Class II Special Controls Guideline: Dengue Virus Serological Reagents

Guideline for Industry and Food and Drug Administration Staff

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

Public Comment

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1. Introduction

This special controls guideline was developed to support the classification into class II (special controls) of dengue (DEN) virus (DENV) serological reagents. DENV serological reagents are devices that consist of antigens and antibodies for the detection of DENV and DEN antibodies in individuals who have signs and symptoms of dengue fever (DF) or dengue hemorrhagic fever (DHF). The detection aids in the clinical laboratory diagnosis of DF or DHF caused by DENV infection.

This document does not address DENV nucleic acid amplification test reagents.

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 DENV serological reagents device 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. Dengue Virus Background

DENVs are a significant cause of febrile illness in the tropical and subtropical regions of the world. The disease is caused by four antigenically related single stranded RNA viruses or serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). DENVs are members of the Flaviviridae virus family. Other members of the Flaviviridae family include yellow fever, Japanese Encephalitis and West Nile viruses. DENV is transmitted to humans by the bite of an infected Aedes mosquito. The incubation period after a mosquito bite ranges from three to eight days before symptoms occur. Primary or classical DF is characterized by high fever and two or more of the following symptoms: severe headache, retro-orbital eye pain, myalgia, maculo-papular rash, arthralgia, lymphadenopathy, and leukopenia, which normally resolves itself in five days. A more severe form of DENV disease is called DHF which in some patients progresses to dengue shock syndrome (DSS). Although DHF may

occur in primary DENV infections, it is usually found in individuals with secondary DENV infections caused by a different DENV serotype. Individuals with DHF display significant thrombocytopenia, which can cause severe hemorrhagic and shock manifestations (DHF/DSS). With medical treatment the case fatality rate is less than 1.0%; however, the case fatality rate can be up to 50% in individuals who are not hospitalized and treated [Ref. 1].

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 DENV 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 for DENV 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 DENV 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 devices identified and classified under 21 CFR 866.3945.

21 CFR 866.3945 – Dengue virus serological reagents

> (a) Identification. Dengue virus serological reagents are devices that consist of antigens and antibodies for the detection of dengue virus and dengue antibodies in individuals who have signs and symptoms of dengue fever or dengue hemorrhagic fever. The detection aids in the clinical laboratory diagnosis of dengue fever or dengue hemorrhagic fever caused by dengue virus.
>
> (b) Classification. Class II (special controls). The special control is FDA's guideline entitled "Class II Special Controls Guideline: Dengue Virus Serological Reagents." See §866.1(e) for availability of the guideline document.

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

5. Risks to Health

FDA has identified the risks of false negative test and false positive test results, both of which can lead to individual and/or public health consequences, as issues of safety and effectiveness associated with this device that require special controls.

Failure of DENV serological reagents devices to perform as indicated or an error in interpretation of the results may lead to misdiagnosis with significant implications on patient management.

A false positive test result for an individual may lead to unnecessary treatment and possibly a less thorough laboratory evaluation for the true cause of illness; in the setting of an outbreak investigation, a false positive result may lead to unnecessary initiation of mosquito vector control measures.

A false negative result may lead to inappropriate use of antibiotics or not being treated with the appropriate intravenous fluids or platelet transfusion, or a false negative result may lead to delays in recognizing the cause of an outbreak and initiating adequate mosquito vector control measures.

The symptoms of dengue infection, i.e., fever, headache, arthralgia, retro-orbital pain, rash, lymphadenopathy and leukopenia, overlap with other causes of acute febrile illnesses. In the absence of clear symptoms or signs that separate DENV infections from other etiologies of febrile illnesses, it is likely that the results of a DENV diagnostic test would strongly influence ascribing the cause of febrile illness to DENV infection.

