reportable range of the assay, should be evaluated. Banked (retrospective) samples are appropriate for these studies as long as the information on the sample population is available to characterize the specimens. Samples from multiple geographic sites or clinical centers should be included. In general pre-dose blood is the preferred sample for TDM of Levetiracetam, but for the purpose of a method comparison, any time of sampling would be acceptable. Factors such as age and time of blood draw with respect to drug administration (e.g., trough, peak) should be noted. Ideally sufficient clinical information should be collected to enable Concentration Dose Response Ratios to be calculated. Often, however, it is not practical to obtain dosing information and sample draw times for stored laboratory specimens. Storage conditions can affect the quantitation of specimens, particularly if they have been stored for an extended period of time. If there is wide variance between the TDM assay and reference method in stored specimens, it is suggested that the specimens be re-assayed utilizing the reference technique before comparing results with the new TDM assay. Such analysis compensates for storage changes that alter drug concentrations.

Appropriate sample size depends on factors such as precision, interference, range, and other performance characteristics of the test. The number of patients should also be large enough so that inter-individual variation would be observed. A statistical justification to support the study sample size should be provided in the protocol description. It is expected that the sample size target, however supported, will include a minimum of 100 samples distributed fairly evenly over a minimum of 50 *individual patients*. However, it is strongly recommended that a greater number of samples should be collected and incorporated into the beta site testing program.

If multiple measurements from individual patients are included, the results should be summarized using appropriate statistical analyses such as Analysis of Variance, Generalized Estimating Equations, or Bootstrapping, to account for correlation of repeat measurements within patients in the study.

For the results to be properly interpreted all relevant information on the sample population should be provided in the package insert. Information on the sample population should include:

- the number of individual patients represented by the samples
- the number of data points

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- the number of clinical sites
- information regarding the time of last dose

Any specific selection (inclusion or exclusion) criteria for samples should be stated together with an indication of whether samples were collected from patients with specific clinical outcomes, or from centers using atypical or novel drug regimens..

The comparator methods used must be clarified, and references to validation of the procedure included. If samples evaluated in the study include both trough and other times of blood draw relative to drug administration, a separate statistical analysis for these groups should be conducted. When providing the results of the method comparison study, the following information should be included:

- Scatterplots of the new assay versus the reference method. The plots should contain all data points, the estimated regression line and the line of identity. Data points in the plot should represent individual measurements.
- A description of the method used to fit the regression line and results of regression analysis including the slope and intercept with their 95% confidence limits, the standard error of the estimate (calculated in the y direction), and correlation coefficient should be included. In cases where parameters are not consistent throughout the reportable range, estimates of more than a single range may be appropriate. If the comparator, as well as the new assay is subject to measurement error, a regression method such as the Deming method may be appropriate, rather than Least Squares.
- To illustrate the degree of inter-individual variations, include graphs of difference in measurements (i.e., new device minus reference HPLC method) versus the reference HPLC method. Appropriate representations include a bias plot of difference in measurements ( $y - x$ ) versus the reference method ( $x$ ), as recommended in CLSI Document EP09-A, or versus the mean of  $y$  and  $x$ , as recommended by Bland and Altman (Bland, 1995).

The points above apply to any reference method. The more information that is available comparing the reference method to the new levetiracetam TDM assay, the easier it is for the reviewer to recognize the validity of the new assay. Providing the information initially in sufficient detail and clarity speeds the review process and we emphasize the importance of clear and frequent communication with the FDA Diagnostics Division during the development of any new TDM assay.

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A variety of clinical circumstances can influence the interpretation of any drug concentration. The purpose of a TDM assay is to provide a tool that can be utilized in conjunction with other clinical parameters and diagnostic procedures to enhance any clinician's ability to provide optimal patient care through the use of Therapeutic Drug Management at any time. More efficient and better the patient's care will result from more readily available TDM assays for the newer AEDs.

#### **(9) Studies at external sites**

Performance of the levetiracetam assay should be evaluated in at least 3 external laboratory sites in addition to that of the manufacturer's site. This may be included as part of the method comparison study described above. Data from individual sites should initially be analyzed separately to evaluate any inter-site variation. Method comparison results from the individual sites can be pooled in the package insert, if it is demonstrated that there are no significant differences in results among sites.

#### **(10) Calibrators**

Provide the following information about the calibrators in the assay kit in the summary report:

- Protocol and acceptance criteria for real-time or accelerated stability studies for opened and unopened calibrators.
- Protocol and acceptance criteria for value assignment and validation, including any specific instrument applications or statistical analyses used.
- Identification of traceability to a domestic or international standard reference material.
- Protocol and acceptance criteria for the transfer of performance of a primary calibrator to a secondary calibrator.

For information about calibrators marketed separately as class II devices under 862.1150, see the guidance "Abbreviated 510k Submissions for *In Vitro* Diagnostic Calibrators."

## **8. References**

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## Appendix: Interference Studies

Interference evaluation/validation	Recommendation
Antiepileptic drugs	<ul style="list-style-type: none"> <li>• Acetazolamide</li> <li>• Carbamazepine</li> <li>• Carbamazepine-10,11-Epoxyde</li> <li>• Clobazam</li> <li>• Clonazepam</li> <li>• Desmethyloclobazam</li> <li>• Desmethylnmethsuximide</li> <li>• Diazepam</li> <li>• Ethosuximide</li> <li>• Ethotoin</li> <li>• 5-ethyl-5-phenylhydantoin</li> <li>• Felbamate</li> <li>• Fosphenytoin</li> <li>• Lamotrigine</li> <li>• Mephenytoin</li> <li>• Methsuximide</li> <li>• Nitrazepam</li> <li>• Oxcarbazepine</li> <li>• Phenytoin</li> <li>• Pregabalin</li> <li>• 10-hydroxycarbamazepine</li> <li>• 2-phenyl-2-ethyl-malonamide (PEMA)</li> <li>• Phenobarbital</li> <li>• p-hydroxyphenobarbital</li> <li>• p-hydroxyphenylhydantoin glucuronide</li> <li>• Primidone</li> <li>• Stiripentol</li> <li>• Tiagabine</li> <li>• Topiramate</li> <li>• Valproic acid</li> <li>• Vigabatrin</li> <li>• 3-keto-Valproic acid</li> <li>• Zonisamide</li> </ul>

*Contains Nonbinding Recommendations*