Guidance for Industry and FDA Staff

Class II Special Controls Guidance Document: Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA

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Division of Microbiology Devices

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Preface

Public Comment

Written comments and suggestions may be submitted at any time for Agency consideration to Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. Alternatively, electronic comments may be submitted to http://www.regulations.gov. Please identify your comments with the docket number listed in the notice of availability that publishes in the *Federal Register* announcing the availability of this guidance document. Comments may not be acted upon by the Agency until the document is next revised or updated.

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Table of Contents

1. Introduction **4**
2. Background **5**
3. Scope **6**
4. Risks to Health **6**
5. Device Description **8**
6. Performance **11**
7. Labeling **21**
8. References **22**

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1. Introduction

The Food and Drug Administration (FDA) has developed this guidance as a special controls guidance to support the classification of enterovirus nucleic acid assays into class II (special controls). An enterovirus nucleic acid assay is intended for the amplification and qualitative detection of enterovirus RNA in human cerebrospinal fluid (CSF). Detection of enterovirus RNA, in conjunction with other laboratory tests such as bacterial Gram stain, bacterial culture, CSF glucose, CSF-blood glucose ratio, CSF protein concentration, and CSF leukocyte count, or other laboratory detection methods for enterovirus, aids in the clinical laboratory diagnosis of enterovirus infection in patients with clinical signs and symptoms of meningitis or meningoencephalitis. The assay includes primers, probes, enzymes (reverse transcriptase and DNA polymerase) and specific enterovirus internal and external controls. Some of these assays are designed for use only with specific instrument systems.

An enterovirus nucleic acid assay for the qualitative detection of enterovirus RNA is used for detecting enterovirus generically. Sponsors of this type of assay should demonstrate that the assay can detect all serotypes (currently 64 serotypes) that have been associated with aseptic meningitis, e.g., enterovirus, coxsackievirus, echovirus, and poliovirus. Positive results with this assay do not rule out other causes of meningitis, including bacteria, mycobacteria, other viruses (e.g., herpes family viruses, arboviruses, mumps virus) and fungi.

This guidance is issued in conjunction with a *Federal Register* notice announcing the classification of enterovirus nucleic acid assays. Designation of this document as a special control means that any firm submitting a 510(k) for an enterovirus nucleic acid assay, will need to address the issues covered in this guidance. The firm must show that its device addresses the issues of safety and effectiveness identified in this guidance, either by meeting the recommendations of this guidance or by some other means that provides equivalent assurances of safety and effectiveness.

This guidance document identifies the classification regulation and product code for an enterovirus nucleic acid assay (Section 3 – Scope). Other sections of this guidance document list the risks to health identified by FDA and describe measures that, if followed by manufacturers and combined with the general controls will generally address the risks associated with these assays and lead to a timely premarket notification review and clearance.

The Least Burdensome Approach

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

2. Background

FDA believes that special controls, when combined with the general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of enterovirus nucleic acid assays for the qualitative detection of enterovirus RNA in cerebrospinal fluid specimens. A manufacturer who intends to market a device of this type should (1) conform to the general controls of the Federal Food, Drug, and Cosmetic Act (the Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific risks to health associated with enterovirus nucleic acid assays, and (3) obtain a substantial equivalence determination from FDA prior to marketing the device.

This document supplements other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87 and other FDA documents on this topic, such as **Premarket Notification: 510(k)**, available on the web at: http://www.fda.gov/cdrh/devadvice/314.html. There are three types of Premarket Notification 510(k)s that may be submitted to FDA: Traditional, Special, and Abbreviated. The Special and Abbreviated 510(k) methods were developed to help streamline the 510(k) review process and are explained in **"The New 510(k) Paradigm – Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance"** (http://www.fda.gov/cdrh/ode/parad510.html). Guidance on the content and format for abbreviated and traditional 510(k)s is available at http://www.fda.gov/cdrh/ode/guidance/1567.html. Information regarding the use of standards can be found in section 514(c)(1)(B) of the Act (21 U.S.C. 360d(c)(1)(B)), and in the FDA guidance, "Use of Standards in Substantial Equivalence Determinations," http://www.fda.gov/cdrh/ode/guidance/1131.pdf. The Special 510(k) is an option for

manufacturers considering modifications to their own cleared devices. Information on how to prepare a Special 510(k) is available at http://www.fda.gov/cdrh/devadvice/3144.html.

3. Scope

The scope of this document is limited to the following device, described in 21 CFR 866.3225 (product code OAI):

21 CFR 866.3225 – Enterovirus nucleic acid assay.

(a) An enterovirus nucleic acid assay is a device that consists of primers, probes, enzymes and controls for the amplification and detection of enterovirus RNA in cerebrospinal fluid (CSF) from individuals who have signs and symptoms consistent with meningitis or meningoencephalitis. The detection of enterovirus RNA, in conjunction with other laboratory tests, aids in the clinical laboratory diagnosis of viral meningitis caused by enterovirus.

(b) Classification. Class II (special controls). The special control is FDA's guidance document entitled "Class II Special Controls Guidance Document: Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA." See § 866.1(e) for the availability of this guidance document.