In the table below, FDA has identified the risks generally associated with the use of *in vitro* diagnostic devices for DENV detection that require special controls. The measures to mitigate these identified issues are in this guideline, as shown in the table below, in combination with proposed subsection 21 CFR 866.3945. 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 to Health and Mitigation Measures

Identified Risks to Health	Mitigation Measures
A false positive test result for an individual may	Device Description

lead to unnecessary treatment and possibly a less thorough laboratory evaluation for the true cause of illness; a false positive result may lead to unnecessary initiation of mosquito vector control measures.	Containing the Information Specified in the Special Control Guideline (Section 6) Performance Characteristics (Section 7) Labeling (Section 8) Postmarket Measures (Section 9)
A false negative test result may lead to inappropriate use of antibiotics or a delay in treatment to prevent death due to dengue hemorrhagic fever or dengue shock syndrome or a false negative result may lead to delay in initiation of mosquito vector control measures.	Device Description Containing the Information Specified in the Special Control Guideline (Section 6) Performance Characteristics (Section 7) Labeling (Section 8) Postmarket Measures (Section 9)
An error in the interpretation of the results	Labeling (Section 8)

6. Device Description Containing the Information Specified in the Special Control Guideline

In your 510(k) submission, you must include a device description that meets the requirements of 21 CFR 807.87(a) and (f) and you must identify the legally marketed predicate device as required by 21 CFR 807.92(a)(3). Furthermore, you must also 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. You may 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 DENV antigens or antibodies in human serum samples.

a. Intended Use

The intended use must specify the nature of the analyte and the target (e.g., DEN IgM antibodies or DEN NS-1 antigens detected by the device), specimen type for which testing will be indicated (i.e., serum), the clinical indication(s) for which the test is to be used, and the specific population(s) for which the test is intended. The intended use must state that the test is qualitative and any specific conditions of use. The intended use must also specifically state as part of the clinical indication whether the

test is to be used in the setting of diagnosing individual patients (e.g., symptomatic individuals who have returned from DEN endemic regions) and/or for diagnosis of individuals during outbreak investigations.

In your 510(k), you must clearly describe the following information related to the intended use of your product:

- The identity of the different DEN serotypes and strains that your device is designed to detect (i.e. strain reactivity).
- How the device test results will be used to aid in laboratory identification of DENV antigens or antibodies in clinical specimens from symptomatic patients.

b. 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.
- Specificity of monoclonal antibodies for the DEN serotypes or flaviviruses of interest.
- Information regarding the rationale for selection of specific antigen targets (e.g., NS-1 or E glycoprotein).
- Optimal parameters for the assay, e.g., pipetting, incubation, washing, and mixing.
- Sample types (e.g., serum specimen), 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 required for 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. This would include sufficient software controls for identifying and dealing with obvious problems in the dataset. 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 an immunoassay procedure detecting DEN antigens or antibodies, such as the following:

- Minimization of false positives due to cross-reactivity with other flaviviruses antibodies or antigens.
- Optimizing your reagents and test procedure for recommended instruments.

In your 510(k), you must provide performance information that supports the conclusion that your design requirements have been met. You must also provide information to verify the design of your reagents (e.g., rationale for selection of specific antibodies or antigens). See Section 7 – Performance Characteristics.

c. Ancillary Reagents

Ancillary reagents are reagents specified in 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 labeling for the assay. For the purposes of this document, ancillary reagents of concern are those that must be specified according to 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 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 requires 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 of 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 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, and other measures.

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

1. You must include, in your 510(k), 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] 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 the special controls applies to your device.

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 must be addressed in order to assure safe and effective use of your test in combination with identified 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 may contact FDA for advice.

d. Testing Methodology

You must provide in your 510(k) submission a detailed description of the principles of operation of 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.

You must include a description for all additional procedures, methods, and practices incorporated into your directions for use (See Section 8 - Labeling) that mitigate risks associated with DEN testing.

e. Specimen Storage and Shipping Conditions

If you recommend specimen storage and/or shipping conditions, you must demonstrate that your device generates equivalent results for the stored specimens at several time points throughout the duration of the recommended storage period and at both ends of your recommended temperature range. You may use the methods described in Clinical and Laboratory Standard Institute (CLSI) document H18-A4, Procedures for the Handling and Processing of Blood Specimens for further guidance [Ref. 2].

f. Interpreting Test Results/Reporting

In your 510(k), you must describe how positive, negative, equivocal (if applicable), or invalid results are determined and how they should be interpreted. In your 510(k) submission, you must indicate the cut-off values for all outputs of the assay.

You must provide the specific cut-off value for defining a negative result of the assay. If the assay has only two possible output results (e.g., positive and negative), this cut-off also defines a positive result of the assay.

