Recommendations for Premarket Notifications for Lamotrigine and Zonisamide Assays

Guidance for Industry and Food and Drug Administration Staff

Document issued on: February 9, 2016

The draft of this document was issued on August 6, 2010

For questions about this document, contact Division of Chemistry and Toxicology Devices at 301-796-6933.



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Food and Drug Administration
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GRZAN

Preface

Public Comment

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Recommendations for Premarket Notifications for Lamotrigine and Zonisamide Assays

Guidance for Industry and Food and Drug Administration Staff

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. Introduction

This guidance document contains recommendations to manufacturers and FDA reviewers concerning information to include in premarket notifications for assays for the anti-seizure drugs, lamotrigine and zonisamide. These assays are intended to quantitatively measure concentrations of the respective drugs in serum or plasma as an aid in the management of patients treated with lamotrigine or zonisamide. The recommendations are based on current review practices as well as information regarding zonisamide and lamotrigine that were submitted to FDA by the Therapeutic Drug Monitoring Roundtable[1]. Some of the general concepts in this guidance may also be helpful in preparing 510(k) submissions for other therapeutic drug assays previously cleared by FDA, and classified within 21 CFR 862 subpart D.[2]

[1] FDA docket: FDA-2004-D-0421.

[2] Guidances specifically for sirolimus, cyclosporine and tacrolimus assays are available at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm077267.htm, and http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm092778.htm. These guidances address additional issues including sample pre-treatment for whole blood samples and cross-reactivities for immunosuppressant assays.

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.

II. Background - Lamotrigine and Zonisamide Assays

Assays that have been cleared for lamotrigine or zonisamide are indicated as an aid in management of individuals treated with these anti-seizure drugs. For example, the assays might be useful for purposes such as helping to diagnose or monitor drug overdose; managing individual patients who might experience one or more drug-drug interactions (DDIs) that can alter the pharmacokinetics of these drugs resulting in changes in drug levels; and providing a relative reference value for an individual after achieving a steady concentration at a specific daily dose judged to be either effective or ineffective[3]. Healthcare providers should exercise caution regarding individual patient management based upon observed reference ranges for zonisamide or lamotrigine published in the literature to date. There is limited documented correlation between serum concentrations and therapeutic effects or toxicity, and overlap has been observed in serum concentrations between responders and non-responders as well as between levels associated with seizure control and adverse reaction. There are few randomized studies investigating the usefulness of routine monitoring of anti-seizure drugs, and there is limited evidence regarding the usefulness of routine monitoring. Nonetheless, therapeutic drug monitoring for these drugs may be helpful in certain situations, such as those noted above.

Healthcare providers should interpret assay results in combination with careful observation of clinical response and with an understanding of the limitations of any specified reference ranges. Sponsors marketing these assays should include discussion of these types of issues in the labeling for zonisamide or lamotrigine assays. (See Section VI-Labeling.)

Assays for lamotrigine or zonisamide are expected to adequately measure the concentration of the specified drug with defined performance characteristics. A false result (falsely high or falsely low) or misinterpretation of results may contribute to inappropriate patient dosing, which in turn could lead to either toxicity or lack of seizure control.

III. Scope

[3] Patsalos et al, "Antiepileptic drugs--best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies." *Epilepsia* 49 (2008)1239-76. (See references within.)

Lamotrigine and zonisamide assays are regulated under 21 CFR 862.3350, Diphenylhydantoin test system, Class II. The product codes for these devices are:
ORH for lamotrigine assays, and
NWM for zonisamide assays.

This guidance addresses lamotrigine or zonisamide assays that employ immunoassay technologies; are for use in central clinical laboratories; and are for use with serum or plasma samples. For assays that do not have these features, different studies or additional studies may be appropriate. [4]

IV. Device Description

Sponsors seeking clearance should include descriptions of the following device features in their premarket notification submission [510(k)]:

- All reagents (analytical and pre-analytical), including controls and calibrators marketed with the assay.
- Assay technology, including specific reactions, and the method of detection.
- Instrumentation, such as specific analyzers, needed to run the assay.

If your device includes software specific to your assay, see "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices," http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm, regarding software information that you should include in your 510(k).

V. Performance Characteristics

A. General Study Recommendations

You should perform the evaluations described in this guidance in a manner consistent with the specific procedures that you recommend to users in the labeling to reflect performance to be expected by the user. For example, samples should be stored as you plan to recommend in the package insert. Calibration processes and quality control measures should generally reflect those that laboratories would use based on recommendations or instructions in your device labeling.

