Guidance for Industry and FDA Staff

Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of In Vitro Diagnostic Device Evaluation and Safety
Division of Microbiology Devices

Preface

Public Comment

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

Additional Copies

Additional copies are available from the Internet at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm. You may also send an e-mail request to dsmica@fda.hhs.gov to receive an electronic copy of the guidance or send a FAX request to 301-847-8149 to receive a hard copy. Please use the document number 1669 to identify the guidance you are requesting.

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Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay

1. Introduction

This guidance document was developed as a special controls guidance to support the classification of respiratory viral panel multiplex nucleic acid assays into class II (special controls). A respiratory viral panel multiplex nucleic acid assay is a qualitative in vitro diagnostic device intended to simultaneously detect and identify multiple viral nucleic acids extracted from human respiratory specimens or viral culture. The detection and identification of a specific viral nucleic acid from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection when used in conjunction with other clinical and laboratory findings. The device is intended for detection and identification of a combination of the following viruses:

(1) Influenza A and Influenza B
(2) Influenza A subtype H1 and Influenza A subtype H3
(3) Respiratory Syncytial Virus (RSV) subtype A and Respiratory Syncytial Virus subtype B
(4) Parainfluenza 1, Parainfluenza 2, and Parainfluenza 3 virus
(5) Human Metapneumovirus (hMPV)
(6) Rhinovirus
(7) Adenovirus

This guidance provides recommendations to manufacturers regarding preparation of premarket notifications for respiratory viral panel multiplex nucleic acid assays and to FDA reviewers. The recommendations in this document are applicable to respiratory viral nucleic acid multiplex assays[1] that employ technologies such as polymerase chain reaction (PCR), reverse-transcriptase polymerase chain reaction (RT-PCR), bead-based liquid arrays and microarrays. This guidance is not intended to address the measurement of individual targets determined by separate assays, which are then reported out simultaneously.

[1] In this guidance, multiplex assays are defined as those assays in which two or more targets are assayed through a common process of sample preparation, amplification and/or detection, and interpretation.

This guidance is issued in conjunction with a *Federal Register* notice announcing the classification of a respiratory viral panel multiplex nucleic acid assay. Designation of this document as a special control means that any manufacturer submitting a 510(k) premarket notification for a respiratory viral panel multiplex nucleic acid assay will need to address the issues covered in this special controls guidance, and if applicable, two other special control guidances identified in the classification regulation (see Section 3, below.) 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 a respiratory viral panel multiplex nucleic acid assay (refer to Section 3). Other sections of this guidance identify risks related to this device type and provide recommendations to address these risks. As described below, depending on the specific analytes comprising the respiratory viral panel (RVP), the device may be subject to additional special controls to address issues associated with detection of particular viruses.

If you want to discuss an alternative means of satisfying the requirement of special controls for this device, you may contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

The Least Burdensome Approach

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to follow the statutory and regulatory criteria in the manner suggested by the guidance and in your attempt to 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 document, **"A Suggested Approach to Resolving Least Burdensome Issues."** It is available on our Center web page at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm085994.htm.

2. Background – Premarket Notifications

A manufacturer who intends to market a device of this generic type must

- 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,
- conform to the special control developed for this device, by addressing the specific risks to health identified in this guidance, and, as applicable, issues associated with

detection of specific analytes identified in the two other special control guidance documents for devices in this classification, described below, in Section 3.

- obtain a substantial equivalence determination from FDA prior to marketing the device. (See also 21 CFR 807.81 and 807.87).

FDA believes that special controls, when combined with the general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of respiratory viral panel multiplex nucleic acid assays.

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, the guidance, **Format for Traditional and Abbreviated 510(k)s**[2] and the section of CDRH's Device Advice webpage, **Premarket Notification 510(k)**.[3] As described in **The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**,[4] a manufacturer may submit a Traditional 510(k), an Abbreviated 510(k), or a Special 510(k). A manufacturer may choose to submit an abbreviated 510(k) when a guidance document exists that is relevant to the device, when special controls have been established, or when FDA has recognized a relevant consensus standard. Manufacturers considering certain modifications to their own cleared devices may lessen their regulatory burden by submitting a Special 510(k). For more information on types of Premarket Notification 510(k)s that may be submitted to FDA, see CDRH's Device Advice webpage, **Premarket Notification 510(k).**

3. Devices within the Scope of this Document

The scope of this document is limited to the following devices described in 21 CFR 866.3980 (product code OCC).

21 CFR 866.3980– Respiratory viral panel multiplex nucleic acid assay. A respiratory viral panel multiplex nucleic acid assay is a qualitative in vitro diagnostic device intended to simultaneously detect and identify multiple viral nucleic acids extracted from human respiratory specimens or viral culture. The detection and identification of a specific viral nucleic acid from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection when used in conjunction with other clinical and laboratory findings. The device is intended for detection and identification of a combination of the following viruses:

(1) Influenza A and Influenza B
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(4) Parainfluenza 1, Parainfluenza 2, and Parainfluenza 3 virus
(5) Human Metapneumovirus
(6) Rhinovirus

[2] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.htm
[3] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/default.htm
[4] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080187.htm

(7) Adenovirus

In addition to this guidance document, for respiratory viral panels that include detection and identification of hMPV, an additional special control guidance is **"Class II Special Controls Guidance Document: Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays."** For respiratory viral panels that include detection and differentiation of Influenza A subtypes, an additional special control guidance is **"Class II Special Controls Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Nucleic Acid Assays."**

The following are the additional product codes that may be applicable for devices cleared under 21 CFR 866.3980

OEM – Human metapneumovirus (hMPV) RNA assay system
OEP – Influenza A virus subtype differentiation nucleic acid assay

This guidance addresses respiratory viral panel multiplex nucleic acid assays that are used in conjunction with the clinical presentation and other laboratory tests (e.g., immunofluorescence, bacterial culture, chest x-rays/radiography) to aid in the diagnosis of respiratory virus infection. This guidance does not address assays intended for use as the sole basis for diagnosis nor does it address assays meant to differentially diagnose viral from non-viral infections. For the assays addressed by this guidance, positive results do not rule out bacterial infection, or co-infection with other viruses, and negative results should not be used as the sole basis for diagnosis, treatment, or management decisions and may need to be confirmed by cell culture. (Refer to the Risks to Health-Section 4 for more information.)