This special controls guidance recommends specific information for mitigating risks identified in the following section (Section 4 - Risks to Health).

This guidance does *not* address devices intended for testing asymptomatic individuals (i.e., screening). Different types of study designs would be appropriate for an intended use that includes screening.

This guidance also does not fully address tests for matrices other than CSF. Additional studies would be called for in these situations.

FDA has developed draft guidance regarding the type of information generally recommended for inclusion in a 510(k) for reagents used in nucleic acid amplification tests. We suggest that manufacturers also consult that guidance when it is finalized [Ref. 1].

4. Risks to Health

Meningitis or meningoencephalitis can be caused by bacteria, viruses and, less commonly in the US, by fungi and protozoans. Bacterial meningitis is the more severe and potentially life-threatening form, whereas viral meningitis, typically caused by enterovirus or herpesvirus, usually self-resolves within 7-10 days [Ref. 2]. About 90% of viral meningitis cases are caused by enteroviruses [Ref. 2, 3], and some authors have suggested that enteroviruses are the most common cause of meningitis in the US, with an estimated 30,000 to 50,000 hospitalizations each

year [Ref. 4]. Taxonomically, enterovirus is classified as those viruses consisting of polioviruses, coxsackieviruses, echoviruses, and enteroviruses [Ref. 5, 6].

Currently, for a patient with a clinical suspicion of meningitis or meningoencephalitis, a spinal tap is performed to determine the etiology of the disease, and patients are treated prophylactically with antibiotics and possibly with antiviral drugs until bacterial and herpesvirus viral culture results become available (2-3 days) and bacterial infection is ruled out [Ref. 7]. A rapid, enterovirus nucleic acid amplification assay for the detection of enterovirus RNA in cerebrospinal fluid specimens in conjunction with other CSF laboratory tests such as bacterial Gram stain, bacterial culture, CSF glucose, CSF blood glucose ratio, CSF protein concentration, CSF leukocyte count, or other laboratory methods for enterovirus detection, can help physicians quickly identify patients with viral meningitis caused by enterovirus, thereby preventing the unnecessary use of antibiotics and the possibility of a repeat spinal tap. As with other diagnostic tests, enterovirus test results should be interpreted in the context of other clinical findings and laboratory tests. If test results are inconsistent with clinical and traditional laboratory findings, patients should be managed according to current practice for meningitis.

Failure of nucleic acid assays for detection of enterovirus RNA to perform as expected, or failure to interpret results correctly, may lead to incorrect patient management decisions:

- A false negative report could lead to delays in providing (or even failure to provide) a definitive diagnosis, and the unnecessary treatment of the patient with antibiotics. A false positive report could lead to a delayed treatment of bacterial meningitis or other forms of meningitis. This delayed treatment due to a false positive result for enterovirus could cause progression of potentially life-threatening bacterial meningitis with subsequent severe morbidity to the patient and potentially even patient death.

- Device failure leading to no result (for example, due to failure of reagents, instrumentation, data management, or software) or an invalid or equivocal result could delay diagnosis, and could require an additional collection of CSF fluid, a procedure which is associated with the risk of infection.

Furthermore, the appearance of new serotypes of enterovirus may affect the performance of an enterovirus nucleic acid amplification assay for the detection of enterovirus RNA in CSF specimens. Primers and probes for detection of enteroviruses are selected for their homology with highly conserved regions within viral RNA segments that are present in most enterovirus serotypes. Primers and probes might not detect new serotypes that appear over time. In addition, test performance can be affected, as the epidemiology and pathology of disease caused by the new enterovirus serotypes could change.

FDA has identified the risks to health generally associated with the use of nucleic acid amplification assays for the detection of enterovirus RNA. Measures recommended to mitigate these identified risks are given in this guidance document, as shown in the table below.

We recommend that you conduct a risk analysis, prior to submitting your premarket notification, to identify any other risks specific to your device. The premarket notification

should describe the risk analysis method. If you elect to use an alternative approach to address a particular risk identified in this document, or have identified risks additional to those in this document, you should provide sufficient details to support the approach you have used to address that risk.

Identified risk	Recommended mitigation measures
Failure of the test to perform properly a. False positive detection of enterovirus b. False negative result c. Delayed results and requirement for new patient specimen collection d. Inability to detect viral RNA from new enterovirus serotypes	Sections 5-6
Failure to properly interpret test results	Section 7

5. Device Description

In your 510(k) submission, you should identify the regulation, the product code, and a legally marketed predicate device. In order to help FDA efficiently review all the aspects of your device compared with the predicate, we recommend you include a table that outlines the similarities and differences between the predicate device and your device.

Key issues in the review of a new device are the specific intended use, the type of specimens tested, the method for nucleic acid isolation, and the technology utilized for amplification and signal detection. You may submit appropriate peer-reviewed literature references relevant to the technology of the device, in addition to the descriptive information, to adequately describe the new device.