So that FDA can correctly interpret test results during review of your 510(k), you should provide clear descriptions of the study designs. In addition, when referring to Clinical and Laboratory Standards Institute (CLSI) guidelines, we recommend you indicate which specific aspects of the guidelines you followed and which you modified (if any). Similarly, we recommend that you

[4] We recommend that you contact OIR for feedback regarding possible regulatory routes and validation strategies if you are considering submitting premarket submissions for new types of lamotrigine or zonisamide assays.

provide a summary of this type of information in the device labeling so device users can correctly interpret results.

When describing studies using spiked or other prepared samples, you should summarize how the samples were prepared, including materials used for spiking or diluting. For studies using patient samples, you should clarify the source of the samples and any sample selection criteria applied.

For analytical evaluations involving estimates of assay bias (e.g., recovery, interference, linearity, matrix evaluations) we recommend you statistically determine the appropriate number of replicates to include at each concentration, based on considerations of factors such as assay repeatability and your acceptance criteria for assay bias.

In your 510(k) you should specify the instrument(s) on which all performance evaluations were conducted. FDA will categorize the CLIA (Clinical Laboratory Improvement Amendment) complexity of your assay in the CLIA database, specifying the instrument(s) on which all evaluations were performed.

B. Precision

You should characterize precision of your test system. We recommend that you follow the guideline "Evaluation of Precision Performance of Quantitative Measurement Methods;" CLSI Document EP5-A2, regarding statistical approach and statement of claims. We recommend you perform this evaluation over a 20 day period to evaluate within-run, between-run, between-day, and any other conditions relevant to your assay.

We recommend that you evaluate precision for three or more drug concentrations. The concentrations should be chosen to span your claimed assay reportable range (i.e., to include a high and a low sample close to assay limits) and to include the potential medical decision point(s). You should perform the precision evaluations using samples in the intended matrix or matrices. We recommend that you include stable samples or pools from patients treated with zonisamide or lamotrigine within the evaluation. Diluted or spiked serum and plasma samples are appropriate to supplement patient samples, especially when a methodology is well-established and no issues regarding pre-analytical steps or new matrices are raised. We recommend that you also run control materials within this evaluation. When feasible, we recommend you also include evaluation of multiple reagent lots measuring the same sample(s).

We recommend that you include the following in the description of your precision evaluation in the 510(k):

- Description of samples tested, including preparation or source of samples, matrix, and target concentrations.
- Site or sites at which the precision protocol was run (e.g., manufacturer's site).
- Number of days, runs, and observations.

- Identification of other factors that were held constant and those that were varied during the evaluation (e.g., number of instruments, calibration cycles, reagent lots, and operators).

- Description of computational methods used (including equations, if they were different or modified from those described in CLSI EP5-A2). All results should be included in the calculations. If you deleted any outliers from your calculations, you should state this and include your justification, for review.

- Results, including mean, coefficients of variation (CV), and standard deviations (SD).

C. Trueness/Bias

You should evaluate bias of your zonisamide or lamotrigine assay in the intended matrix. Typically, this involves recovery studies in which known amounts of a reference material[5] (whose concentration is known independently of your assay) are spiked into drug-free clinical samples or pools and the samples are measured using the new assay. Sample matrices used in this evaluation should mimic patient samples as closely as possible. Therefore we recommend spiking into drug-free patient serum or plasma. (We do not recommend using only calibrator or control material in this evaluation, or spiking the drug only into zero calibrator or control materials.) We recommend that you evaluate recovery for replicate samples at a minimum of three concentrations spanning the reportable range. If additional levels are tested across the entire assay range, then it may be possible to perform your linearity and recovery evaluations together as one study; see Section V.D-Linearity, below.

In cases in which the 510(k) submission includes calibrators, we recommend spiking the same reference materials similarly into both calibrator and serum samples in order to demonstrate whether there is any matrix effect for the calibrator material relative to clinical samples.

Some immunoassays may exhibit a "high dose hook effect" in which there is a decrease in response of the assay at high concentrations. You should extend the studies beyond the reportable range to the highest concentrations that might possibly be encountered in clinical settings in order to evaluate whether the device exhibits a high dose hook effect.

We recommend that you include the following in the description of your recovery evaluation:

- Matrix, and source or preparation of the samples and materials used for spiking.