If the assay has an equivocal zone, you must provide ranges (limits) for the equivocal zone and recommendations for how the user should follow up the equivocal results. If your interpretation of the initial equivocal results requires retesting, your 510(k) must address:

- Whether retesting should be done by the same assay or a different method.
- Whether retesting should be repeated from the same preparation or a new patient specimen.
- An algorithm for defining a final result by combining the initial equivocal result and the results after retesting if retesting is done by the same assay as the initial testing. (This algorithm must be developed before the pivotal clinical studies that evaluate the clinical performance of the assay).

If the assay can have an invalid result, you must describe how an invalid result is defined. If internal controls are part of the determination of invalid results, you must provide recommendations on the interpretation of each possible combination of control results for defining the invalid result. You must provide recommendations for how to follow up any invalid result, i.e., whether the result should be reported as invalid or whether retesting is recommended. If retesting is recommended, you must provide information similar to that for retesting of equivocal results (i.e., whether retesting should be repeated from a new aliquot of the same sample or a new patient specimen).

In addition, you must describe how you monitor performance results over time to identify changes in performance due to antigenic changes in the DENV, emergence of a new DENV strain, or due to prevalence changes from the existing prevalence at the time your product was evaluated.

7. Performance Characteristics

a. General Study Recommendations

Your 510(k) submission must include detailed descriptive information regarding the studies that you conducted to establish each of the performance characteristics outlined below.

Prospective clinical studies must be done to determine the performance of your device in conditions similar to the proposed intended use. In general, for both clinical studies and reproducibility studies, you must conduct testing at three (3) sites, representative of the intended user (e.g., clinical laboratory sites).

Although it is anticipated that serum or plasma will be the only appropriate sample type for DEN serological assays, if a sample matrix other than serum, e.g., urine or saliva, is recommended, you must evaluate your assay performance with each possible specimen type that you intend to be used with your assay.

You must provide appropriate specific information in your 510(k) submission describing the protocols used during your assay development in order for FDA to accurately interpret acceptance criteria and data summaries contained in your application during our review. When referring to CLSI (Clinical and Laboratory Standards Institute) protocols or guidelines, indicate which specific aspects of the protocols or guidelines were followed.

We recommend that you contact FDA prior to initiating your clinical studies program to obtain feedback regarding your planned studies and the intended uses that are planned for inclusion in your 510(k) submission.

b. Analytical Studies

You must establish the following performance characteristics for your DEN immunoassay in your 510(k):

(1) Analytical Sensitivity

(a) Limit of Detection

At the present time there are no known International Standards or Preparations for DEN antibody or antigens. It is recommended that the sponsor contacts FDA to discuss potential approaches to determine the LoD of your assay.

(b) Analytical Reactivity

You must test a well characterized reference panel of samples by your immunoassay, if available. For example, the WHO-CDC reference panel was created by a joint effort of the United Nations International Children's Emergency Fund/United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases and the Pediatric Dengue

Vaccine Initiative and could be tested to establish the analytical reactivity of your immunoassay [Ref. 3].

(c) Strain Reactivity

A panel of well characterized DEN samples must be tested by your immunoassay to establish reactivity with different strains of DENVs. The four (4) DEN serotypes antibodies and antigens must also be tested by your assay.

You may cite literature and/or other evidence for DEN strains excluded from your study; also, additional DEN strains may be appropriate for inclusion based on clinical and epidemiological trends at the time the device is being developed.

The results of strain reactivity testing (i.e., strains that are and are not detected by the assay) must be listed in the product labeling.

(2) Analytical Specificity

(a) Cross-Reactivity:

You must test for potential cross-reactivity against the pathogens listed in Table 2 that can cause febrile illness. In particular, studies must be conducted to characterize performance in the presence of antibodies to other flaviviruses (e.g., St. Louis encephalitis, West Nile, yellow fever, Japanese encephalitis), alphaviruses (e.g., eastern equine encephalitis), and other viruses and bacteria that cause fever and rash symptoms (e.g., enteroviruses, herpes simplex). For antigen cross-reactivity, the same microorganisms must be tested at medically relevant viral and bacterial levels (usually 10^6 cfu/mL or higher for bacteria and 10^5 pfu/mL or higher for viruses). The identities and titers of viral and bacterial isolates used for cross-reactivity studies must be confirmed prior to testing.