You should include the following descriptive information to adequately characterize your nucleic acid amplification assay for the detection of enterovirus RNA:

A. Intended Use

Your 510(k) must include labeling that describes the intended use of your product. (See 21 CFR 807.87(e).) The intended use should include the patient population to be tested, specimen types for which testing will be indicated (e.g., CSF), and any specific conditions of use. You should ensure that all elements of the intended use are clearly stated, particularly regarding the enterovirus serotypes the device is intended to detect (e.g., enterovirus, echovirus, coxsackievirus, poliovirus).

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

- The identity, phylogenetic relationship, or other recognized characterization of the serotypes of enterovirus that your device is designed to detect.

- How the device test result is intended to be used in a diagnostic algorithm, and other measures that would be needed for a laboratory identification of enterovirus RNA in suspected aseptic meningitis.

- Clinical and epidemiological parameters that are relevant to a patient case diagnosis (e.g., bacterial Gram stain, bacterial culture, CSF glucose, CSF-blood glucose ratio, CSF protein concentration, and CSF leukocyte count) [Ref. 5-8].

Note: Recognized laboratory methods for the definitive identification of enteroviruses are available. The use of multiple specimen types for diagnostic truth determination might be called for, please see CLSI M41-A Table 3, CSF, stool, respiratory swabs and fluids [Ref. 8].

B. Reagents and Other Device Components

When describing reagents and other device components in your 510(k), we recommend that you follow general guidance provided in other FDA guidance documents. FDA has developed a draft guidance regarding Nucleic Acid Amplification Testing, which will be particularly relevant when it is finalized [Ref. 1]. Additionally, you should describe your design requirements for the device that address or mitigate risks associated with primers, probes, instruments, and controls used in a nucleic-acid based test procedure to detect viral RNA segments from enteroviruses. (The performance data in the 510(k) should support the conclusion that design requirements have been met.) Examples include:

- Designing your reagent for use in a closed tube test system (e.g., self containing cartridge), to minimize false positives due to amplicon or carryover contamination.

- Developing methods for extraction and purification that yield suitable quality and quantity of viral RNA from CSF for use in the test system with your reagents.

- Optimizing your reagents and test procedure for recommended instruments.

We recommend that you include illustrations or photographs of any non-standard equipment or methods, if applicable.

You should provide appropriate literature to support that a conserved RNA segment within any enteroviral serotype genome will be detected.

Ancillary reagents

Ancillary reagents are those reagents that a manufacturer specifies 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

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 Brand X DNA amplification enzyme, and use of any other DNA amplification enzyme may alter the performance characteristics of your device from that reported in your labeling, then Brand X DNA amplification enzyme is an ancillary reagent of concern for the purposes of this document.[1]

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 should 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 should address the elements 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 should 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. Using your risk assessment as a basis for applicability, you should 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.
   - 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.

[1] 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. If you are unsure whether this aspect of the special controls applies to your device, we recommend you consult with the FDA.

- Any other issues that must 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 should 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 FDA for advice.

C. Testing Procedures Using your Device

In your 510(k), you should provide a detailed description of the principles of operation, including those for detecting and differentiating nucleic acids from enterovirus, for your device. You should specifically describe testing conditions, procedures, and controls designed to safeguard against conditions that can cause false positive and false negative results. These include, but are not limited to:

- Overall design of the testing procedure, including control elements incorporated into the recommended testing procedures. Control materials should approximate the lower range of clinically relevant viral RNA levels and should be extracted as a clinical sample.

- Description of, or recommendations for, any internal controls (e.g., internal controls that monitor contamination, extraction efficiency, and amplification inhibition).

- Features and additional controls that monitor procedural errors or factors (e.g., degradation of the master mix) that adversely affect amplification and detection.

We recommend that you include a description of all additional procedures, methods, and practices incorporated into your directions for use (See Section 7 - Labeling) that mitigate risks associated with testing for enteroviruses.

D. Interpreting Test Results/Reporting

In your 510(k), you should describe how positive, negative, equivocal, or invalid results are determined and how they should be interpreted. In addition, you should describe how you monitor results over time to identify changes in performance due to biological changes within the virus serotype classifications, or changes in performance when the prevalence changes from the existing prevalence at the time your product is evaluated.

6. Performance

A. General Study Recommendations

In your 510(k) you should provide detailed descriptive information on the studies you conducted to establish each of the performance characteristics outlined below. In general, for the analytical studies to establish precision and for the clinical studies, we recommend that you conduct testing at 3 sites, representative of where you intend to market the device (e.g., clinical laboratory sites.)

You should evaluate performance of your assay, for each specimen type that you recommend for your assay.

In order to accurately interpret acceptance criteria or data summaries during the review, we recommend that you provide appropriate specific information concerning protocols. This information is also important to aid users in understanding information in your labeling. For example, when referring to CLSI (Clinical and Laboratory Standards Institute) protocols or guidelines, we recommend that you indicate which specific aspects of the protocols or guidelines were followed.

We recommend that you contact the Division of Microbiology Devices in the Office of *In Vitro* Diagnostic Device Evaluation and Safety (OIVD) to obtain feedback regarding your planned study and the clinical claims you intend to support. Sponsors may contact OIVD to obtain feedback concerning study plans.