- Target/expected concentrations of the samples and the methods by which these were determined, independently of the new assay.

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[5] In this context, the term “reference material” refers to material whose properties are sufficiently homogeneous, stable and well established to be used for the calibration of a measuring system, the assessment of a measurement procedure, or for assigning values to materials.

- Number of replicates measured, and calculations or statistical methods used.
- Results of the new assay measurements relative to target (expected) results. Results should be represented separately for each concentration level tested.

D. Linearity

You should demonstrate linearity of your assay by evaluating samples with concentrations distributed across the entire reportable range. Patient samples or pools containing elevated drug concentrations diluted with drug-free patient samples more closely resemble native patient samples, and we recommend this approach if there is reason to suspect that linearity with spiked samples might not sufficiently mimic performance patient samples. If this is not a concern and you are planning to combine your recovery and linearity evaluations into one study, spiked samples in the appropriate matrix may be preferable. You should evaluate sufficient concentration levels (e.g. 7 to 9 levels) across your entire claimed measuring range. We recommend you prepare samples to be evenly distributed across the claimed range.[6] You should include a non-zero sample at (or below) your claimed assay lower limit and a sample at or above the assay claimed upper limit to demonstrate that linearity holds across the entire assay range.

We recommend that you include the following in the description of your linearity evaluation:

- Sample matrix, and method of preparation or origin.
- Number of samples and replicates evaluated.
- Calculations and statistical methods used.
- Results, including the known sample concentrations, and the concentrations measured with your assay for each sample. You should also describe how you determined the expected or target concentration (e.g., based on standardized materials; based on the high sample measured with your assay and the dilution factor, etc.)
- Results of linear regression analysis.

If you recommend to users that samples outside the range can be diluted, you should describe your validation of the procedure. Samples in the intended matrix spiked to high concentrations

[6] Preparation of sample dilutions by consecutive 1:1 dilutions of a high sample may not be sufficiently well-distributed throughout the assay range, especially near the lower end of the assay range; therefore we do not recommend this approach. In addition, we do not recommend the approach of preparing multiple dilution series', each spanning only part of the assay range because this may not allow for demonstration of continuous linearity across the entire claimed assay range.

can be used for this purpose. The (high) target/expected concentration should be determined independently of the new assay.

E. Lower Limits of the Assay

You should evaluate assay performance at the claimed lower limits of your assay, including precision and bias relative to reference materials. You may be able to accomplish this by including samples at the claimed lower limits within your precision and recovery studies. Alternatively, we recommend you refer to the CLSI document, "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; EP17-A2," regarding definitions and the minimal experimental design regarding lots, days, dilutions, replicates, etc. To estimate bias of samples in an evaluation for the limit of quantitation, you should evaluate recovery of reference materials by your assay (similar to recovery studies described in Section C, above).

In your 510(k), you should describe your study design for determining the lower limits of the assay, including the types of information listed above in the bullets in Section V.B-Precision, and Section V.C-Trueness/Bias, above. You should describe results for both precision and accuracy at this lower limit.

F. Endogenous and Exogenous Interference

You should characterize the effects of potential interferents on assay performance. We recommend that you follow the guideline "Interference Testing in Clinical Chemistry; Approved Guideline," CLSI Document EP7-A2, regarding types of interferents to consider for testing and study designs, including specified test concentrations (of analyte and interferent) and statistical considerations.[7] Studies should be designed so that both positive and negative bias may be detected. We recommend you determine the appropriate number of replicates so that testing is performed with sufficient power to detect any clinically significant interference and with a sufficient confidence level to recognize when there is no significant interference.

For all interference testing, we recommend you describe the following in your 510(k):

- Potential interferents and concentrations at which they were tested.
- The concentrations of lamotrigine or zonisamide in the samples and how samples were prepared (e.g., by spiking drug-free serum sample pools).
- Definitions and/or calculations for computing interference (and cross-reactivity where applicable).
- Results of test and control sample measurements and calculated interference (or cross-reactivity as relevant).

[7] For testing collection tubes/anticoagulants, see Section V.G-Matrix Comparison.

Common examples of compounds to test are listed below. In addition, manufacturers should carefully consider other substances that may potentially interfere based on knowledge of the chemistry of the procedure, the indications for use, and structure of the analyte. Structural databases may be helpful for consideration of the latter.

