Class II Special Controls Guideline: John Cunningham Virus Serological Reagents

Guideline for Industry and Food and Drug Administration Staff

Document issued on: January 23, 2014

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of *In Vitro* Diagnostics and Radiological Health
Division of Microbiology Devices

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Preface

Public Comment

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Table of Contents

1. Introduction 1
2. John Cunningham Virus - Background 1
3. Premarket Notifications - Background 2
4. Scope 2
5. Risks to Health 3
6. Device Description 4
6.1 Device Components 4
6.2 Ancillary Reagents 5
6.3 Test Methodology 6
7. Performance Characteristics 8
7.1 General Study Recommendations 8
7.2 Analytical Studies 8
7.2.1 Assay Cut-off 8
7.2.2 Precision Testing 8
7.2.3 Interference 10
7.2.4 Cross reactivity 10
7.2.5 Matrix Comparison Studies 11
7.2.6 High Dose Hook Effect 11
7.2.7 Reagent Stability 12
7.2.8 Specimen Collection and Handling Conditions 12
7.2.9 Other Analytical Studies 12
7.3 Clinical Performance 12
7.3.1 Expected Values 12
7.3.2 Clinical Studies 13
7.4 Detectability and Comparative Performance 15
8. Labeling 16
8.1 Instructions for Use 16
8.2 Quality Control 16
8.3 Warnings and Precautions 16
9. References: 18

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1. Introduction

This document was developed to support the classification of the John Cunningham Virus (JCV) serological reagents for the risk stratification for progressive multifocal leukoencephalopathy (PML) development into class II (special controls). JCV serological reagents are devices that consist of antigens and antisera used in serological tests to identify antibodies to JCV in serum and plasma. The identification aids in the risk stratification for the development of PML in multiple sclerosis and Crohn's disease patients undergoing natalizumab therapy. These devices are for adjunctive use, in the context of other clinical factors for the development of progressive multifocal leukoencephalopathy.

This document does not address JCV serological reagents for any indication other than the risk stratification for the development of PML or JCV nucleic acid amplification reagents. Please contact the Division of Microbiology Devices in the Office of *In Vitro* Diagnostics and Radiological Health for further information on these assay submissions.

This guideline identifies measures that FDA believes will mitigate the risks to health associated with these devices and provide a reasonable assurance of safety and effectiveness. Firms submitting a 510(k) for a JCV serological reagents will need either to (1) comply with the particular mitigation measures set forth in the special controls guideline or (2) use alternative mitigation measures, but demonstrate to the Agency's satisfaction that those alternative measures identified by the firm will provide at least an equivalent assurance of safety and effectiveness.

2. John Cunningham Virus - Background

JCV is a small, non-enveloped double-stranded DNA human polyomavirus. It has a very restricted host range and, at present, is only known to infect and cause disease in humans. It is very common in the human population as a majority of adults are seropositive for

antibodies to JCV [Ref. 1, 2, 3, 4]. Infections with JCV are usually clinically asymptomatic and self-limiting in the immunocompetent population. JCV may lead to the development of PML, a rare demyelinating disease of the human brain caused by lytic infection of the oligodendrocytes with the virus. JCV causes PML only in severely immunosuppressed individuals through complex, poorly understood interactions between various host and viral factors [Ref. 5, 6, 7]). The development of PML is very rare even among most immunosupressed individuals. Immunomodulatory biologic therapies such as natalizumab have been associated with increased cases of PML. Since JCV infection is a necessary precursor for PML development, the ability to identify patients who are infected with JCV can be a useful tool to stratify patients for the risk of PML development among individuals undergoing natalizumab therapy [Ref. 8]. The detection of anti-JCV antibodies in the blood determines exposure to JCV.

3. Premarket Notifications - Background

FDA concludes that special controls, when combined with the general controls of the Federal Food, Drug & Cosmetic Act (the FD&C Act), are necessary to provide reasonable assurance of the safety and effectiveness of JCV serological reagents A manufacturer who intends to market a device of this type must (1) conform to the general controls of the FD&C Act, including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific issues of safety and effectiveness identified in this guideline, and (3) obtain a substantial equivalence determination from FDA prior to marketing the device.