B. Controls

When conducting the performance studies described below, we recommend that you run appropriate external controls every day of testing for the duration of the analytical and clinical studies. Examples of appropriate external controls include vaccine or prototypic vaccine strains, low pathogenic viruses, and inactivated viruses. You may contact OIVD's Division of Microbiology Devices at FDA for further information regarding controls. For devices based on nucleic acid technology, we generally recommend that you include the following types of controls:

Negative Controls

Blanks or no template control

The blank, or "no-template" control, contains buffer or sample transport media and all of the assay components except nucleic acid. These controls are used to rule out contamination with target nucleic acid or increased background in the amplification reaction. It may not be needed for assays performed in single test disposable cartridges or tubes.

Negative sample control

The negative sample control contains non-target nucleic acid or, if used to evaluate extraction procedures, it contains the whole organism. It reveals non-specific priming or detection and indicates that signals are not obtained in the absence of target sequences. Examples of acceptable negative sample control materials include:

- Patient specimen from a non-enterovirus infected individual
- Samples containing a non-target organism (e.g., cell line infected with non-enterovirus)

- Surrogate negative control, e.g., alien encapsidated RNA [Ref. 9]

Positive Controls

Positive control for complete assay

The positive control contains target nucleic acids, and is used to control the entire assay process, including RNA extraction, amplification, and detection. It is designed to mimic a patient specimen and is run 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:

- Cell lines infected with an enterovirus strain
- Packaged enterovirus RNA

Positive control for amplification/detection

The positive control for amplification/detection contains purified target nucleic acid at or near the limit of detection for a qualitative assay. It controls the integrity of the patient sample and the reaction components when negative results are obtained. It indicates that the target is detected if it is present in the sample.

Internal Control

The internal control is a non-target nucleic acid sequence that is co-extracted and co-amplified with the target nucleic acid. It controls for integrity of the reagents (polymerase, primers, etc.), equipment function (thermal cycler), and the presence of inhibitors in the samples. Examples of acceptable internal control materials include human nucleic acid co-extracted with the enterovirus virus and primers amplifying human housekeeping genes (e.g., RNaseP, β-actin). The need for this control is determined on a device case-by-case basis [Ref. 10].

C. Performance Studies

We recommend that you establish the following performance characteristics for your enterovirus (EV) assay in your 510(k):

1. Analytical sensitivity.

Limit of Detection

We recommend that you determine the limit of detection (LoD) for each of the five EV species CVA6 (A), CVA9 (B), CVA17 (C), EV70 (D) and PV1 (poliovirus) using limited dilutions of regrown and retitered viral stocks. The study should include serial dilutions of a representative of each of the five serotypes and 3-5 replicates for each dilution made in pooled EV negative human sample or equivalent matrix. You should report the LoD as the level of virus that gives a 95% detection rate. The LoD should be confirmed by preparing at least 20 additional replicates at the LoD concentration and demonstrating that the virus was detected 95% of the time. Recommended reference methods for LoD determination are the tissue culture infectious dose$_{50}$ (TCID$_{50}$) and plaque assay. Since the nucleic acid based

devices detect not only the infective viral particles but the total viral RNA present in the tested specimen an additional reference method quantifying nucleic acids (e.g. genome copy equivalent or ug/mL of viral RNA) can also be included.

We recommend that you determine the LoD for each analyte in the most commonly used or most challenging matrix tested by the device. We suggest that you refer to CLSI document EP17-A [Ref. 11], when designing your studies.

Analytical Reactivity (Inclusivity)

We recommend that you demonstrate that the test can detect all EV serotypes that your primer and probes are designed to detect. The dilutions should be made in pooled EV negative human sample or equivalent matrix. All virus identities and titers should be confirmed.

2. Analytical Specificity

Cross-reactivity

We recommend that you test for potential cross-reactivity with other relevant microorganisms, including bacteria, viruses and parasites. In particular, you should characterize performance of the test in the presence of whole microorganisms that may present similar clinical symptoms and may be confused with EV infection, e.g., EBV, HSV-1, HSV-2, HHV-6, HHV-7, Adenovirus-2, Measles, Mumps, Parainfluenza 1-4, Influenza A, Influenza B, VZV, CMV, Group B *Streptococcus, Haemophilus influenzae* B, *H. influenzae* non-B, *Escherichia coli, Neisseria meningitides, Citrobacter freundii,* and *Citrobacter koseri.* In addition, high concentrations of both intact white blood cells and total RNA isolated from white blood cells should be evaluated for cross-reactivity. We recommend that you test medically relevant levels of viruses and bacteria (usually 10^6 cfu/ml or higher for bacteria and 10^5 pfu/ml or higher for viruses). We recommend that you confirm the virus and bacteria identities and titers.

Interference

We recommend that you conduct a comprehensive interference study using medically relevant concentrations of the interferent to assess the potentially inhibitory effects of substances encountered in the indicated human specimens.

Potentially interfering substances include, but are not limited to, the following: other viral or bacterial agents which may be co-infecting the patient, 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. We recommend that you test interference at the assay cut-off determined for the utilized EV serotype. We also recommend that you 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. 12] for additional information.