Examples of endogenous compounds:

- Bilirubin
- Triglycerides
- Cholesterol
- Uric acid
- Rheumatoid factor
- Albumin
- Gamma globulin
- Human anti-mouse antibodies, HAMA
- Hemoglobin

Examples of types of commonly co-administered drugs:

- All antiepileptic drugs (and relevant metabolites when available).
- Available antipsychotic and antidepressant drugs.
- Common tranquilizers and hypnotics.
- Commonly prescribed antibiotics.
- Common over-the-counter drugs.

In accordance with CLSI guideline EP7-A2, we recommend that you test up to levels at least three times that of the highest acute peak concentration reported following therapeutic dosage.

Specificity for parent compound

You should characterize cross-reactivity with drug metabolites. For lamotrigine assays, primary known metabolites you should test include N-2 glucuronide, N-5 glucuronide, N-2 methyl metabolite, and N-2 oxide. For zonisamide assays, metabolites to test include N-acetyl zonisamide (NAZ) and 2-sulfamoylacetyl phenol (SMAP). The highest metabolite concentration should challenge the assay; the concentration should be at least three times higher than the peak

metabolite concentrations found in patient samples, including samples from patients with conditions that may cause metabolite accumulation. We recommend that you include samples negative for parent drug, as well as samples containing parent drug concentrations near the potential medical decision point(s).

G. Matrix Comparison

You should evaluate performance of your assay with all serum and plasma matrices that you recommend for use with your assay, including anticoagulants or types of collection tubes specified in your labeling. As noted in Section V.I-Method Comparison, below, you should identify the matrix for samples used in the method comparison study. If you recommend use of your assay with additional matrices, anticoagulants or collection tubes, which were not fully evaluated in your method comparison study, you should submit matrix comparison evaluations. In any case, you should check that the collection tubes you recommend to users are FDA cleared for TDM use.

You should compare results of patient samples in the test matrix to results of patient samples with the same drug concentration in the control matrix. (The control matrix in this case is the one that is fully validated in the method comparison study.) We recommend that you compare a minimum of 15-20 samples at multiple concentrations spanning the assay range. In many cases, it is acceptable to test drug-free patient samples or pools that were collected in the appropriate collection tube type and then spiked with zonisamide or lamotrigine. In designing the study you should consider the timing of spiking (e.g. before pre-analytical processing) to best mimic patient samples. The approach of spiking samples is appropriate in cases in which (1) the predicate assay (with the same technology and intended use) has been cleared for use with the matrix or tube type in question, and (2) there are no known issues regarding performance for samples in that matrix.

If you choose to include claims for use of your assay with particular matrices or tube types that were not previously cleared for zonisamide or lamotrigine immunoassays, you should include a more comprehensive study rather than relying solely on spiked samples, so that the evaluation incorporates any effect of pre-analytical steps. For example, samples from patients treated with zonisamide or lamotrigine may be drawn into “control” tubes and “new matrix” tubes. Studies of this type described in the literature may be used as supporting information if the study is well-designed and the assay used for testing is similar to your assay. You should describe the study design including sample types and preparation, calculations or statistical methods, and specific collection tubes used. Results should include recovery relative to the control matrix for each concentration level tested, as well as results of linear regression analysis. You should also clarify how you determined that assay precision is not affected by sample matrix.

Because tube components may sometimes affect assay results, we encourage manufacturers to clarify in the package insert the specific types of tubes used in the method comparison and matrix comparison studies.

H. Specimen Storage Recommendations

You should describe in your 510(k) the study design and results supporting the conditions for specimen storage and transport that you recommend in your package insert. The study should include assessment of whether the assay maintains acceptable precision and accuracy when patient specimens are stored at the limits of the storage times and temperatures (including freeze/thaw cycles if applicable). We recommend you present results in terms of concentration measured and percent recovery for the various time points and explain any trends observed.

I. Method Comparison

You should compare patient sample measurements using your assay to those measured with a previously cleared assay. It is important to use unaltered patient samples from the intended use population because these reflect the relevant proportions of free and bound drug, metabolites, and other drugs commonly co-administered to patients treated with the target drug. Banked (retrospective) samples are typically appropriate for these studies. We recommend you provide general information concerning known overall sample characteristics, selection methods, and criteria (see bullets below). Samples should be distributed throughout the claimed analytical measurement range of your assay. We recommend you ensure that the storage and handling conditions at which samples are held during the time that elapses between measurements with each of the separate assays does not contribute to measurement bias. Ideally samples should be measured by the two methods within a short time span. We recommend you follow the CLSI guideline EP-9A2, "Method Comparison and Bias Estimation Using Patient Samples" regarding your study design.