Table 2. Microorganisms for Cross-Reactivity Studies

Test Organism
West Nile virus
Japanese encephalitis virus
Saint Louis encephalitis virus
Yellow fever virus
Hepatitis A virus
Hepatitis B virus
Hepatitis C virus
Epstein Barr virus
Borrelia burgdorferi
Leptospirosis
Cytomegalovirus
Rheumatoid Factor
Anti-Nuclear Antibodies
Eastern equine encephalitis virus
Chikungunya virus
Influenza A and B virus
Measles virus

(b) Interference

You must conduct a comprehensive set of interference studies with your device. Potentially interfering substances include, but are not limited to, other constituents of the specimen of choice (e.g., white blood cells, protein, whole blood, hemoglobin, and controls or reagents spiked into the specimen for control purposes). You must test interference at or near the assay cut-off. You must evaluate each interfering substance at its potentially highest concentration ("the worst case"). If no significant clinical effect is observed, no further testing is necessary. We recommend you refer to the CLSI document, "Interference Testing in Clinical Chemistry," EP7-A2 [Ref. 4] for additional information. Other potentially interfering substances include, but are not limited to, the following:

Table 3. Substances for Interference Studies

Substances
Bilirubin
Cholesterol
Lipids
Heparin
Na Citrate
EDTA
Albumin
Hemoglobin

(3) Precision

(a) Within-Laboratory Precision/Repeatability

You must conduct intra-assay, inter-assay and inter-lot precision studies. You must test sources of variability such as operators, days, and assay runs by testing for a minimum of 12 days (not necessarily consecutive) with three (3) replicates of each sample per run, two runs and two operators per day. The test panel must consist of at least four samples spanning the entire range of medically relevant analyte concentration. For your inter-lot evaluation you must include at least three lots (as appropriate). Your repeatability study report must include the following information: number of days and runs, number of lots, number of operators, and acceptance criteria applied to the studies. You may perform these studies in-house, i.e., within your own company facility.

For qualitative tests without instrumentation needed (e.g., immunochromatographic tests or lateral flow devices), repeatability studies are usually not necessary.

(b) Between-Laboratory Precision/Reproducibility

The protocol for the reproducibility study may vary slightly depending on the assay format although the sample panel must be the same as described for repeatability studies cited below. In general, you must use the following approach to reproducibility studies:

- Evaluate the reproducibility of your test at three testing sites (this may include two external sites and one in-house site or three external sites).
- Use a five day testing protocol, including a minimum of two runs per day, (unless the assay design precludes multiple runs per day), and three replicates of each panel member per run.

- Have at least two operators at each facility perform the test each day. You must provide training only to the same extent that you intend to train users after marketing the test.

For your testing you must include at least 2 – 3 DEN serotype antibodies or antigens at a minimum of four levels that include analyte or output concentrations close to the assay cut-off, i.e.:

- A "negative" sample: a sample with an analyte concentration below the clinical cut off such that results of repeated tests of this sample are negative 100% of the time.
- A "high negative" sample: a sample with an analyte concentration below the clinical cut-off such that results of repeated tests of this sample are negative approximately 95% of the time (i.e., results are positive approximately 5% of the time).
- A "low positive" sample: a sample with a concentration of analyte just above the clinical cut-off such that results of repeated tests of this sample are positive approximately 95% of the time.
- A "moderate positive" sample: a sample ideally reflecting a clinically relevant concentration. At this concentration one can anticipate positive results approximately 100% of the time, e.g., approximately two to three times the concentration of the clinical cut-off.

You may refer to the CLSI document EP15-A2 [Ref. 5], EP05-A2 [Ref. 6], and EP12-A2 [Ref. 7] for guidance on reproducibility study design.

c. Controls

You must run appropriate controls each time analytical and clinical studies are conducted. You may contact FDA for further information regarding appropriate controls. In general, you must include the following types of controls:

(1) Negative Controls

A negative sample may serve as a negative control; the negative control is used to rule out contamination or increased background in test reactions.

(2) Positive Controls

The positive control must contain DEN antigens or antibodies at levels close to the cutoff of the assay and must be used to control the entire assay process including extraction (if appropriate). The positive control must be designed to mimic a patient specimen and is run as part of the assay or as a separate assay concurrently

with patient specimens at a frequency determined by a laboratory's Quality System (QS). Examples of acceptable positive assay control materials include:

- Specimens from a DEN infected or convalescent individual.
- Commercial DEN antibodies or antigens that have been processed and calibrated.