3. Precision

Within-Laboratory Precision/Repeatability

We recommend that you conduct within-laboratory precision studies for devices that include instruments or automated components. You may perform these studies in-house, i.e., within your own company.

We recommend that you test sources of variability (such as operators, days, assay runs) for a minimum of 12 days (not necessarily consecutive), with 2 operators, each performing 2 runs per day, and 2 replicates of each sample per run. These test days should span at least two calibration cycles, if the calibration cycle is shorter than 2 months. We recommend that the test panel consist of 3-6 samples (1-2 viral strains) at three levels of viral load that include:

- A "high negative" sample (C_5 concentration): 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 (and results are positive approximately 5% of the time).

- A "low positive" sample (C_{95} concentration): 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 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 clinical cut-off).

When the limit of blank (LoB) is used as a cutoff, then the concentration C_{95} is the same as the limit of detection (LoD) and the zero concentration (no analyte present in sample) is C_5 if LoB is established with Type I error of 5%.[2] For details, see EP17-A [Ref. 11]. CLSI documents EP5-A2 [Ref. 13] and EP12-A [Ref. 14] contain further information about designing and performing precision studies.

Reproducibility

As a general guide, we recommend the following protocol to evaluate reproducibility, although specifics may vary depending on the assay format.

- Evaluate the reproducibility of your test at 3 testing sites (for example, at least two external sites and one in-house site).

- 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.

[2] Type I error is the probability of having truly negative samples (those with zero concentration) give values that indicate presence of analyte. Usually, Type I error is set at 5% or less.

- Each day, have at least two operators at each facility perform the test. We recommend that, for rapid testing or point-of-care (POC)[3] devices, you include a larger number of devices in your evaluation, in order to best represent the settings in which the devices will be used.

- Use the same sample panel as described in the repeatability study above.

The CLSI document, EP15-A2 [Ref. 15], contains additional information on reproducibility study design.

4. Carry-Over/Cross-contamination Studies (for multi-sample assays and devices that require instrumentation)

We recommend that you demonstrate that carry-over and cross-contamination does not occur with your device (including the nucleic acid extraction method). In a carry-over study, high positive samples should be used in series alternating with high negative samples in patterns dependent on the operational function of the device. You should include at least 5 runs with alternating high positive and high negative samples during the carry-over study. The high positive sample in the carry-over study should be high enough to exceed 95% or more of the results obtained from specimens of diseased patients from the intended use population. The high negative samples should contain the analyte concentration below the cut-off such that repeat testing of this sample is negative approximately 95% of the time. The carry-over effect can then be estimated by the percent of negative results for the high negative sample in the carry-over study compared with 95%. For details, see [Ref. 16].

5. Specimen Collection, Handling, Storage, and Shipping Conditions

If you recommend specimen collection, transport, and storage conditions, you should demonstrate that your device generates equivalent results using specimens that are handled in the same manner as recommended in the device package insert. For the specimen storage conditions you should demonstrate that your device generates equivalent results for the stored specimens at several time points throughout the duration of the recommended storage and at both ends of your recommended temperature range. If viral transport medium (VTM) is recommended for storage or shipping, you should conduct appropriate studies to demonstrate that the device will perform as described when the specimen is preserved in VTM [Ref. 7].

6. Nucleic Acid Extraction Methods

You should conduct analytical and clinical studies to demonstrate the efficiency and reproducibility of your recommended nucleic acid extraction procedure(s) for use for CSF or other specimen types. These analytical studies should include determination of the Limit of

[3] Point-of-care tests, also known as bedside or near-patient tests, are tests intended to be performed in an alternate site, outside a central laboratory environment, generally nearer to, or at the site of, the patient.

Detection (LoD) for each claimed specimen type using known infectious virus concentrations, e.g., plaque forming units (pfu) or 50% Tissue Culture Infectious Dose ($TCID_{50}$), as well as reproducibility studies for each claimed specimen type. Recommendations for conducting the LoD study are provided under "Limit of Detection" (see limit of detection, Section 6.c.1, above). The reproducibility evaluation for the nucleic acid extraction should be conducted at three sites (e.g., two external and one in-house site) in the matrix specified in your labeling, at virus concentrations near the clinical cut-off. We recommend you 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. The test panel should consist of 3-6 samples (containing 1 to 2 clinically significant serotypes [strains, types, or serotypes as appropriate]) at three viral load levels that include. See Section 6.c.3., above for recommendations concerning the reproducibility study.

If you choose to obtain clearance for multiple extraction methods, you should demonstrate the LoD and reproducibility for each method. With the assumption that the extraction method introduces minimum variability to the overall assay performance you may wish to consider combining the extraction method variable with each site performance variable. For example, if you recommend three different extraction methods, you can design a reproducibility study by evaluating one of the three extraction methods at each testing site: test extraction method A at site 1, method B at site 2, and method C at site 3. If the results generated from the test panel mentioned above do not show significant differences, no further reproducibility studies are needed. However, if the initial extraction equivalency studies from the three sites indicate statistically significant differences in assay performance, the reproducibility study should be expanded to include testing each extraction method at three study sites (e.g., site 1 extraction method A, site 2 extraction method A, and site 3 extraction method A).