Appropriate sample size for this evaluation may depend on factors such as assay precision, interference, and assay range. The number of patients should also be large enough to observe any effects from inter-individual variations. Consistent with CLSI document EP9-A2, we recommend testing a minimum of 100 patient samples across the measuring range of the assay. We recommend you provide information supporting the study sample size in the 510(k).

If you choose to include multiple specimens from some of the individual patients you should summarize your results with appropriate statistical analyses to account for correlation of repeat measurements within patients in the study.[8]

We recommend that you include the following when describing the method comparison study design:

- Selection methods used to obtain the samples. You should describe the origin of the sample population as a whole and any specific criteria (inclusion or exclusion) you

[8] We do not recommend selecting multiple samples from a small number of patients in order to achieve the adequate sample size since this may not evaluate the effect of inter-individual variability.

applied in obtaining samples. We also recommend you describe summary information of the sample population as a whole, such as age range (e.g., adults) and time of blood draw with respect to drug administration (e.g., pre-dose), as well as sample matrix. If samples were from clinical studies or sites using specific or atypical drug regimens or sampling times, you should indicate this.

- The number of data points and number of samples measured. If multiple specimens were selected from individual patients, we recommend you indicate the number of patients represented by the sample population.
- The sites at which the study was conducted (e.g., manufacturer's site).
- The comparator method used and the type of site at which that method was performed (e.g., manufacturer's site, clinical laboratory).
- The time over which samples were measured using the new test method and the number of calibration cycles performed.
- Statistical methods used to analyze the data.
- All data should be included in analyses. If any data were omitted from any of your analyses, this should be described for review and justified.

You should include analyses of data based on single measurements of the new device in the 510(k) and the labeling since this may best depict variability of results under conditions that will be used for reporting test results (i.e., results based on one measurement). If samples were measured in duplicate on the new device, you can address this by performing an analysis using the first replicate of each set of measurements taken with the new device. If you collected duplicate measurements with each system (as recommended in CLSI EP9-A2), we recommend you also include these results since this may provide a better estimate of bias relative to the predicate device.

We recommend you include the following when presenting results of your method comparison evaluation in the 510(k):

- Range of data (the highest and the lowest value by the comparator).
- The method used to fit the linear regression line (ordinary least squares, weighted regression, Deming, Orthogonal regression) and a scatter plot of new assay versus the comparator method illustrating the line of best fit along with the line of identity.
- Results of regression analysis including the slope and intercept with 95% confidence intervals, the standard error of the estimate (calculated in the y direction – rather than orthogonally), and correlation coefficient should be included. If the comparator method is subject to measurement error, then Deming regression may be appropriate in stead of ordinary least squares regression. We recommend you present method comparison results based on single measurements of each sample with your new assay.
- A bias/difference plot of difference in measurements (i.e., new method minus the comparator method on the y-axis) versus the drug concentrations (on the x-axis). We

recommend you also present related summary results including the mean bias and standard deviation.

- A line listing of all measurements from both methods, for reference.

J. Calibrators and Control Materials

This section summarizes information we recommend you include in your 510(k) to describe calibrator and control materials you plan to market for zonisamide and lamotrigine assays.[9]

1. General Description

You should describe the components of the calibrators and control materials, including analyte concentrations, matrix, and preservatives, as well as any specific components that may call for special handling (e.g., preservatives such as sodium azide.) You should also describe if any matrix effects are observed, as compared to patient specimens. The latter can be incorporated into the recovery studies (see Section V.C-Trueness/Bias, above) by spiking drug-free patient samples and zero-level calibrator samples with equal amounts of the drug in question.

When blood products are used as components, each donation of human blood or blood component intended for use in preparing a product must be tested for communicable disease agents as specified in 21 CFR 610.40.

2. Traceability and Value assignment

You should describe the material to which your calibrator is traceable and aligned. We recommend that calibrators should be traceable to the best reference material available (for example, USP material) when it is available. You should include sufficient information to clearly characterize the accuracy of the assigned value of the marketed calibrator (relative to the material to which it is traceable). [10]

The following types of information may be helpful to present for this purpose:

- A summary of your process for value assignment and verification.