(3) Internal Control

The internal control monitors the integrity of the reagents, whether the assay procedure was followed correctly, and the presence of inhibitors in the samples. Examples of acceptable internal control materials include anti-mouse IgG antibodies, biotin, streptavidin, and anti-peroxidase. It may only be needed for assays performed in single test disposable cartridges or tubes.

d. Specimen Collection and Handling

You must specify the specimen type(s) that your assay is intended to measure. A specimen has to be collected from the appropriate anatomical site or source at a time in the course of disease when DENV antigen or antibody is likely to be isolated from the specimen.

The quality and quantity of the target analyte can be highly dependent on factors such as specimen source, collection method, handling (e.g., transport and storage times and temperatures). Testing results you provide in your 510(k) must validate that the device maintains acceptable performance (e.g., accuracy, reproducibility) under all the conditions recommended in your labeling. For example, you must assess the effect of recommended storage times and temperatures (including freeze-thaw cycles) on sample stability using an analysis of specimen aliquots stored and/or transported under your recommended conditions of time and temperature. You must state your acceptance criteria for all specimen collection and handling conditions and stability parameters.

Follow all applicable state and federal biosafety guidelines for collecting and handling specimens for pathogen identification. For standard precautions in handling of specimens, refer to the most current editions of the related Clinical and Laboratory Standards Institute (CLSI) documents [Ref. 8].

e. Assay Cut-off

Your 510(k) submission must explain how assay cut-off(s) were determined (also see Section 8.g - Test Results). Selection of the appropriate cut-off can be justified by the relevant levels of sensitivity and specificity based on Receiver Operating Curve (ROC) analysis of pilot studies with clinical samples; details regarding ROC analysis are included in CLSI document GP-10A [Ref. 9]. The performance of your device using the pre-determined cut-off (and equivocal zone, if applicable) must be validated in an independent population consistent with the defined intended use of your device (also see Section 7.f - Clinical Studies).

f. Clinical Studies

You must conduct clinical studies to determine the performance of your device for the specific intended uses of your assay. The approach to specimen collection may differ depending on whether the intended use is as an aid in the diagnosis of specific individuals or as an aid in the investigation of suspected DENV outbreaks. For the diagnosis of individual patients, specimens must be prospectively collected and tested from individuals from the intended use population (i.e., patients with signs and symptoms consistent with DF or DHF). Fresh samples are preferred for these studies although it may be possible to supplement fresh samples with prospectively collected archived specimens[2]. To use prospectively collected archived specimens to evaluate your device you must demonstrate that sample freezing or other preservation techniques do not affect analyte stability, that appropriate archives are selected, and that appropriate measures are taken to identify and remove or mitigate any biases in the studies. If you evaluate the assay using specimens that were archived, you must ensure that the specimens are not selectively utilized (i.e., that all specimens are tested). Samples must be masked during testing to avoid possible bias. If both fresh and archived/frozen samples are tested, you must analyze the data for these two sample groups separately.

The protocol for each clinical study performed must be included in the 510(k) submission. Sponsors are strongly encouraged to discuss study protocols with FDA prior to initiation of clinical studies.

The following issues must also be addressed during the design of your clinical studies:

(1) Study Protocol

Clinical study protocols must be completed and reviewed by the principal investigators prior to the study's initiation. At a minimum, protocols must include complete patient inclusion and exclusion criteria, the type and number of specimens needed, study procedures, and a detailed statistical analysis plan. Copies of the original study protocols, protocol modifications, and any other relevant study information must be included in your 510(k) submission.

We encourage sponsors to contact FDA to request a review of their proposed study protocols and the selection of specimen type as part of the pre-Submission review process. This is particularly recommended in a situation where different intended uses of the test may be studied or sponsors are planning to submit a 510(k) submission for the first time.

(2) Specimen Type(s)

Serum and plasma specimens are appropriate sample matrices for this device. Contacting FDA is indicated if other sample types will be studied. Specimens must be collected sequentially from all patients at each study site who meet the specific study inclusion criteria. The total number of samples you must include in your study will

depend on anticipated assay performance and the prevalence of DEN disease in the study population.