In addition to the analytical studies (LoD and Reproducibility), each extraction method (instrument) should be utilized in at least one clinical site during the clinical trials to generate clinical performance data. If results from the expanded reproducibility testing indicate a significant difference in efficiency among the extraction methods, the data from each clinical testing site (using a different NA extraction method) are not considered equivalent and should not be pooled, but rather analyzed separately. As a result, additional prospective clinical samples may be needed in order to obtain a statistically significant number of prospective samples for each claimed extraction method.

When the limit of blank (LoB) is used as a cutoff, then the concentration C_{95} is the same as the limit of detection (LoD) and the zero concentration (no analyte present in sample) is C_5 if LoB is established with Type 1 error of 5%. For details, see EP17-A [Ref. 11]). CLSI documents EP5-A2 [Ref. 13] and EP12-A [Ref. 14] contain further information about designing and performing precision studies.

7. Prevalence (Expected Values)

You should establish the prevalence of EV in an endemic population with signs and symptoms consistent with meningitis or meningoencephalitis. You should assay a statistically relevant number of specimens that are representative of the intended use including the specified sample

matrix. You should provide these results based on your new device performance, and summarize the distribution of the population according to age group (infants <1, children 1-5, 6-10, 11-15, 16-21, and adults ≥ 21 years) and gender. Since this device is not intended for use in screening blood or tissue donors, samples from these donors should not be used for this study.

8. Clinical studies

You should conduct prospective clinical studies to determine the performance of your device for all the specimen types you claim in your labeling. You should prospectively collect the specimens from individuals with signs and symptoms consistent with clinical suspicion of meningitis or meningoencephalitis. You should describe the protocol of each clinical study (including the inclusion and exclusion criteria, study endpoints, acceptance criteria), and a description of how the studies support the proposed intended use. You should include a sufficient number of samples so that results will be statistically and clinically meaningful. Archived samples may be useful to provide specimens from patients who have symptoms of meningitis, and from whom fresh specimens may not be readily available (e.g., CSF from very young patients). When using the archived specimens, selection protocols should be used to minimize bias, and appropriate archives should be selected. Furthermore, samples should be masked to avoid testing bias. If both fresh and archived frozen samples are tested, we recommend that you analyze the data separately. For archived samples, results should be represented as percent agreement.

We recommend that you assess and compare the performance of your device to a predetermined algorithm that uses composite reference methods. Additionally, your device should also be compared to EV viral culture. The composite reference methods should include laboratory results such as:

1. Methods that provide clinical evidence consistent with meningitis, for example, laboratory results such as CSF Gram stain, CSF bacterial culture, CSF glucose, CSF-blood glucose ratio, CSF total protein concentration, CSF leukocyte count. Results from additional specimen types, e.g., stool specimen may also be part of the composite reference method.

2. Detection of an EV genome in CSF by two different well-characterized and validated nucleic acid amplification tests (NAAT). The NAAT primers pairs should generate amplicons from different genomic regions. One of the NAAT assays should provide sequence information. Bi-directional sequencing should be performed on both strands of the amplicon and the generated sequence should be of an acceptable quality (quality score of 40 or higher as measured by PHRED or similar software packages) and should match the reference or consensus sequence [Ref. 10, 17].

You should establish the analytical performances of the NAAT followed by sequencing analysis reference assays by performing analytical sensitivity (LoD) and reactivity studies as described in the "Performance" section of this special control guidance (Section 6.c., above).

You should evaluate literature and/or other validation data for establishing and describing the performances of the NAAT followed by sequencing analysis reference assays prior to clinical testing. You should include in your 510(k) validation data from literature to justify your selection of primers for the NAAT followed by sequencing analysis reference assays. You should also include primer sequences, target region of the genome, BLAST results, and sequence alignments. This information is important to demonstrate that the reference methods target conserved regions of the EV genome and react broadly in detecting EV serotypes. As a part of the pre-market application, primer sequences, target region of the genome, BLAST results, and sequence alignments should also be submitted to the FDA. Appropriate controls, including a positive control that has a low genomic copy number of the virus, a negative control and an independent inhibition control measure, should be incorporated into each of the NAAT followed by sequencing analysis reference assays.

You may contact the Division of Microbiology Devices within the OIVD for further information regarding establishing a predetermined algorithm that uses composite reference methods.

Study Protocol

We recommend that you develop a detailed study protocol that includes patient inclusion and exclusion criteria, type and number of specimens needed, directions for use, and a statistical analysis plan that accounts for variances to prevent data bias. We recommend that you include this, and any other relevant protocol information, in your 510(k).

We encourage sponsors to contact the Division of Microbiology Devices to request a review of your proposed studies and selection of specimen types. This is referred to as the pre-IDE process. We particularly encourage manufacturers to seek this type of discussion when samples are difficult to obtain or sponsors are planning to submit a premarket notification for the first time.

Specimen Type(s)

The total number of samples you should include in your study for substantiating a claim for the detection of EV will depend on the prevalence of the virus and on the assay performance.