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[9] Additional FDA guidance documents generally addressing calibrators and controls include "Abbreviated 510(k) Submissions for In Vitro Diagnostic Calibrators; Final Guidance for Industry" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm092800.htm) and "Assayed and Unassayed Quality Control Material" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079179.htm) .

[10] The term accuracy in this context is related to both trueness and precision of measurement.

- The uncertainty of measurement of the product calibrator assigned values.[11]
- Evaluation of calibrator recovery relative to the higher level standard to which the calibrator is traceable.

You should also describe how you assign values for control materials.

3. Stability

You should describe your design and results for stability studies under closed and opened conditions for calibrator and control materials. "Closed" conditions refer to the shelf-life conditions you recommend to end-users prior to opening the product. "Opened" refers to the time after first opening the vial, which may include on-board or other recommended storage and use conditions. For long term studies for which results are not yet available, acceptance criteria may be provided in place of results.

We recommend that you present results or criteria for stability in terms of analyte concentration recovery since this is most understandable for FDA review. One approach is to measure a panel of samples, or control materials that are kept under known stable conditions, while calibrating the system at the various test time points, using the calibrators under evaluation. Another approach may be to compare measurements of calibrators under test conditions to measurements of the same calibrator material kept under known stable conditions at the various time points. We recommend that the other assay reagents should be kept under known stable conditions during calibrator stability studies. You should include testing of samples at multiple concentrations across the assay range and at multiple time points.

If you present results or acceptance criteria for calibrator stability in terms of control material recovery, please note that acceptable criteria for control ranges are often much wider than those that would be acceptable for calibrator stability. The results or acceptance criteria you specify for calibrator stability should ensure continued accuracy commensurate with performance claimed in the package insert.

We recommend that you include descriptions of the following for both opened and closed calibrator or control material stability testing:

- Test samples and control/reference samples measured.
- Materials used for calibration.
- Time intervals of testing.

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[11] See International Standards Organization, 2003 "*Metrological Traceability of Values Assigned to Calibrator and Control Materials,*" ISO Document 17511.

- Temperature (and any other relevant environmental conditions).
- Results (and/or acceptance criteria when long-term results are not available). If you conclude that no change is detected, you should also clarify in the 510(k) the difference (in terms of recovery) that your study was designed to detect.
- Expiration dating currently specified in the product label.

K. Studies at External Sites

For fully automated immunoassays that use standard technologies, studies using the new system performed at the manufacturer's site are typically sufficient. For assays that may be complex to perform (e.g. complex, non-automated analytical or pre-analytical procedures), we recommend that you evaluate performance at two external clinical laboratory sites in addition to that of the manufacturer. This study could be included as part of the method comparison study described above and should also include precision information from each site. Data from individual sites should initially be analyzed separately to evaluate any inter-site variation. The data summary may be pooled in the labeling if you demonstrate that there are no significant differences in results among sites.

VI. Labeling

Labeling for in vitro diagnostic devices must satisfy the requirements found in 21 CFR 809.10. Specific recommendations for zonisamide and lamotrigine assays, regarding some of these requirements, are described below.

Specimen collection and storage:

You must include instructions for specimen collection and preparation for analysis. 21 CFR 809.10(b)(7). We recommend that you include the following:

- Discussion of any limitations or instructions related to the specimen type, including the appropriate matrix, anticoagulants, preservatives, or collection tubes.
- Instructions concerning preserving integrity of the specimen, such as required temperatures or materials for collection, transport, storage (short and long term), and assay procedural steps. Storage conditions that you recommend to the user should be based on the conditions you have validated for your test system. You should clearly define acceptance criteria that you apply (e.g., x% recovery) in determining the recommended storage conditions. Information on storage conditions based on literature can be cited if it is applicable to your test system.
- Discussion of the importance of consistency and accurate recording of time of blood draw with respect to the last dose if this is relevant for interpretation of results.

Assay procedure

You must provide a step by step outline of recommended procedures from reception of the specimen to obtaining results. 21 CFR 809.10(b)(8). We recommend that you include the following as part of the procedure:

- A validated procedure for dilution, if you instruct users to dilute samples with values above the highest calibrator.
- Steps, based on procedures you have validated for your test system, that users can take to minimize the effect of carryover or other causes of bias or imprecision.
- Any special handling instructions for reagents.