This guideline identifies the classification regulation and associated product codes for JCV serological reagents. In addition, other sections of this guideline list the risks to health and describe mitigation measures that, if followed by manufacturers and combined with the general controls, will address the risks associated with these devices and will generally lead to a timely premarket notification [510(k)] review. This document, will supplement other FDA documents regarding the specific content requirements of a premarket notification submission for JCV serological reagents. For additional information regarding 510(k) submissions, refer to 21 CFR 807.87 and the Center for Devices and Radiological Health (CDRH) Device Advice: Comprehensive Regulatory Assistance.[1]

4. Scope

The scope of this document is limited to the devices identified and classified under 21 CFR 866.3336 (product code OYP (Anti-JCV antibody detection assay)):

21 CFR 866.3336 John Cunningham Virus serological reagents

[1] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm.

(a) *Identification*. John Cunningham Virus serological reagents are devices that consist of antigens and antisera used in serological assays to identify antibodies to John Cunningham Virus in serum and plasma. The identification aids in the risk stratification for the development of progressive multifocal leukoencephalopathy in multiple sclerosis and Crohn's disease patients undergoing natalizumab therapy. These devices are for adjunctive use, in the context of other clinical risk factors for the development of progressive multifocal leukoencephalopathy.

(b) *Classification.* Class II (special controls). The special control for this device is the FDA guideline document entitled "Class II Special Controls Guideline: John Cunningham Virus Serological Reagents." For availability of the guideline document, see §866.1(e).

This document does not apply to John Cunningham Virus nucleic acid amplification reagents or John Cunningham Virus serological reagents for any indication other than the risk stratification for PML development. Please contact the Division of Microbiology Devices in the Office of *In Vitro* Diagnostics and Radiological Health for further information on nucleic acid amplification devices.

5. Risks to Health

FDA has identified the following risks to health associated with the use of JCV serological reagents. Failure of the JCV serological reagents to perform as indicated or an error in the interpretation of the results may lead to improper patient management.

False positive results may lead a physician and patient to elect not to use natalizumab therapy, due to perceived increased risk of developing PML. The patient may have a reduced quality of life due to continuing multiple sclerosis or Crohn's disease symptoms.

False negative results may lead to an under-estimation of the patients risk for developing PML. This is because prior infection with JCV is a known precursor of PML development. If the patient receives a false negative result they would be at a higher than the anticipated risk of developing PML. Treatment with natalizumab therapy may predispose a patient to an increased risk of developing PML.

An error in the interpretation of the results may lead a physician and patient to elect to use or not to use natalizumab therapy due to a misperceived risk of developing PML.

Under this guideline, manufacturers who intend to market a device of this type must conduct a risk analysis prior to submitting a premarket notification to identify any other risks specific to their device. The premarket notification must describe the risk analysis method used. If you elect to use an alternative approach to mitigate a particular risk identified in this guideline, or if you or others identify additional potential risks from use

of a device of this type, you must provide sufficient detail regarding the approaches used to mitigate these risks and a justification for your approach.

Table 1 – Identified Risks and Mitigation Measures

Identified Risks	Mitigation Measure
False positive results	Device Description (Section 6) and Performance (Section 7)
False negative results	Device Description (Section 6) and Performance (Section 7)
Failure to perform as indicated or an error in the interpretation of the results	Labeling (Section 8)

6. Device Description

You must identify the applicable regulation and the product code(s) for your device; you must include a table that outlines the similarities and differences between the predicate device (or another legally marketed device for the same intended use) and your device. We encourage you to reference appropriate peer-reviewed articles that support the use of your device for its intended diagnostic use and the specific test principles incorporated into the device design. You must describe each of these device elements in detail.

In addition, you must include the following descriptive information to adequately characterize your device for the detection of anti-JCV specific antibodies in human serum or plasma samples.

6.1 Device Components

You must provide a detailed description of the assay components included with the reagent kit. Your description must include:

- A description of the antibodies measured or detected by your device including the class and subclass of the detected antibody, e.g., IgM, IgG, or total Immunoglobulins.
- A description of the antigen source and specificity, characterization and purification methods, and results. If your device uses a recombinant antigen, you must specify the vector used and address potential cross reactive effects.
- A detailed description of the JCV strain used for the antigen preparation (including the source of the isolate, e.g., from a PML or non PML patient), the target gene from which the antigen is generated, the epitopes present on the antigen, and the rationale for your selection. You must indicate if the virus strain used has any PML mutations and the consequences that this may have on the assay performance. We recommend that your strain selection is

such that it allows detecting exposure to multiple JCV epitopes that are common to all JCV isolates.