For devices detecting EV RNA, we recommend that you include a sufficient number of prospectively collected samples, for each specimen type you claim to generate a result of at least 90% sensitivity with a lower bound of the two-sided 95% CI greater than 80%. All EV RNA detecting devices should demonstrate specificity with a lower bound of the two-sided 95% CI greater than 90%.

If you have questions regarding the choice of appropriate specimen type(s) and numbers, please contact the Division of Microbiology Devices.

Study Sites

We recommend that you conduct your studies at a minimum of three different geographical sites representing the testing environment where the device will ultimately be used (e.g., clinical laboratory) by individuals who will use the test in clinical practice. At least one of the study sites should be a US site. One of the study sites may be in-house.

Clinical investigations of unapproved and un-cleared in vitro diagnostic devices are subject to the investigational device exemption (IDE) provisions of Section 520(g) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360j) and implementing regulations. You should consider how 21 CFR part 812 (IDEs) applies to your particular study and refer to part 50 (informed consent), and part 56 (institutional review boards review) for other applicable requirements. Clinical investigations of significant risk devices require the submission of an IDE application to FDA for review and approval, in accordance with 21 CFR part 812. You may also refer to the "Information Sheet Guidance for IRBs, Clinical Investigators, and Sponsors, Significant risk and Nonsignificant Risk Medical Device Studies" at http://www.fda.gov/oc/ohrt/irbs/devrisk.pdf, "Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" at http://www.fda.gov/cdrh/oivd/guidance/1588.pdf and "In Vitro Diagnostic (IVD) Device Studies-Frequently Asked Questions "at http://www.fda.gov/cdrh/oivd/guidance/1587.pdf.

Study Population

We recommend that you conduct your studies on individuals presenting with signs and symptoms of meningitis or meningoencephalitis. We recommend that you include a meaningful number of samples from each age group. We recommend that you present the data stratified by age (e.g., neonates, 2 months to 21 years, adults 22 years old and older) in addition to the overall data summary table.

Presentation of Results

In your 510(k) you should describe how the samples were selected, and any reasons that samples were excluded.

We recommend that you initially analyze and present data from each study site separately to evaluate any inter-site variation and include results of the analysis in the 510(k). It may be possible to pool clinical study results from the individual sites in the package insert if you can demonstrate that there are no significant statistical or clinical differences in the results or populations among sites.

We also recommend that you analyze and present data separately for prospective clinical samples evaluated against "Clinical Diagnosis Truth" (determined by a predetermined algorithm that uses a composite reference method); banked prospectively collected clinical samples evaluated against "Clinical Diagnosis Truth"; prospective clinical samples evaluated against viral culture; and banked prospectively collected clinical samples evaluated against viral culture.

We recommend that you provide line data, including appropriate daily external control testing data during all clinical studies. You may supply this information electronically using Microsoft EXCEL, delimited text files, or SAS files.

7. Labeling

Final labeling for IVD devices for direct detection of enterovirus RNA in human CSF specimens, like other devices, are subject to statutory requirements for labeling (sections 502 and 201(n) of the Act; 21 USC §§ 352, 321(n)). A nucleic acid amplification assay for detection of enterovirus RNA must include specific labeling, including adequate instructions, warnings, and precautions (21 CFR 809.10). Recommendations below are aimed at assisting you to prepare labeling that satisfies these requirements. They are also recommended as measures to mitigate the risks identified previously in this guidance to help ensure safe and effective use of these devices, particularly when the results of a nucleic acid amplification assay for the detection of enterovirus RNA might be inconsistent with other indications of meningitis [Ref. 6].

Your labeling should clearly describe the identity, phylogenetic relationship, or other recognized characterization of enteroviruses that your device is designed to detect, and the associated clinical aspects of human infection.

Intended Use

In addition to specific elements that describe the analyte detected, your intended use should specify indications for testing CSF specimens from patients with signs and symptoms of meningitis or meningoencephalitis and that the assay should only be used in conjunction with other laboratory testing and clinical observations. FDA also recommends that your statement of intended use be clarified by a warning statement such as: "Positive results do not preclude bacterial or other types of infection and should not be used as the sole basis for treatment or other patient management decisions."

You should place a bolded CAUTION box under the Intended Use and on the kit outer box label to state as follows:

CAUTION: The results obtained with [name of sponsor's assay] should be used only as an adjunct to clinical observation and other information available to the physician. Positive [name of sponsor's assay] results do not rule out other causes of meningitis, including bacteria, mycobacteria, other viruses (e.g. herpes family viruses, arboviruses, mumps virus, etc.) and fungi.

Directions for use

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

Precautions, Warnings, and Limitations

You should clearly describe any assay limitations in the labeling. This section should include the appropriate limitations and warnings that a physician needs to know prior to ordering the test. We recommend that you incorporate directions for reporting results into the Results section, including a reminder to report results to state or local public health departments, if applicable.