Quality control and calibration procedures

The step by step outline of the procedure must include details of kinds of quality control procedures and materials required, as well as details of calibration. See 21 CFR 809.10(b)(8)(v) and 21 CFR 809.10(b)(8)(vi). You should advise users of the specifics of calibration and quality control procedures necessary to ensure your stated performance claims. We recommend that you include a statement in your package insert that laboratories should follow federal, state, and laboratory guidelines for quality control. When you specify ranges for your quality control material, you should clarify how these were determined and indicate that laboratories should determine their own acceptable ranges based on their own acceptance criteria. We also recommend that you identify the material to which your calibrator is traceable. If you do not market these materials for your assay, you should include recommendations for appropriate quality control materials.

If your 510(k) includes calibrator or control materials to be sold separately from your assay, you should include the separate labeling for these components.

Warnings and Precautions

You must include a statement of warnings or precautions for users as established in the regulations contained in 16 CFR part 1500 and any other warnings appropriate to the hazard presented by the product. See 21 CFR 809.10(a)(4).

You should specify any reagents that require special handling by the user (e.g., infectious reagents, caustic reagents). You should describe the biological source of reagents (e.g., enzymes, antibodies).

When blood products are used as components, you should include a statement in your package insert that the animal/human source components containing any blood-product derived material has been tested by FDA approved (or equivalently recognized) assays and found to be negative for HIV type 1 (HIV-1) and type 2 (HIV-2), as well as for hepatitis B surface antigen and antibody to hepatitis C virus (HCV), and found to be negative (not repeatedly reactive). You should also clarify that, because no testing can offer complete assurance that these or other infectious agents are absent, this material should be handled using good laboratory practices to avoid skin contact and ingestion.

Limitations

You must include a statement of limitations of the procedure. See 21 CFR 809.10(b)(10). You should identify any factors known to affect results and describe the effect on results (e.g., highly lipemic samples may cause falsely low results).

It may be appropriate in some cases to recommend that it is generally good practice to use the same assay and matrix consistently for individual patients.

Reference ranges

You must indicate how reference ranges were established, including identifying the population on which it was established. See 21 CFR 809.10(b)(11). At this time, we recommend that you state that therapeutic ranges are not well-established for zonisamide and lamotrigine and include an explanation such as the following:

> Considerable overlap has been observed between serum levels in responders and non-responders as well as between levels associated with seizures and adverse effects, and there is not a clear relationship between lamotrigine serum concentration and clinical response across patients. Optimal ranges may vary among patients. Caution should always be exercised regarding individual patient management based upon observed reference ranges; assay results should always be interpreted in combination with careful observation of clinical response.

We recommend that, rather than citing a reference range based on a single study, you cite and summarize balanced and representative studies in the literature concerning serum concentration ranges that have been observed in those studies to be associated with seizure control or with adverse effects. When summarizing studies, we recommend that you include relevant points, such as the sampling time (relative to time of drug dose), the assay methodology, and the nature of the population tested in that study (e.g., newly diagnosed). If studies have shown lack of correlation, we recommend you discuss this as well. We ask that you include in your 510(k) copies of references you use to support statements concerning ranges cited in your labeling. You should clarify that reference ranges should only imply a lower limit below which therapeutic response is relatively unlikely and an upper limit above which toxicity is relatively likely to occur in the specific populations studied. Clinicians using proposed ranges should be aware that because of individual variation some patients may achieve therapeutic benefit with serum drug concentrations outside the range or may experience toxicity with levels below the lower limit of the reference range.

We recommend that you refer to the drug package insert regarding pertinent pharmacokinetic information including: time to steady state, appropriate sample draw times (with respect to dose), and clinical conditions or co-administered drugs that may affect pharmacokinetics. You should discuss, or refer to the drug label, regarding information such as time to steady-state, or recommended sampling times.

Performance Characteristics

You must include specific performance characteristics of the assay. See 21 CFR 809.10(b)(12). We recommend that you summarize the study design and results for each performance characteristic discussed in Section V–Performance Characteristics. You should specify the instrument(s) used for the performance testing. Your representation of study designs and results in the package insert should include information relevant to aid the user in understanding test performance. The reportable range claimed in the package insert should be based on the assay range for which you have validated precision, linearity, and method comparison. The CLSI guidelines, referred to in Section V–Performance Characteristics, include instructions and examples for statements of claims in the labeling. We recommend you follow these formats in your labeling. You should include data plots of the method comparison study. If your assay is for use with more than one instrument, you should specify in the package insert the instrument used to evaluate the performance characteristics represented in the package insert.