A respiratory viral panel multiplex nucleic acid assay may include the use of instrumentation for clinical multiplex test systems. Instrumentation for clinical multiplex test systems is regulated under 21 CFR 862.2570. Guidance for such instrumentation is available in the FDA Guidance for Industry and FDA Staff, **"Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems."**[5] If your respiratory viral panel multiplex nucleic acid assay uses instrumentation for clinical multiplex test systems, the instrument needs to be cleared for use with your assay. You may submit the information for both the assay and the instrumentation within one 510(k), or the instrument manufacturer may submit, in conjunction with the assay premarket notification, a separate 510(k) for instrumentation only. The amount of information needed for clearance of the instrument depends on whether or not the instrument has been cleared before for a similar intended use, and whether or not there are software modifications to accommodate the new assay.

4. Risks to Health

Respiratory illness caused by various commonly circulating respiratory viruses (e.g., Influenza A, RSV) can cause high morbidity and mortality, particularly in at-risk populations such as the elderly and the very young. Therefore, FDA has identified potential risks to health associated with this assay, i.e., issues that may impact safety or effectiveness of a respiratory viral panel multiplex nucleic acid assay. These include failure of the device to

[5] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm077819.htm

perform as indicated, leading to inaccurate results or lack of results, and incorrect interpretation of results; both of these potential risks may lead to incorrect patient management decisions.

Failure of the test to perform as indicated:
A false positive result could lead to unnecessary or inappropriate treatment for the misidentified viral illness, as well as delayed treatment of the actual infection, which may potentially be a more serious infection caused by bacteria or other pathogens. A false negative result could lead to failure to provide a diagnosis and the correct treatment, and may contribute to unnecessary treatment. A lack of result could lead to delayed diagnosis and inadequate treatment. Additionally, for assays that both detect Influenza A and differentiate between Influenza A subtypes, if a specimen yields a positive test result for Influenza A, but produces negative test results for all specific influenza A subtypes intended to be differentiated (i.e., H1 or H3), then local, state or federal public health authorities should be notified to determine whether the specimen represents a novel strain of Influenza A, in accordance with the MMWR notice (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5613a4.htm and http://www.cste.org/ps/2007pdfs/novelfluanndssjan10final23.pdf).[6] Therefore, inaccurate results for influenza types and subtypes included in the respiratory viral panel may lead to inappropriate public health responses, such as unnecessary infection control and prevention actions, or delayed recognition of an outbreak or cluster of influenza A virus.

Failure to interpret results correctly:
A respiratory panel multiplex nucleic acid assay is intended to aid in the diagnosis of respiratory viral infection when used in conjunction with other clinical and laboratory findings. Therefore, failure to interpret assay results in the context of the other laboratory results and the clinical presentation could also lead to inappropriate or delayed treatment. For example, positive assay results do not rule out bacterial co-infection, or co-infection with other viruses, and the virus(es) detected by the assay may not necessarily be the cause of the clinical symptoms or disease. In addition, detection of the nucleic acid of a viral agent may not necessarily indicate active infection. In the case of co-infections, there is a risk of one virus out-competing the virus that is the cause of the high risk infection (e.g., RSV viral pneumonia in infants) and reporting just the lower risk virus (e.g., Rhinovirus), which may not be the cause of the clinical symptoms. For reasons such as these, additional testing (e.g., bacterial culture, immunofluorescence, chest x-rays/radiography) is needed in order to obtain the final diagnosis of respiratory viral infection.

This special control guidance makes recommendations for mitigating the following specific sources of error that can lead to the risks associated with this type of assay:

Inaccurate results (i.e., false positive or false negative results) or lack of result may be attributed to the following:

- Failure or improper use of reagents, instrumentation, data management, or software included with the assay.

[6] Centers for Disease Control and Prevention. 2006-07 Influenza vaccine composition in, "MMWR Recommendations and Reports: Prevention and Control of Influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP)." 2006;55(RR10):1-42. http://www.cdc.gov/flu/professionals/vaccination/composition0607.htm

- Failure or improper use of ancillary reagents or problems with the quality of ancillary reagents.
- Improper testing when performed by laboratory personnel lacking expertise in molecular testing.

False positive results can be caused by the following:

- Persistence of specific viral RNA or DNA sequences in vivo, independent of virus viability, because nucleic acid tests do not distinguish non-viable viruses from infective viral particles (that are discerned by cell culture).
- The potential of assay primers and probes to cross-react with nucleic acid sequences from viruses detected by the assay, other pathogens which may be present in patient specimens, or other endogenous nucleic acids sequences.
- In the case of an open assay system, possibilities of cross-contamination and amplicon contamination if proper control measures are not implemented.

False negative results can be caused by the following:

- Analyte degradation due to improper storage or transport of specimens and extracted nucleic acid, or inadequate extraction of the nucleic acid material.
- The propensity of respiratory viruses to mutate or the emergence of new subtypes. Primers and probes are generally selected for their homology with highly conserved regions within viral RNA or DNA segments. Primers and probes might fail to react with some of the new subtypes or variants that are discovered or have mutated over time, reducing assay performance. The high mutation rates for certain viruses (e.g., influenza A) may affect the assay's ability to detect and identify claimed viruses.
- Competitive inhibition or interference by other substances present in patient specimens or introduced into the analytical system during sample processing / handling.

Failure to properly interpret test results due to:

- Possibility of bacterial co-infection.
- Inaccurate interpretation and reporting of testing results when testing is performed by laboratory personnel lacking expertise in viral diagnosis.
- In the case of co-infections, reporting out the result of a lower risk virus which is out-competing the virus which might be the cause of the high risk infection (i.e., RSV in infants, viral pneumonia)
- Misinterpretation due to performance variability related to seasonal virus prevalence and specific patient populations.