(3) Study Sites

For the intended use of individual patient diagnosis, you must conduct your studies at a minimum of three different geographical sites representing testing environments where the device will ultimately be used (e.g., clinical laboratories) and by laboratory personnel likely to perform the test in clinical practice. At least one of the study sites must be located in the United States. It is recommended that sponsors discuss appropriate study sites for the intended use of outbreak investigation with FDA prior to initiating studies since these studies are more likely to use prospectively archived specimens. [3]

(4) Study Population

DEN serological assays must be used with individuals that present or have recently presented with signs of DF or DHF. DF symptoms include sudden onset of fever, severe headache, pain behind the eyes, body and muscle aches and joint pain, nausea, and vomiting. Sometimes severe forms of the disease called DHF or DSS may occur after 3-5 days of fever. DHF is characterized by increased vascular permeability, hypovolemia and abnormal blood clotting mechanisms. DHF is a potentially more deadly condition, similar to DF, but after several days the patient becomes irritable, restless, and sweaty, and the condition can progress to plasma leak and hemorrhage.

(5) Reference Methods

You must assess and compare the performance of your device to an appropriate reference method or a predetermined algorithm based on composite reference methods (i.e., where the results of more than one assay are included as part of the reference method e.g., DEN IgM enzyme-linked immunosorbent assay (ELISA) and/or hemagglutination inhibition (HAI). RT-PCR is likely to be most appropriate).

The reference or composite methods must be well-characterized and validated. You must provide published literature or laboratory data in support of the validation for differentiation of the DEN serotypes. Validation must include LoD and analytical reactivity data. The LoD of the reference assay must be similar to the analytical sensitivity of the submitted device

[3] In this document, we define prospectively collected archived specimens as specimens collected sequentially from all patients meeting study inclusion criteria; specimens must not be selectively included based on known results, and all testing must be conducted with investigators completely blinded to any previous results or patient characteristics. Specimens must be as fresh as possible or appropriately stored.

If you compare your device to an RT-PCR assay, you must follow up with bi-directional sequencing of target amplicons for DEN serotype identification. Sequencing must be performed on both strands of the amplicon (i.e., bi-directional sequencing), must demonstrate that the generated sequence is at least 200 base pairs of an acceptable quality (e.g., a quality score of 40 or higher as measured by PHRED or similar software packages), and must demonstrate that it matches the reference or consensus sequence [Ref. 10 & 11].

It is recommended that you contact the FDA for further information regarding the use of nucleic acid amplification test (NAAT) reference assays and establishing a predetermined algorithm that uses composite reference methods.

(6) Presentation of Clinical Study Results

Analysis must be based on the intended use (i.e., the unit of analysis must be either by individual specimen or by testing an individual's acute and convalescent specimens).

Study analysis must account for all samples collected. Comparisons of device performance against the reference standard must be included as 2 cell by 2 cell tables. Additional analyses must be included for device performance relative to patient characteristics (e.g., subject age, time of specimen collection relative to illness onset, study site, etc). In studies that combine fresh specimens and archived specimens, analyses must compare performance on each specimen type separately and then combined.

All study data must be included in the 510(k) submission as Microsoft Excel, delimited text, or as SAS transport files. Data files must include appropriate annotations or separate codebooks and must include all primary and derived variables (e.g., the result of the clinical reference algorithm for determining DEN diagnosis). Description of the statistical methods applied to the data set must be sufficiently detailed to allow interpretation of the lower estimates of the positive agreement that may be acceptable, and may be discussed with FDA prior to initiating clinical studies.

8. Labeling

DENV serological reagents, like other devices, are subject to statutory requirements for labeling (including sections 201(n) and 502(a) of the FD&C Act; 21 USC § 321(n) and 352(a)). These IVD devices must provide adequate directions for use and adequate warnings and precautions (Section 502(f) of the FD&C Act; 21 USC § 352(f)). Specific labeling requirements for all IVD devices are set forth in 21 CFR 809.10; also see 21 CFR 801.119 where it is stated that IVDs labeled in accordance with 21 CFR 809.10 are deemed to satisfy section 502(f)(1) and 21 CFR part 801.