Limitations:

In addition to any limitations and warnings that are relevant to your specific assay, you should provide the following types of statements in the Limitations section:

- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte targets (viral sequences) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
- Positive results should be interpreted in conjunction with other laboratory findings (e.g., CSF glucose, CSF Gram stains, CSF protein, CSF leukocytes, etc.), and clinical signs or symptoms. Positive results do NOT rule out other non-EV causes of meningitis. In rare instances, meningitis can be caused by co-infection of a viral and bacterial or other agent. Negative results (e.g., no enteroviral nucleic acid detected) do not exclude other types of viral (e.g., HSV) or bacterial infection.
- Cross-reactivity of this assay with human rhinoviruses may occur, but rhinoviruses should not be present in human cerebrospinal fluid and are not a recognized cause of meningitis.
- Additional testing for enterovirus or bacterial infection may be required.
- Results that are positive for enterovirus RNA do not definitively identify an enteroviral serotype.
- False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may occur if inadequate numbers of virus are present in the specimen.
- Negative results should not be reported if the internal controls fail.
- Positive and negative predictive values are highly dependent on prevalence. False positive test results are likely when prevalence of disease due to enterovirus is low or non-existent in a community, or outside the enteroviral season.
- New serotypes of enterovirus may not be detected.

Prevalence

You should include the prevalence of the test with an explanation of the result. You should also summarize the study used to determine the prevalence, including the number of samples, age, gender, and demographics of the population.

Performance Characteristics

You should include in the package insert a summary of the study designs and the results of the studies described in Sections 6 that would aid users in interpreting test results. This includes clinical and analytical performance characteristics.

8. References

[1] Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens - Draft Guidance for Industry and FDA Staff, DRAFT GUIDANCE, issued on: December 8, 2005; available at http://www.fda.gov/cdrh/oivd/guidance/1560.html

[2] Viral (Aseptic) Meningitis. http://www.cdc.gov/ncidod/dvrd/revb/enterovirus/viral_meningitis.htm. Centers for Disease Control and Prevention: Respiratory and Enteric Viruses Branch. 10/15/2004.

[3] Tunkel AR, Scheld WM. Acute Meningitis. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases, 6th Ed. Philadelphia:Elsevier, 2005:1083-6.

[4] Romero JR, Rotbart HA. Enteroviruses. In: Murray PR, Baron EJ, eds. Manual of Clinical Microbiology 8th edition. Washington, DC: American Society for Microbiology, 2003:1427-1438.

[5] Modlin JF. Coxsackieviruses, Echoviruses, and Newer Enteroviruses. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases, 6th Ed. Philadelphia: Elsevier, 2005: 2148-2161.

[6] Sawyer MH, Rotbart HA. Viral Meningitis and Aseptic Meningitis Syndrome. In: Scheld WM, Whitley RJ, Marra CM, eds. Infections of the Central Nervous System, 3rd ed. Philadelphia, PA: Lippincott, Williams & Wilkins, 2004: 75-93.

[7] Tunkel AR. Approach to the Patient with Central Nervous System Infection. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases, 6th Ed. Philadelphia: Elsevier, 2005:1079-1083.

[8] Clinical and Laboratory Standards Institute. Viral Culture; Approved Guidelines. CLSI document M41-A [ISBN 1562386239]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898; USA 2006; Sections 6.1 – 6.2.

[9] Pasloske BL, Walkerpeach CR, Obermoeller RD, Winkler M, and DuBois DB. Armored RNA Technology for Production of Ribonuclease-Resistant Viral RNA Controls and Standards. J. Clin. Microbiol. 1998; 36:3590-3594.

[10] Clinical and Laboratory Standards Institute. 2006. Molecular Diagnostic Methods for

Infectious Disease; Approved Guideline. CLSI document MM3-A2 [ISBN 1-56238-596-8] Clinical and Laboratory Standards Institute, Wayne PA.

[11] Clinical and Laboratory Standards Institute. 2004. Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI document EP17-A [ISBN 1-56238-551-8] Clinical and Laboratory Standards Institute, Wayne PA.

[12] Clinical and Laboratory Standards Institute. 2005. Interference Testing in Clinical Chemistry; Approved Guideline. EP7-A2. Clinical and Laboratory Standards Institute, Wayne PA.

[13] Clinical and Laboratory Standards Institute. 2004. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. EP5-A2. Clinical and Laboratory Standards Institute, Wayne PA.

[14] Clinical and Laboratory Standards Institute. 2002. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. EP12-A. Clinical and Laboratory Standards Institute, Wayne PA.

[15] Clinical and Laboratory Standards Institute. 2008. User Verification of Performance for Precision and Trueness; Approved Guideline-Second Edition. EP15-A2. Clinical and Laboratory Standards Institute, Wayne PA.

[16] Haeckel R. Proposals for the Description and Measurement of Carry-over Effects in Clinical Chemistry. Pure Appl. Chem. 1991; 63:302-306.

[17] Clinical and Laboratory Standards Institute. 2004. Nucleic Acid Sequencing Methods in diagnostic Laboratory Medicine; Approved Guideline. CLSI document MM9-A [ISBN 1-56238-558-5] Clinical and Laboratory Standards Institute, Wayne PA.