In the table below, FDA has identified the risks to health generally associated with the use of this device. Measures recommended to mitigate the identified risks are described in this guidance document, as shown in the table below. Additional measures to mitigate the risks associated with hMPV and Influenza A subtypes testing are described in the associated guidance documents (see Section 3, above) and should also be addressed when applicable to

your test. You should conduct a risk analysis prior to submitting your premarket notification to identify any other risks specific to your device. Risks may vary depending on the type of nucleic acid assays used, the intended use of the test, the sample type, and how the result will be used. The premarket notification should describe the risk analysis method. If you elect to use an alternative approach to address the risks identified in this document, or have identified risks additional to those in this document, you should provide sufficient detail to support the approach you have used to address that risk.

Identified risk	Recommended mitigation measures
Inaccurate results (i.e., false positive or false negative results), or lack of results	Labeling (Section 7) Performance Characteristics (Sections 6) Device Description (Section 5)
Failure to properly interpret test results	Labeling (Section 7)

5. Device Description

In your 510(k) submission, you should identify the regulation, the product code(s), and a legally marketed predicate device. We recommend that you include a table that outlines the similarities and differences between the predicate and your device.

You should include the following descriptive information to adequately characterize your respiratory viral panel multiplex nucleic acid assay.

5.A Intended Use

The intended use should specify the virus types and/or subtypes the test detects and identifies, the nature of the analyte (e.g., RNA, DNA), specimen types for which testing will be indicated, the clinical indications for which the test is to be used, and the specific population(s) for which the test is intended. The intended use should state that the test is qualitative, whether analyte detection is presumptive, and any specific conditions of use.

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 respiratory viruses that your device is designed to detect.

- How the device results might be used in a diagnostic algorithm and other measures that might be needed for a laboratory identification and diagnosis of the respiratory virus infection.
- Any relevant literature references that describe in detail important information related to features of the viruses in the panel that may affect the performance characteristics of the device (e.g., cross-reactivity with other viruses, mutation frequency).

5.B Test Methodology

You should describe in detail the methodology used by your device. For example, you should describe the following elements, as applicable to your device:

- Test platform (e.g., RT-PCR, bead arrays).
- Specificity of probes for the pathogen sequences of interest.
- Information and rationale for selection of specific target sequences and the methods used to design primers and probes.
- Limiting factors of the assay (e.g., saturation level of hybridization, maximum cycle number).
- Sample type (e.g., swabs, aspirates, and viral culture media), collection and handling methods.
- Reagent components provided or recommended for use, and their function within the system (e.g., buffers, enzymes, fluorescent dyes, chemiluminescent reagents, other signaling/amplification reagents).
- The potential for specific and non-specific probe cross-hybridization.
- Internal controls and a description of their specific function in the system.
- External controls that you recommend or provide to users.
- Instrumentation required for your device, including the components and their function within the system.
- Types of output generated by the instrumentation and system parameters (e.g., measurement ranges).
- The computational path from raw data to the reported result (e.g., how raw signals are converted into a signal). 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, if available.

When applicable, you should describe design control specifications for your device that address or mitigate risks associated with primers, probes and controls used in a nucleic-acid based multiplex test procedure to detect viral RNA or DNA segments from respiratory viruses, such as the following examples:

- Prevention of probe cross-contamination for multiplexed tests in which many probes are handled during the manufacturing process.
- Correct placement and identity of assay features (e.g., probes).
- Minimization of false positives due to contamination or carryover of sample.
- Use of multiple probes to enable detection of virus variants appearing due to mutations within the target nucleic acid segment(s), or variants within a designated novel respiratory viral strain (or lineage).
- Developing or recommending validated methods for nucleic acid extraction and purification that yield suitable quality and quantity of viral nucleic acid for use in the test system with your reagents. You should address suitable validated extraction method(s) for different specimen types your assay claims in its intended use.

In your 510(k), you should provide performance information that supports the conclusion that your design requirements have been met.

5.C Ancillary Reagents

Ancillary reagents are those reagents that an RVP 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.[7]

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.

[7] 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.

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 RVP, and any other issues that could present a risk of obtaining incorrect results with your RVP.

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 (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 RVP.
    - 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.

5.D Controls

Controls should approximate the composition and nucleic acid concentration of a sample in order to adequately challenge the system, as well as address reproducibility around the cut-off.

You should describe the following concerning quality control and calibration:

- The nature and function of the various controls that you include with, or recommend for, your system. These controls should enable the user to determine if all steps and critical reactions have proceeded properly without contamination or cross-hybridization.

- Your methods for value assignment (relative or absolute) and validation of control and calibrator material, if applicable.
- The control parameters that could be used to detect failure of the instrumentation to meet required specifications.

We recommend that you consult with FDA when designing specific controls for your device. Controls should provide information about (1) sample quality, (2) nucleic acid quality, and (3) process quality. We generally recommend that you include the following types of controls:

5.D.i Negative Controls

Blank 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. This control is used to rule out contamination with target nucleic acid or increased background in the amplification reaction. It may not be applicable 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 not targeted by the assay. 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-respiratory virus infected individual
- Samples containing a non-target organism (e.g., cell line infected with non-respiratory virus)
- Surrogate negative control, e.g., packaged RNA

5.D.ii Positive Controls

Positive control for complete assay

The positive control is designed to mimic a patient specimen, contains target nucleic acids, and is used to control the entire assay process, including nucleic acid extraction, amplification, and detection. Acceptable positive assay control materials include cell lines infected with a non-pathogenic strain of virus detected by the assay in the appropriate matrix mimicking the assay specimen type.

Positive controls are run as a separate assay, concurrently with patient specimens, at a frequency determined by a laboratory's quality system. Some examples of acceptable external positive assay controls include:

- Cell lines infected with a non-pathogenic strain of virus detected by the assay
- Vaccine or prototypic vaccine strains
- Low pathogenic viruses
- Inactivated viruses
- Packaged viral RNA (in the case of RNA respiratory viruses)

Positive control for amplification/detection

The positive control for amplification/detection can contain 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.

5.D.iii 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 (e.g., polymerase, primers), equipment function (e.g., thermal cycler), and the presence of inhibitors in the samples. Examples of acceptable internal control materials include human nucleic acid co-extracted with the influenza 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.