- A description of any shared homology of your selected antigen with other polyomaviruses and its effect on the assay performance.
- A detailed description of the specific controls and calibrators to be used in the assay and a description of the primary purpose for the quality control material. The source, antibody levels of the controls, matrix effects, acceptance criteria for the control performance, and how the controls were established.
- A description of the capture reagent, e.g., antigen capture or antibody capture.
- A detailed description of the secondary detection reagent (conjugate) used by your device, including the antibody specificity (depending on the assay design, e.g., all human Ig classes, IgG, IgM.), the efficiency of binding, nature of the antibody (polyclonal or monoclonal), antibody source, purification, instructions for use, and any factors that may lead to potential cross reactivity.
- An explanation of any potential for the antigen and antibody reagents in your device to react with similar polyomaviruses. Measures must be undertaken in the device design to minimize the impact of this potential cross reactivity and described in your submission.

6.2 Ancillary Reagents

Ancillary reagents are reagents specified by you in your device labeling as "required but not provided" in order to carry out the assay as indicated in its instructions for use and to achieve the test performance claimed in the device labeling. For the purposes of this document, ancillary reagents of concern are those specified according to manufacturer and catalog or product number, or other specific designation, in order for your device to achieve its labeled performance characteristics. For example, if your device labeling specifies the use of a specific brand of reagent (e.g., 'Brand X Extraction Buffer or other buffers shown to be equivalent'), and use of any other extraction buffer may alter the performance characteristics of your device from that reported in your labeling, then Brand X Extraction Buffer or other buffers shown to be equivalent are ancillary reagents of concern for the purposes of this document.[2]

By contrast, if your device relies on the use of 95% ethanol and any brand of 95% ethanol will allow your device to achieve the performance characteristics provided in your labeling, then 95% ethanol is not an ancillary reagent of concern for the purposes of this document.

If the instructions for use for your device specify one or more ancillary reagents of concern, you must address how you will ensure that the results of testing with your device

[2] Even if you establish that one or more alternative ancillary reagents may be used in your assay, each of those named alternatives may still be an ancillary reagent of concern. We recommend you consult with FDA if you are unsure whether this aspect of special controls applies to your device.

and these ancillary reagents, in accordance with your instructions, will be consistent with the performance established in your premarket submission. Your plan may include application of quality systems approaches, product labeling, or other measures.

In order to address this aspect of the special control, your 510(k) submission must address the elements described below. FDA will evaluate whether your plan will help to mitigate the risks presented by the device to establish its substantial equivalence.

(1) Your 510(k) must include a risk assessment addressing the use of ancillary reagents, including risks associated with management of reagent quality and variability, risks associated with inconsistency between instructions for use provided directly with the ancillary reagent and those supplied by you with your assay, and any other issues that could present a risk of obtaining incorrect results with your assay.

(2) Using your risk assessment as a basis for applicability, you must describe in your 510(k) how you intend to mitigate risks through implementation of any necessary controls over ancillary reagents. These may include, where applicable:

- User labeling to assure appropriate use of ancillary reagents (see "Labeling" for further discussion).
- Plans for assessing user compliance with labeling instructions regarding ancillary reagents.
- Material specifications for ancillary reagents.
- Identification of reagent lots that will allow appropriate performance of your device.
- Stability testing.
- Complaint handling.
- Corrective and preventive actions.
- Plans for alerting users in the event of an issue involving ancillary reagents that would impact the performance of the assay.
- Any other issues that should be addressed in order to assure safe and effective use of your test in combination with named ancillary reagents, in accordance with your device's instructions for use.

In addition, you must provide testing data to establish that the quality controls you supply or recommend are adequate to detect performance or stability problems with the ancillary reagents.

If you have questions regarding identification, use, or control of ancillary reagents, you should contact the Division of Microbiology Devices in the Office of *In Vitro* Diagnostics and Radiological Health.