Your 21 CFR 809.10(b) compliant labeling for DENV serological reagents must also include the information described below. This labeling information helps to mitigate the risks identified previously in this guideline to ensure safe and effective use of these devices. All requirements in 21 CFR 809.10 must be addressed in device labeling even if not mentioned below.

a. Intended Use

The intended use must specify that the device is an aid in the diagnosis of DENV infection. You must also specify the DENV serotypes detected by your assay and any additional specific confirmatory measures that are needed to be taken to confirm your test result if your test is presumptive. The intended use must specify the sample type and the specific population for which the test is intended.

b. Device Description

In the device description, you must briefly describe the assay methodology and rationale used in this type of device.

c. Procedure

This section must include a general description of the entire analysis procedure from the collections of patient samples to result reporting.

d. Directions for Use

You must provide clear and concise instructions that delineate the procedures for using the device, and the types of controls that will minimize risks of inaccurate results. Instructions must encourage the use of additional control measures and testing of control materials to ensure use in a safe and effective manner.

e. Warnings, Precautions, and Limitations

In addition to any other limitations and warnings that are relevant to your specific assay, you must include statements such as the following under Limitations, as applicable:

- That the device cannot differentiate between different flaviviruses antibodies or antigens.

- Serological test results are presumptive and require confirmation by Plaque Reduction Neutralization Test or by other methods recommended in the current CDC guidelines for diagnosing this disease.

- Testing should only be performed on patients with clinical symptoms consistent with Dengue fever or Dengue hemorrhagic fever.

- This test is not intended for prenatal screening, or for the general population screening without symptoms consistent Dengue fever. The test is not FDA cleared for the screening of blood or plasma donors.

- The performance of this test has not been established for monitoring treatment of dengue.

- The positive predictive value depends on the likelihood of the virus being present.

- Results from immunosuppressed patients must be interpreted with caution.

- Serological cross-reactivity across the flavivirus group is common (i.e., between West Nile, St. Louis encephalitis Murray Valley encephalitis, Japanese encephalitis, and yellow fever viruses).

- IgM antibodies to dengue may persist for months after infection has resolved.

- Positive results should be interpreted in the context of clinical and other laboratory findings and may not indicate active dengue virus induced disease.

- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.

f. Specimen Collection

You must provide guidance on what type of specimens should be used and the optimal time or window for collecting them (antigen or antibody). You must state if there are any inappropriate specimens that should not be used in this assay. You must also provide recommendations for specimen storage, number of freeze/thaw cycles, and transport conditions that are optimal/acceptable for your device. For virus or antigen testing, it must also be noted in labeling that samples should be collected as soon as possible after the onset of symptoms. Samples for antibody testing are usually collected at days 3-5 after onset of fever.

g. Interpretation of Test Results

The interpretation of test results section in the package insert must list all possible assay outputs and determinations of the presence or absence of DENV antigen or antibody and the expected result of the assay controls. If internal controls are part of the determination of valid positive and negative results, you must provide the interpretation of each possible control result and a recommendation for how to follow up any invalid or indeterminate result.

If your assay has an equivocal zone, you must provide the interpretation and the recommendation for how to follow up the equivocal result. (e.g., whether the equivocal result should be reported as such, or whether testing should be repeated). If your

interpretation of the results requires repeat testing of an invalid or equivocal result, you must provide the recommendation whether testing should be repeated and how repeat testing should be performed (e.g., on the same or a different specimen from the same patient).

Final assay results must be reported as positive, negative, or equivocal (as appropriate). Depending on test performance or other device-specific factors, additional qualification may be necessary.

9. Postmarket Measures

As part of your good manufacturing practices performed as part of complying with the Quality Systems regulations under 21 CFR Part 820, you must annually obtain and analyze postmarket data to ensure the continued reliability of your device for detecting different DENV strains and serotypes that may evolve over time. This is particularly true if new DENV strains emerge, or if DENVs that are less common at the time of your device clearance become more prevalent. Postmarket data must address the clinical performance of your device with new DENV strains.

To demonstrate how you will address this aspect of the special control, you must provide a plan with your 510(k) that describes how you intend to assure that the performance characteristics of your device remain unchanged over time. This plan is likely to include periodic testing of highly prevalent DENV strains at defined time intervals with your device. FDA will evaluate whether this plan will help to mitigate the risks presented by the device and therefore help to provide reasonable assurance of the safety and effectiveness of the device.

10. References

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