5.E Interpreting Test Results/Reporting

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

- In particular, you should provide the cut-off value for defining a negative result of the assay. If the assay has only two output results (negative/positive), this cut-off also defines a positive result of the assay.
- If the assay has an equivocal zone, you should provide cut-off values (limits) for the equivocal zone.
- If your interpretation of the initial equivocal results requires re-testing, you should provide (1) a recommendation whether re-testing should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen, and (2) an algorithm for defining a final result by combining the initial equivocal result and the results after re-testing (note that this algorithm should be developed before the pivotal clinical study that evaluates the clinical performance of the assay).
- If one of the reported outputs of your assay can be an equivocal result, you should provide the interpretation and recommendation for how the user should follow-up the equivocal results for each pathogen on your panel.
- If the assay has an invalid result, you should describe how an invalid result is defined. If internal controls are part of the determination of invalid results, you should provide the interpretation of each possible combination of control results for defining the invalid result. You should provide recommendations for how to follow up any invalid result, i.e., whether the result should be reported as invalid or whether re-testing is recommended. If re-testing is recommended, you should provide information similar to that for the re-testing of equivocal results (i.e., whether re-testing should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen).

6. Performance Characteristics

In your 510(k), you should detail the study design you used to evaluate each of the performance characteristics outlined below.

If your product labeling calls for the use of ancillary reagents, the premarket performance testing submitted to support your 510(k) should use the ancillary reagents that your instructions for use reference. The performance claims you establish through premarket testing, which will be reflected in your labeling under 21 CFR 809.10(b), should be based on the particular test configuration you describe in your labeling.

Evaluation of assay performance should include appropriate controls for the duration of the analytical and clinical studies. This includes any positive and negative controls provided with your assay as well as appropriate external controls recommended but not necessarily provided with the assay.

6.A Preanalytical Factors

Consideration of preanalytical factors is critical for high-quality respiratory viral panel nucleic acid tests. In your 510(k), you should address the following issues regarding preanalytical factors.

6.A.i Specimen Collection and Handling

You should specify the specimen type(s) your assay is intended to measure. Appropriate specimen types depend on a variety of factors, including the site of infection and the viral nucleic acid to be detected. Specifically, a specimen has to be collected from the appropriate anatomical site or source at the time in the clinical progression of the disease state during which the organism will be present at that site. Appropriate specimen types for viral panel nucleic acid tests are respiratory tract specimens (e.g., nasopharyngeal swab (NPS), nasal aspirate (NA), nasal swab, and nasal wash).

The quality and quantity of extracted target can be highly dependent on multiple factors such as specimen source, collection method, handling (e.g., transport and storage times and temperatures). Testing results you provide in your 510(k) should validate that (1) your system provides adequate and appropriate nucleic acid for all analytes detected by your assay (i.e., different virus types and subtypes, viral DNA and RNA), and (2) the device maintains acceptable performance (e.g., accuracy, reproducibility) under all the various conditions you recommend in your labeling. For example, you should assess the effect of recommended storage times and temperatures on sample stability and recovery using an analysis of specimen aliquots stored and/or transported under your recommended conditions of time and temperature. You should state your acceptance criteria for all specimen stability parameters.

Specimens for pathogen identification should be collected and handled using all applicable state and federal biosafety guidelines. For standard precautions for handling

of specimens, refer to the most current editions of the related Clinical and Laboratory Standards Institute (CLSI) documents. [8]

6.A.ii Fresh vs Frozen Samples (stability)

Sensitivity for detection of some viruses changes for frozen specimens as compared to fresh. In developing your test, we recommend you consider, and when appropriate evaluate, whether this is a concern for any of the pathogens detected by the particular multiplex panel (i.e., they should not be frozen and thawed). You should assess the effect of repeated freeze/thaw cycles on the yield of the viral nucleic acid and its influence on the assay performance.

6.A.iii Nucleic Acid Extraction

Different extraction methods may yield nucleic acids of varying quantity and quality, and therefore the extraction method can be crucial to a successful result. Purification of viral nucleic acids can be challenging because biological samples may contain low viral titers in the background of human genomic DNA, as well as high levels of proteins and other contaminants. Additionally, the lysis conditions may differ depending on issues such as whether the assay measures both RNA and DNA viruses, sample type, etc.

For these reasons, you should evaluate the effect of your chosen extraction method on the performance of the assay with respect to satisfactory nucleic acid quantity and quality for the intended use of the assay. In addition, you should evaluate your assay's analytical and clinical performance characteristics for each virus using the entire pre-analytical process (including extraction procedures) that you recommend for use with your assay. This should include demonstrating the reproducibility and Limit of Detection (LoD) of your assay with each recommended extraction procedure. In addition, external site studies (including reproducibility and clinical studies) should include the extraction procedures prescribed in your labeling.

You should perform these evaluations whether you intend to actually provide reagents in your test kit for extraction and preparation of nucleic acid, or whether you simply instruct users concerning appropriate reagents.

If you include or recommend multiple extraction methods for use with your assay, you should demonstrate extraction quality and efficiency, as well as analytical and clinical performance of your assay with each extraction method and each virus. Specifically, you should demonstrate LoD and reproducibility for each method. You may be able to combine 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 of three testing sites: test extraction method A at site 1, method B at site 2, and method C at site 3. However, if the studies from the three sites indicate statistically significant

[8] *Biosafety in Microbiological and Biomedical Laboratories* 1999. Richmond, J.Y. and McKinney, R.W. eds. HHS Publication Number (CDC) 93-8395; and CLSI. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue. CLSI document M29-A. Wayne, PA: Clinical and Laboratory Standards Institute; 1997.

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, B and C, site 2 extraction method A,B and C, and site 3 extraction method A,B and C).

In addition to the analytical studies (LoD and Reproducibility at external sites), each extraction method 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 nucleic acid extraction method) are not considered equivalent and should not be pooled, but rather should be analyzed separately. As a consequence, additional prospective clinical samples may be called for in order to support the claimed extraction method.