6.3 Test Methodology

You must describe in detail the methodology used by your device. This must include describing the following elements as applicable to your device:

- The specific test methodology to be used, e.g., immunoassay or immunochromatographic procedure.
- The type of the immunoassay to be used, e.g., competitive or non competitive.
- A description of the detectable label used by the immunoassay, e.g., enzymatic or fluorescent.
- Limiting factors of the assay, e.g., pipetting, incubation, washing, and mixing.
- Specimen types, collection, and handling methods.
- Reagent components provided or recommended for use, and their function within the system (e.g., solid support, buffers, fluorescent dyes, chemiluminescent reagents, substrates, conjugates, other reagents).
- Instrumentation involved in the use of your device, including the components and their function within the system.
- The computational path from raw data to the reported result (e.g., how raw signals are converted into a value) if appropriate. It would also include adjustment for background and normalization, if applicable.
- Illustrations or photographs of non-standard equipment or methods as appropriate.

When applicable, you must describe design control specifications for your device that address or mitigate risks associated with your immunoassay procedure detecting anti-JCV antibodies, such as the following:

- Minimization of false positives due to contamination or cross reactivity.
- Developing or recommending validated methods for antigen protein extraction and purification.
- Optimizing your reagents and test procedures.

In your 510(k) submission, you must provide a detailed description of the principles of operation for your device. You must specifically describe testing conditions, procedures, and controls designed to provide safeguards for conditions that can cause false positive and false negative results, or that may present a biosafety hazard. These include, but are not limited to:

- Description of, or recommendations for, any external controls and/or internal controls (e.g., sample negative controls and/or internal controls that monitor assay performance).
- Overall design of the testing procedure, including control elements incorporated into the recommended testing procedures.
- Features and additional controls that monitor procedural errors or factors (e.g., degradation of reagents) that adversely affect assay performance and detection.

7. Performance Characteristics

7.1 General Study Recommendations

You must provide data and statistical evaluation sufficient to determine if the device is substantially equivalent in terms of performance characteristics. You must provide data to substantiate claims of intended use or demonstrate clinical significance, and to validate use of a new technology, as appropriate. In general, testing sites must be representative of where the submitter intends to market the device, e.g., clinical laboratory.

7.2 Analytical Studies

In your 510(k), you must detail the study design you used to evaluate each of the performance characteristics outlined below. All analytical performance studies must be conducted using the final version of your device.

7.2.1 Assay Cut-off

You must provide data to explain how your assay cut-off was established and clinically validated. In your 510(k), you must describe how positive, negative, invalid, or equivocal (if applicable) results are determined and how they should be interpreted. In your 510(k) submission, you must provide the values of cutoffs of a signal for all outputs of the device. As part of doing this you must:

- Provide the cutoff value for defining a negative result of the device. If the device has only two outputs (negative/positive), this cutoff also defines a positive result of the device.
- If the device has an equivocal zone, describe how the cutoff was established and provide cutoff values (limits) for the equivocal zone.
- If your interpretation of the initial equivocal results requires re-testing, or application of a confirmatory assay, provide an algorithm for defining a final result by combining the initial equivocal result and the result of the confirmatory assay (note that this algorithm should be developed before initiating the clinical study).
- If one of the final outputs of your device can be an equivocal result, provide the interpretation and recommendation how to follow up after obtaining the equivocal result.
- If the device has an “invalid” result, provide an explanation of how an invalid result is defined. If internal controls are part of the determination of invalid results, you must provide the interpretation of each possible combination of control results. Provide recommendations on how to follow up any invalid result.

7.2.2 Precision Testing

You must provide data evaluating the precision (i.e., repeatability/reproducibility) of your device. The Clinical and Laboratory Standards Institute (CLSI) documents, "Evaluation

of Precision Performance of Clinical Chemistry Devices" (CLSI document EP05-A2) [Ref. 9], “User Verification of Performance for Precision and Trueness” (CLSI document EP15-A) [Ref. 10], and "User Protocol for Evaluation of Qualitative Test Performance" (CLSI document EP12-A2) [Ref. 11], include recommendations that may be helpful for developing experimental design, calculations, and a format for presenting results of the precision studies. You must identify the sources of assay variability and include them in the precision study. Analysis of the data must be performed with numerical values of your device (for example, signal to cutoff values) and with qualitative values (e.g., positive, negative).