You should provide your recommendations for assuring specimen adequacy for the different specimen types for which your assay is indicated. For example, the quality of the nucleic acid can be assessed using internal controls that determine presence and/or quality of the nucleic acid.

6.A.iv Well-to-Well Cross Contamination with Automated Extraction Systems

If automated systems are used or recommended for nucleic acid extraction, you should include a check of potential well-to-well cross contamination as part of the performance qualification of the extraction instrument. You should provide a software hazard analysis for automated extraction systems as part of your 510(k). A validation study of the extraction process can be designed in a grid such that a nucleic acid-containing sample with a concentration at the highest anticipated clinical level is surrounded on all sides by a "no template control". The results should demonstrate that well-to-well cross-contamination does not occur.

6.A.v Performance Study Quality Controls

Evaluation of assay performance should include appropriate controls for the duration of the analytical and clinical studies. This includes any positive and negative controls provided with your assay as well as appropriate external controls recommended but not necessarily provided with the assay.

The external positive control contains target nucleic acids and is used to control the entire assay process including nucleic acid extraction, amplification, and detection. It is designed to mimic a patient specimen and is run as a separate assay, concurrently with patient specimens.

6.B Analytical Performance

The following are analytical performance characteristics you should demonstrate for your assay in your 510(k).

6.B.i Limit of Detection (LoD)

LoD is defined as the lowest concentration of analyte that can be consistently detected (typically in ≥95% of samples tested under routine clinical laboratory conditions) in a defined type of sample. By definition, this concentration must yield an assay value

that can be reproducibly distinguished from values obtained with samples that do not contain the analyte.

Determination of LoD for multiplex assays follows the same principles as for single analyte assays (described in CLSI document EP17-A *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*; 2004). During the validation of a test system, you should determine the LoD for each specimen type and each analyte that will be tested in the respiratory viral panel multiplex assay. This can be accomplished by limiting dilutions of infected cell samples into non-infected cell samples. You should confirm the titer of the viruses prior to use in the study, using tissue culture infectious dose 50 (TCID50) units, or alternatively, colony forming units/mL (CFU/mL), or plaque forming units/mL (PFU/mL). The method for LoD determination includes the re-growth and re-titering of viral stocks. We recommend that you prepare serial dilutions using appropriate pooled negative sample matrixes as diluents that include 3-5 replicates for each dilution. You should report the LoD as the level of virus that gives a 95% detection rate. Depending on the assay, it might not be necessary to perform a separate LoD determination at the whole range of concentrations for every single specimen type; LoD should be determined for at least the most common and most problematic ones. The LoD may be confirmed by preparing at least 20 additional replicates at the LoD concentration and demonstrating that the virus is detected 95% of the time. You should also evaluate the sensitivity of the test for the influence of microorganism diversity. You should apply the whole process of the test system from sample preparation to amplicon detection when evaluating assay LoD.

6.B.ii Analytical Reactivity (Inclusivity)

You should demonstrate reactivity for different respiratory viral strains that the assay has been designed to detect (i.e., by probing conserved nucleic acid regions). You should collect data for relevant strains of every virus detected by the assay, although the number of tested strains may vary depending on the specific test target for a particular viral type or subtype. All virus identities and titers should be confirmed.

We recommend that you demonstrate that the test can detect additional clinically relevant virus strains representing temporal and geographical diversity for each claimed analyte at viral levels at or near the LoD. For example, if your assay detects and identifies different influenza types and subtypes, we recommend that you demonstrate that the test can detect at least ten virus strains representing temporal and geographical diversity for each claimed influenza subtype at viral levels at or near the LoD. Suggested strains for LoD and analytical reactivity studies are shown in Table 1 below. If vaccine strains are included, they should represent recent flu seasons. The information on the current vaccine strains is available from the Centers for Disease Control and Prevention (CDC) at http://www.cdc.gov/flu/professionals/vaccination/composition0708.htm. Vaccine strains may vary from one influenza season to another.

To further illustrate, if your assay detects Adenovirus, we recommend testing between 10-15 different serotypes. Some of the serotypes you should consider testing are: Type 1, 2, 3, 4, 5, 6, 7, 11, 14, 16, 21, 34, 35, and possibly some others like type 10, 13, 18, 31, 40, 41. If you are performing sequencing of your clinical specimens to

establish which Adenovirus groups can be detected by your assay, groups generally retrieved in respiratory specimens are B:1, B:2, C, and E.

For any other viruses detected and identified by your respiratory viral panel multiplex assay, the cross-reactivity study table (Table 3 in the analytical specificity section below) provides some suggested viral types to include in reactivity studies, as well as cross-reactivity studies, depending on which virus types and subtypes your device detects. For virus types and subtypes for which is it difficult to obtain sufficient samples to demonstrate detection of numerous strains, we recommend that you contact the FDA to discuss your study.

Table 1. Influenza strains recommended for reactivity studies.

Type	Subtype	Influenza Viral Strain
A	H1N1-like	A/New Caledonia/20/1999
A	H3N2-like	A2/Wisconsin/67/2005 or Ag equiv A/Hiroshima/522005
B		B/Malaysia/2506/2004 or Ag equiv B/Ohio/1/2005
A	H1N1	A/PR/8/34
A	H1N1	A/FM/1/47
A	H1N1	A/NWS/33
A	H1N1	A1/Denver/1/57
A	H1N1	A/New Jersey/8/76
A	H3N2	A/Port Chalmers/1/73
A	H3N2	A/Hong Kong/8/68
A	H3N2	A2/Aichi2/68
A	H3N2	A/Victoria/3/75
A	H1	A/NY/55/2004
A	H3	A/Hawaii/15/2001
B		B/Lee/40
B		B/Allen/45
B		B/GL/1739/54
B		B/Taiwan/2/62
B		B/Hong Kong/5/72
B		B/Maryland/1/59
B		B/Florida/2006
A	H5N1	Human and /or Avian
A	H5N2	Avian
A	H7N2	Human and /or Avian
A	H7N7	Human and /or Avian
A	Other subtypes	Human and/or animal species

6.B.iii Analytical Specificity

You should determine analytical specificity for your respiratory viral panel multiplex assay for all viruses, through evaluations of the complete system, from nucleic acid extraction through amplification and detection. You should prepare the samples used for the analytical specificity in a matrix equivalent to the claimed specimen types. These studies should be performed for each specimen type used in the assay. In general, you should evaluate both interference and cross-reactivity for your device. Where applicable, you should evaluate potential for non-specific amplification, non-specific hybridization, and cross-hybridization of your device.