You must conduct within-laboratory precision testing (i.e., at the manufacturer’s site). If the device is intended to be performed in a single laboratory, include multiple operators from that laboratory. Evaluate repeatability (within-run imprecision), between-run, between-day, and between-operator components of impression.

If the device is intended to be performed in more than one laboratory, you must conduct the reproducibility study at three sites (at least 2 external sites). For each of the three sites, evaluate precision at each site separately and provide components of imprecision as repeatability (within-run imprecision), between-run, between-day, between-operator. In addition, provide precision for the combined data with appropriate components of imprecision and between-site component.

You must use patient samples, your assay calibrator(s), and the quality control materials that you supply or recommend for your device for this characterization. You must evaluate precision at relevant concentrations, including levels near medical decision points (e.g., at limit of detection). The test panel must consist of at least three samples:

- “High negative” sample: a sample with the virus concentration below the clinically established cutoff such that results of repeated tests of this sample are negative approximately 95% of the time and positive approximately 5% of the time (C5 concentration).
- “Low positive” sample (close to limit of detection): a sample with a concentration of analyte just above the cut-off such that results of repeated tests of this specimen are positive approximately 95% of the time.
- “Moderate positive” sample: a sample with a concentration at which one can anticipate positive results approximately 100% of the time (e.g., approximately two to three times the concentration of the clinically established cutoff).

You must include the following items in your 510(k):

- Detailed description of the factors included in the precision studies (number of days, number of operators, number of instruments, reagent lots, calibration cycles, etc.).
- Provide a description of the study design including which factors were held constant and which were varied during the evaluation.

For each sample in the precision study, we recommend that you present:

- The mean value of numerical values.
- Variance of components with total imprecision (standard deviation and percent coefficient of variation (CV)).
- Percents of positive (above the cutoff) and negative (below the cutoff) results for each site separately and for all sites combined.
- Percent of invalid results for each site separately and for all sites combined.

7.2.3 Interference

You must characterize the effects of potential interferents on your assay performance. Examples of experimental designs, including guidelines for selecting interferents for testing, are described in detail in CLSI EP07-A2 [Ref. 12]. Potential sources of endogenous interference can include compounds normally found in serum and plasma, including, but not limited to, hemoglobin, lipids, bilirubin, γ-globulin, cholesterol, total protein (albumin), and ascorbic acid. You must assess the potential interference with exogenous agents such as concomitant medications that may be used by the intended use population.

You must include the following items:

- Types and levels of interferents tested
- Level of antibody in the sample, including a description of how the levels of antibodies were determined
- Number of replicates tested
- Criteria or method for computing interference
- Point estimate of the observed interference effect (percent recovery) as the percent difference between the means of the test and control samples

You must identify any observed trends in bias (i.e., negative or positive) and indicate the range of observed recoveries in the presence of the particular interferent. This approach is more informative than listing average recoveries alone. You must establish and state your criteria or level for determining non-interference prior to the initiation of the study.

You may not need to perform additional interference testing with potential interferents of your assay that have already been identified in literature or by other sources. In these cases, it may be appropriate to address additional potential interferents with appropriate citations in the labeling.

7.2.4 Cross reactivity

You must include data on assay analytical specificity by measuring the cross reactivity of your device with antibodies to other genetically related or clinically relevant viruses, microbial agents causing neurological manifestations, or microbial agents likely to be

present in immunocompromised patients. For example, evaluation of potential cross reactivity with antibodies to the following agents:

- Antibodies to other polyomaviruses, such as BKV
- Antibodies to HSV, HIV, CMV, VZV, HHV6, EBV
- Antibodies to *Streptococcus agalactiae, Escherichia coli, Listeria monocytogenes, Chlamydia trachomatis, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Ureaplasma urealyticum, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Chryseobacterium meningosepticum, Mycobacterium tuberculosis, Viridans streptococci*, gram-negative bacilli, other gram-positive organisms, *Treponema pallidum,* Lactobacillus, and anaerobes, *Candida albicans* and other fungi, such as *Pneumocystis jiroveci*

In your 510(k) submission, you must explain how the presence of a particular cross reactant will be determined and describe your method of evaluating the potential cross reactivity and the pre-determined acceptance criteria. Additional cross reactivity testing or alternative testing must be supported with a valid scientific justification. We recommend that you contact the Division of Microbiology Devices within the Office of *In Vitro* Diagnostics and Radiological Health to discuss alternative cross reactivity studies.

7.2.5 Matrix Comparison Studies

At least forty paired plasma and serum samples must be evaluated in the matrix comparison studies; the numerical values of serum samples must span the interval of numerical values of the device. For evaluation of systematic difference between numerical values of serum and plasma samples, Deming or Passing-Bablok regression must be performed. For more details about regression analysis, see CLSI document EP09-A2-IR "Method Comparison and Bias Estimation Using Patient Samples" [Ref. 13]. The scatter plot of results must be presented along with a linear regression line and a diagonal line. For linear regression analysis, present slope and intercept with 95% confidence intervals. Using the linear regression equation, provide an estimation of a systematic difference at the cutoff point(s).

If duplicate measurements for serum and plasma samples were obtained, provide estimation of repeatability for each sample type. If the repeatability is found to be dependent on the numerical values of serum samples, the interval may be divided into subintervals and the repeatability will be evaluated for each subinterval separately. For comparing the repeatability of the plasma and serum samples, ratios of two standard deviations with 95% confidence interval (based on F distribution) should be calculated. In addition, you must provide a comparison of the qualitative results (e.g., positive, negative) for the serum and plasma samples in a two-by-two table.

7.2.6 High Dose Hook Effect

You must demonstrate that excess analyte does not cause a hook (prozone) effect or explain why the evaluation is not applicable for your device design.

7.2.7 Reagent Stability

You must describe your study design for determining the stability of the reagents, and, if applicable, a description of test conditions and results. For each study, you must describe your acceptance criteria and how you selected them. The CLSI document, "Evaluation of Stability of In Vitro Diagnostic Reagents" (CLSI document EP25-A) [Ref. 14] includes recommendations that may be helpful for developing experimental design, calculations, and a format for presenting results of the stability studies.

7.2.8 Specimen Collection and Handling Conditions

You must substantiate statements in your labeling about specimen storage and transport by assessing whether the specimen can maintain acceptable performance with your device (e.g., reproducibility at the cut-off) over the storage times and temperatures recommended to users. For example, an appropriate study may include an analysis of aliquots stored under the conditions of time, temperature, storage, shipping, or number of freeze/thaw cycles that you recommend to users of the device. You must describe the criteria for an acceptable range of recoveries under the recommended storage and handling conditions [Ref. 15].

7.2.9 Other Analytical Studies

Conducting any additional analytical performance studies that are appropriate for your device design is recommended. When applicable, we recommend that you test panels consisting of well-characterized anti-JCV serum or plasma samples if available. We recommend that you contact the Division of Microbiology Devices in the Office of *In Vitro* Diagnostics and Radiological Health if you intend to perform such studies.

7.3 Clinical Performance

The data from your clinical studies must support the indications for use and claims for your device. The clinical validation studies must use patient samples that are obtained from the intended use population. You must describe the protocol of each clinical study, including the inclusion and exclusion criteria, study design, and statistical analysis methods.

7.3.1 Expected Values

You must establish the expected values (observed positivity rates) of JCV antibodies in a patient population(s) representative of the intended use population and sample type of your device. You must provide these results based on your device (rather than a predicate device). You must provide demographic information about study population(s), such as age, sex, and geographical area. You must present percents and numbers of

positive, negative, and equivocal (if applicable) results stratified by age group, sex and geographical area. Blood donors must not be used for this study.

7.3.2 Clinical Studies

In your submission you must provide data for the evaluation of your device to address the following points:

- Whether the positive anti-JCV result generated by your device is a risk factor for PML development in intended use patients;
- Whether the anti-JCV results generated by your device bring additional information about risk of PML beyond the currently known risk factors.

The performance of your device is described by the sensitivity and specificity, the positive and negative likelihood ratios, or by a risk of PML for the positive test result, a risk of PML for the negative test results and a pre-test risk of PML. A positive test result is considered a risk factor, if the risk of PML for a positive result is higher than the pre-test risk of PML, or if the risk of PML for a negative test result is lower than the pre-test risk of PML.