Interference

You should demonstrate that your assay can specifically detect the target organism in the presence of relevant interferents. These studies should include other organisms, homologous sequences, and contrived samples with high background levels of human DNA. The following sections describe specific types of interference studies you should perform in order to assess potential interference for your respiratory viral panel multiplex assay. CLSI document EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline*; 2005 provides additional information about how to design interference studies.

a) Interfering substances

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 respiratory specimens. Potentially interfering substances to test include those that may pre-exist in the specimen (e.g., blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms) as well as those that may be introduced during specimen collection and sample preparation. Examples of potentially interfering substances are presented in Table 2, below. We recommend that you test the effect of each interferent on detection of each virus type in your respiratory viral panel multiplex assay. The amount of virus in the sample should be at the specific cutoff concentration. 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.

You should also assess other commonly prescribed or over-the-counter medications and their metabolites, as appropriate. Since spiking experiments may not necessarily be an accurate model of the *in vivo* scenario, alternative experimental designs such as assessing the effect of medications in patients included in the clinical studies received may need to be considered, as appropriate.

Table 2. Substances Recommended for Interference Studies

Substance	Active Ingredient
Mucin: Bovine submaxillary gland, type I-S	Purified mucin protein
Blood (human)	
Nasal sprays or drops	Phenylephrine, Oxymetazoline, Sodium chloride with preservatives
Nasal corticosteroids	Beclomethasone, Dexamethasone, Flunisolide, Triamcinolone, Budesonide, Mometasone, Fluticasone
Nasal gel	Luffa opperculata, sulfur
Homeopathic allergy relief medicine	Galphimia glauca, Histaminum hydrochloricum
FluMist©	Live intranasal influenza virus vaccine
Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol
Anti-viral drugs	Zanamivir
Antibiotic, nasal ointment	Mupirocin
Antibacterial, systemic	Tobramycin

b) Interference by other microorganisms

You should evaluate your assay for interference by microorganisms that are not tested by your assay. We recommend that you spike your RVP test specimens with other microorganisms that are expected to be present in anatomical sites to be sampled for testing, or known to commonly cause similar symptoms as specific viruses included in the respiratory viral multiplex assay. Spiking allows for determination of the maximum amount of a contaminating substance the assay can tolerate without causing interference or adversely affecting the test result.

c) Competitive Interference by viral panel analytes

You should assess the effects of clinically relevant co-infections with each of the pathogens probed by the assay. The selection of viruses that you consider for these studies should be based on combinations of viruses that are known to occur or are widespread (for example, Influenza A and RSV).

If one of the targets/analytes of a respiratory viral panel multiplex assay is expected to be present at a high level, the detection of another target present at low levels could potentially be impaired. To assess whether this would be the case, we recommend you evaluate competitive interference with one target at the LoD concentration and another target at a very high concentration. This can be done either during the LoD evaluation or, alternatively, as a part of reproducibility or interference studies.

Cross-reactivity

The following sections describe specific types of cross-reactivity studies in order to assess potential cross-reactivity in the absence of the respiratory viral panel multiplex assay target organisms.

a) Cross-reactivity with other viruses in the panel

Section 6.B.ii above discussed testing characterized samples for each target probed by the multiplex panel to establish reactivity (inclusivity) of the assay for that particular pathogen. You should also utilize similar types of samples to evaluate (and rule out) cross-reactivity between the virus and the primers/probes designed to detect the other viruses and subtypes that are a part of the respiratory viral panel. While the virus concentration in the inclusivity study should be near the assay cutoff, the concentration in the cross-reactivity studies should be at the highest anticipated clinical level.

b) Cross-reactivity with pathogens or targets that are not part of the respiratory viral panel

To determine whether your multiplex assay cross-reacts with analytes other than the ones it is designed to measure, we recommend assessing a panel of closely related organisms. It is important to consider analytes that have a probability of presenting at the target population specimen collection site for each sample type. For example, you should establish that your viral detection assay primers/probes do not cross-react with human or bacterial nucleic acids, which are likely to be present in the specimen. We recommend that you test for cross-reactivity with respiratory pathogens not included in your panel, and other microorganisms with which respiratory specimens of the majority of the population may have been infected, e.g., Epstein Barr Virus (EBV) and cytomegalovirus (CMV). 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.

Microorganisms recommended for cross-reactivity studies are listed in Table 3 below; pathogens listed in this table should be used in addition to several Influenza A and Influenza B subtypes listed in Table 1, Influenza strains recommended for reactivity studies.

Table 3. Microorganisms recommended for cross-reactivity studies.

Organism	Type
Adenovirus	Type 1-5,7,11,14,22,31,35
Cytomegalovirus	
Herpes simplex virus Type 1	
Varicella-zoster virus	
Enterovirus	Coxsackie, echovirus, poliovirus

Epstein Barr Virus	
Human parainfluenza	Type 1-4
Coronavirus	HKU1, 229E, OC43, NL63
Measles	
Human metapneumovirus	1A, 1B, 2A, 2B
Mumps virus	
Respiratory syncytial virus	Type A, Type B
Rhinovirus	Type 1A (groups A, B, C, E, F)
Bordetella pertussis	
Chlamydia pneumoniae	
Corynebacterium sp.	
Escherichia coli	
Hemophilus influenzae	
Lactobacillus sp.	
Legionella sp	
Moraxella catarrhalis	
Mycobacterium tuberculosis avirulent	
Mycoplasma pneumoniae	
Neisseria meningitides	
Neisseria sp.	
Pseudomonas aeruginosa	
Staphylococcus aureus	Protein A producer
Staphylococcus epidermidis	
Streptococcus pneumoniae	
Streptococcus pyogenes	
Streptococcus salivarius	

Additionally, we recommend testing cross-reactivity of your RVP assay with vaccines such as live attenuated influenza virus vaccine (Nasal-Spray Flu Vaccine), considering there may be reactive patient testing results from individuals that have received the vaccine.

We encourage you to present cross-reactivity testing data for devices detecting multiple pathogens in the format shown in Table 4.

Table 4. Data Presentation Example.

EXAMPLE		*Reference Reagent, Results Positive (+) or Negative (-) for Reactivity*						
Organism	Strain	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Adenovirus	Type 1	+	-	-	-	-	-	-
	Type 3	+	-	-	-	-	-	-
	Type 5	+	-	-	-	-	-	-
	Type 6	+	-	-	-	-	-	-
	Type 7	+	-	-	-	-	-	-

	Type 10	+	-	-	-	-	-	-
	Type 13	+	-	-	-	-	-	-
	Type 14	+	-	-	-	-	-	-
	Type 18	+	-	-	-	-	-	-
	Type 31	+	-	-	-	-	-	-
	Type 40	+	-	-	-	-	-	-
	Type 41	+	-	-	-	-	-	-

Influenza A	Aichi (H3N2)	-	+	-	-	-	-	-
	Mal (H1N1)	-	+	-	-	-	-	-
	Hong Kong (H3N2)	-	+	-	-	-	-	-
	Denver (H1N1)	-	+	-	-	-	-	-
	Port Chalmers (H3N2)	-	+	-	-	-	-	-
	Victoria (H3N2)	-	+	-	-	-	-	-
	New Jersey ($H_{SW}N1$)	-	+	-	-	-	-	-
	WS (H1N1)	-	+	-	-	-	-	-
	PR (H1N1)	-	+	-	-	-	-	-
Influenza B	Hong Kong	-	-	+	-	-	-	-
	Maryland	-	-	+	-	-	-	-
	Mass	-	-	+	-	-	-	-
	Taiwan	-	-	+	-	-	-	-
	GL	-	-	+	-	-	-	-
	Russia	-	-	+	-	-	-	-
RSV	Long	-	-	-	-	-	-	+
	Wash	-	-	-	-	-	-	+
	9320	-	-	-	-	-	-	+

6.B.iv <u>Cut-off</u>

You should explain how the cut-off for each target/analyte was initially determined as well as how it was validated (see also Section 5.E). The cut-off should be determined using appropriate statistical methods. To support the cutoff you determined you may provide for example, a result distribution, 95th and 99th percentiles, percents of the non-negative (positive or equivocal) results, and so on, for the clinical samples without any respiratory viruses in your pilot studies. 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 the pilot studies with clinical samples (for details about ROC analysis, see CLSI document GP10-A *Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics*

(ROC) Plots; Approved Guideline; 1995). If the assay has an equivocal zone, you should explain how you determined the limits of the equivocal zone. The performance of your device using the pre-determined cut-off (and equivocal zone, if applicable) should be validated in an independent population consistent with the defined intended use of your device (the pivotal clinical study discussed in Section 6.D, below).

6.B.v Precision (Repeatability/Reproducibility)

You should provide data demonstrating the precision (i.e., repeatability and reproducibility) of your system. The CLSI documents, EP5-A2*, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*; 2004, and EP12-A2, *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline*: 2008, include guidelines that may be helpful for developing experimental design, computations, and a format for establishing performance claims.

We recommend you establish the precision for each target/analyte using samples that have a range of analyte concentrations that span the detection limits of your assay. Ideally, all sources of assay variability in the precision study should be identified. In general, any variable that changes from day to day or week to week should be examined for its impact on assay precision. While some sources of variability can be evaluated in an in-house precision study, the site-to-site reproducibility study should include an evaluation of the major sources of variability described below, for each virus:

- Extraction-to-extraction reproducibility: samples used in reproducibility testing are processed from clinical specimens (e.g., nasopharyngeal swabs) at the test site, using the procedure you plan to recommend in the test labeling.
- Between-instrument reproducibility
- Site-to-site and operator-to-operator reproducibility: include three or more sites (at least two external sites and one in-house site) with multiple operators at each site. Operators should reflect potential users of the assay in terms of education and experience. You should provide training only to the same extent that you intend to train users after marketing the test.
- A minimum of three-to-five non-consecutive days to cover day-to-day variability of each analyte tested by the respiratory viral panel (if applicable, spanning two instrument calibration cycles).
- A minimum of two runs per day (unless the assay design precludes multiple runs per day) and two replicates of each panel member per run is recommended to assess between-run component as well as within-run and within-day imprecision in your reproducibility study.
- Lot-to-lot reproducibility: including evaluation of multiple product lots (e.g., multiple lots of assay reagents and ancillary reagents, multiple lots of primers and probes for RT-PCR, multiple lots of beads or arrays), and multiple instruments.
- Every analyte the test can detect should be represented by the test samples, For each analyte (target) detected by the respiratory viral panel, we recommend including at least three levels of viral load, including analyte or output concentrations close to the assay cut-off:

1. 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).
2. 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.
3. A "moderate positive" sample: a sample ideally reflecting clinically relevant concentration[9]. 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).

Note: When the limit of blank (LoB) is used as a cut-off, 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%[10].

- We also recommend that you evaluate the reproducibility of samples with clinically relevant co-infections using low concentrations (around the assay cut-off) of one of the viruses expected in co-infection and high concentrations of the other, and vice versa.

In the study design description in your 510(k), you should identify which factors (e.g., instrument calibration, reagent lots, and operators) were held constant and which were varied during the evaluation, and describe the computations and statistical analyses used to evaluate the data.

In general, for qualitative tests, variance components should be estimated using the appropriate statistical method for all the factors considered in the precision study, as well as overall. Particularly for qualitative tests that have an underlying quantitative output, the component of precision is often measured for each source of variation, as well as the total variation, using analysis of variance. For each sample in the precision study, you should provide the mean value with variance components (standard deviation and percent CV). In addition, for each sample, provide percents of values above and below the cut-off and percent of invalid results for each site separately and for all sites combined (if applicable, provide percents of equivocal results for each sample in the precision study for each site and for all sites combined).