The probability of PML for the positive test results is equal to the product of the positivity rate among the PML subjects and the pre-test risk of PML divided by the positivity rate among all subjects in the intended use population.

An increase in the risk of PML for the positive test result compared to the pre-test risk of PML is equal to the ratio of the positivity rate among the PML subjects and the positivity rate among all subjects. The estimates of sensitivity and specificity are considered as binomial proportions; therefore, one can consider the probability of PML as a constant in the calculations.

If the positivity rate of the device for the subjects with PML is statistically higher than the positivity rate of the device in the intended use population, the device will be considered statistically informative. In order to demonstrate that your device does so, you must provide the following data:

- Results of your device for samples from confirmed PML patients collected prior to clinical diagnosis of PML. For each PML patient, you must provide information about time of sample collection and the status of other risk factors. Calculate the percent of positive results by your device for the PML patients (sensitivity) with a 95% confidence interval. For calculation of confidence intervals for the proportions, a score method described in the CLSI document EP12-A2 is recommended [Ref. 11].
- Results of your device for samples collected from patients representing the intended use population. Provide demographic characteristics and other relevant clinical information for these patients. Calculate the percent of

positive results by your device in the intended use population with 95% confidence interval.

- The calculated ratio of the positivity rate for the subjects with PML and the positivity rate in the intended use population with 95% confidence interval. Your data must demonstrate that the positivity rate for the subjects with PML is statistically higher than the positivity rate in the intended use population and these positivity rates are clinically acceptable.
- Device performance based on statistical modeling by considering your estimates of sensitivity (positivity rate of your device for the PML), positivity rate of your device in the intended use population, and incidence of PML. Data of your modeling and the estimates of the risks and a relative risk along with 95% confidence intervals will be used by FDA to complete Table 2 and Table 3:

Table 2

	Number with PML	Number without PML	Total Number Patients Treated
Device Positive			
Device Negative			
Total			

Table 3

	Device Performance
Risk of PML for Positive Result	
Risk of PML for Negative Result	
Relative Risk	

In addition, you must provide data demonstrating that device results bring additional information about the risk of PML beyond the currently known risk factors: prior immunosuppresant therapy and duration of treatment with natalizumab. For this, you may provide data to determine relationships between the positivity rate of your device and duration of treatment and between the positivity rate of your device and prior immunosuppression therapy status.

7.4 Detectability and Comparative Performance

You must determine the detectability of anti-JCV antibodies by comparative performance to a valid predicate device using clinical samples representative of the intended use population. You must test prospectively collected samples. The sample panel must include samples that span the assay detection range. Positive samples must include at least 30-50% of samples close to the cutoff of the test device. The total number of sample types you include in your study for substantiating a claim for detection of antibodies to JCV will depend on the prevalence of the antibodies to JCV in your clinical study patient population and on your device's performance. All JCV antibody devices must demonstrate positive and negative percent agreement results with a point estimate of at least 95% or alternatively, a clinically relevant percentage. The lower bound of the 95% (two-sided) confidence interval must be greater than 90%. All raw line data from your studies must be submitted for review. You must supply this information electronically using Microsoft Excel, delimited text files, or SAS files. You must provide your calculated results in a two cell by two cell table. We recommend that you contact the Division of Microbiology Devices in the Office of *In Vitro* Diagnostics and

Radiological Health for sample size determination and in situations where prospective samples close to the assay cutoff are not available.

8. Labeling

Your 21 CFR 809.10 compliant labeling must also include the following information.

8.1 Instructions for Use

You must provide clear and concise instructions that delineate the technological features of the specific device and how the device is to be used for testing patients. Instructions must encourage local/institutional training programs designed to familiarize users with the features of the device and how to use it in a safe and effective manner.

8.2 Quality Control

You must provide a description of quality control (QC) recommendations in the labeling and specify what your quality control material will measure. Your labeling must provide instructions for use that include conducting daily testing of all quality controls. You must address both initial and repeat QC results with an explanation of the action taken for all out-of-range test results. The same QC materials must be recommended in the device package insert. The QC section of the package insert must also include the following statement "QC procedures should be performed in conformance with applicable state and/or federal accreditation requirements".

8.3 Warnings and Precautions

Your labeling must include the same warnings or precautions for users as established in the regulations contained in 16 CFR part 1500 and any other warnings appropriate to the hazards presented by the product; a statement "For In Vitro Diagnostic Use", and all the procedural warnings and precautions appropriate to the device. You must address issues concerning safe use of your assay with statements or information such as the following:

- The statement "Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly."
- The statement "All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and

decontaminated or disposed of with proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2." [Ref.16]

- The statement "After adding patient or control specimens, the test tubes or plates should be considered potentially infectious and handled accordingly."
- Provide detailed information on any hazardous materials (e.g., preservatives) included in the reagents, the recommended precautions for handling, and mitigations in case of accidents.
- The statement "Follow only protocols described in the package insert. Modifications of the protocol may give erroneous results."
- The statement "Cross-contamination of patient specimens can cause erroneous results." Provide instruction to avoid mixing of samples and minimize contamination.
- The statement "Bacterial contamination of serum or plasma specimens or reagents can produce erroneous results." Provide instructions and recommendations for aseptic sample collection.

8.4 Interpretation and Reporting of Test Results

You must provide an adequate description of expected results if the test provides other than quantitative results. 21 CFR 809.10(b)(9). Interpretation of results must be presented in a tabular format. Results should not be reported in instances where performance has not been established.

In your presentation of the results you must describe how positive, negative, equivocal (if applicable), or invalid results are determined and how they should be reported and interpreted. In your results table in the labeling you must provide an example of the actual output results that your device produces and the appropriate interpretation and detailed recommendations for retesting and reporting. For example, it must be clearly stated that a negative result indicates that anti-JCV antibodies were not detectable in the sample tested.

In addition to the guidance on the result interpretation, you must address potential result misinterpretation concerns for anti-JCV testing to aid in the risk stratification of PML development in the limitations section of the device labeling. This is particularly important since there is no reference standard or method to accurately determine the presence of anti-JCV antibodies or exposure to JC virus. The end user should clearly understand that if the anti-JCV test fails to detect antibodies to JC virus it does not rule out exposure to JCV. The lack of detectable antibodies could be attributed to several factors related to the test performance or the host immune response. The user must be made aware of alternative approaches or follow up recommendations for negative results to enable appropriate patient management decisions.

The limitations must clearly explain the situations that may lead to false results and provide examples of possible misinterpretation of the results and their consequences. The following are examples of the limitations that must be considered:

- The statement "All results from this and other serological tests should be correlated with clinical history, epidemiological data, and other data available to the attending physician in evaluating the patient."
- The statement "A single positive result only indicates previous exposure to JC virus; the level of antibody response may not be used to determine active infection or disease stage."
- A statement explaining that false negative results may occur and explain that negative test results do not rule out the possibility of exposure and potential for developing PML[Ref. 4, 17]. Then provide a statement with the false negative rate of your assay and an explanation of how it was estimated.
- A statement explaining that false positive results may occur. Then provide a statement with an estimate of the false positive rate of your assay as applicable and an explanation of how it was determined.
- A statement explaining that the false positive rate and the false negative rate of the anti-JCV test can not be accurately estimated as there is no accepted reference method for determining JC virus serological status.
- A statement providing examples of situations that may lead to a false result such as potential cross reactants as demonstrated in your studies or from scientific literature, e.g., "Gamma globulin is known to cross react with this assay. Patients undergoing γ globulin therapy may have erroneous results."
- A statement listing potential interferents that may contribute to erroneous results.
- The statement "An anti-JCV positive result is not diagnostic for PML and the test result should not be used for the diagnosis of PML."
- A statement explaining that some patients may not have detectable antibody levels in a particular situation, which may lead to a false negative result. Then provide a statement delineating known conditions relevant for the intended use population, e.g., testing should not be performed for at least two weeks following plasma exchange due to removal of antibodies from the serum.
- The statement "A negative result does not eliminate the risk of developing PML and does not rule out prior exposure to JCV. A negative result should not be used as the sole basis for diagnosis, treatment, or management decisions."
- A statement explaining that the performance of the device using sample types other than sample type for which performance was evaluated has not been established. Then provide a statement clearly indicating any populations for which the performance of this device has not been established.

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