6.B.vi Carryover Studies and Cross-contamination Studies (for multi-sample assays and devices that require instrumentation)

For multi-sample assays and devices that require instrumentation, we recommend that you demonstrate that carryover and cross-contamination do not occur with your device. In a carryover and cross-contamination study, we recommend that high

[9] Sample with a typical concentration of the infected subjects in the intended use population (for example, a median value of the concentrations from the infected subjects)

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

positive samples be used in series alternating with high negative samples in patterns dependent on the operational function of the device. We recommend that at least five runs with alternating high positive and high negative samples be performed. We recommend that the high positive samples in the study be high enough to exceed 95% or more of the results obtained from specimens of diseased patients in the intended use population. We recommend that the high negative samples contain the analyte concentration below the cut-off such that repeat testing of this sample is negative approximately 95% of the time. The carryover and cross-contamination effect can then be estimated by the percent of negative results for the high negative sample in the carry-over study compared with 95%.

6.B.vii Stability Studies

You should describe your study design for determining the real-time stability of the reagents and instruments, and if applicable, a description of stress test conditions and results. For each study, you should describe your acceptance criteria values and how you selected them.

6.C Instrumentation and Software

For instruments and systems that measure and sort multiple signals, and other complex laboratory instrumentation that has not been previously cleared, refer to the guidance document **"Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems,"** for details on the types of data you should provide to support instrument clearance.

If your system includes software, you should submit software information detailed in accordance with the level of concern (See: **"Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices"**[11]**).** You should determine the level of concern prior to the mitigation of hazards. In vitro diagnostic devices of this type are typically considered a moderate level of concern, because software flaws could indirectly affect the patient and potentially result in injury because the healthcare provider and patient do not get accurate information.

Below are additional references to help you develop and maintain your device under good software life cycle practices consistent with FDA regulations.

- General Principles of Software Validation; Final Guidance for Industry and FDA Staff; available on the FDA Web site at: http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM085371.pdf.
- Guidance for Off-the-Shelf Software Use in Medical Devices; Final; available on the FDA Web site at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073778.htm.
- 21 CFR 820.30 Subpart C – Design Controls of the Quality System Regulation.

[11] http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM089593.pdf

- ISO 14971-1; Medical devices - Risk management - Part 1: Application of risk analysis.
- AAMI SW68:2001; Medical device software - Software life cycle processes.

6.D Clinical Performance Studies

6.D.i Clinical Study Design

You should conduct prospective clinical studies using specimens from individuals representing the intended use population, i.e., those with signs and symptoms consistent with respiratory tract infection, to determine the performance of your device for all respiratory virus types and subtypes as well as all specimen types you claim in your labeling. In your 510(k), 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. We recommend that you include samples from each age group in your clinical studies and present the data demonstrating the performance of your test stratified by age (e.g., less than 5, 6- 21, 22-59, and greater than 60 years old) in addition to the overall data summary table. Fresh samples are preferred. However, analysis of prospectively collected archived specimens[12] may be acceptable if you can demonstrate that freezing or other preservation techniques do not alter the performance of the device in comparison to testing of fresh specimens, if appropriate archives are selected, and appropriate measures are taken to identify and remove or mitigate any biases in the study set. If you evaluate the assay using specimens that were archived after performing viral culture on fresh specimens, you should ensure that the specimens are not utilized selectively (i.e., you should still test all specimens in a prospective manner). 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 of these two groups separately. We encourage you to contact the FDA to request a review of your proposed studies.

The clinical dataset should consist of clinical samples collected from at least three different clinical sites in different geographical locations. Preferably, studies would be conducted within the U.S. population. If some of the studies are conducted outside the U.S, you should document the relevance of your studies to U.S. clinical practice and demographics.

In rare cases when a particular respiratory virus has been shown to have a low prevalence in the intended use population (e.g., some parainfluenza virus (PIV) types), it may be appropriate to supplement prospectively collected specimens with banked specimens known to contain a particular virus type or subtype (i.e. pre-selected

[12] In this guidance, we define *prospectively collected archived specimens* as specimens collected sequentially from all patients meeting study inclusion criteria and representing assay intended use population (i.e., not pre-selected specimens with known results) coming in to a clinical testing facility between two pre-determined dates (e.g., from the beginning to the end of one flu season), so there is no bias and prevalence is preserved. These specimens should be appropriately stored (e.g., frozen at -70^{o}C) and, as noted in the text, the sponsor should show that there is no change in device performance due to banking/freezing/storage of the specimens.

banked specimens)[13]. In such cases, results from this population should be presented separately from the prospectively collected specimen results, and performance calculated as positive percent agreement and negative percent agreement.

We recommend that you collect all relevant clinical and laboratory information available for your clinical study patients. This should include age (children, adults, geriatric population), days since onset of symptoms, gender, patient population (i.e., outpatient, ER, hospitalized, immuno-compromised), signs and symptoms and indications for testing, any medications taken or administered, viral culture and definitive identification of isolated viruses using antibodies, any additional test results for diagnosing disease status and severity (e.g., radiography, bacterial culture, or gram stain), and a final diagnosis if available (e.g., influenza-like illness (ILI), bronchitis, bronchiolitis, pneumonia). The clinical information appropriate for consideration may vary with the study group of interest.

6.D.ii Reference Methods

You should compare your assay's performance to the established gold standard reference methods of viral culture or an FDA-cleared direct specimen fluorescent antibody (DSFA) assays. Viral culture should be performed on freshly collected specimens. For respiratory viruses that are difficult to culture, a composite (multi-test) reference method (a predetermined algorithm that combines results of few tests) can be used as a comparator method. This method should include a separate well-characterized nucleic acid amplification method (e.g., PCR) followed by bidirectional sequencing. The nucleic acid amplification method used in the composite reference method should be targeted to the different genomic regions (i.e., incorporate different viral target) from the ones probed by your assay. You should provide published literature or laboratory data in your submission in support of the primers used for amplification. We recommend that you perform the sequencing reaction on both strands of the amplicon (bidirectional sequencing) and 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 that it matches the reference or consensus sequence.

When using viral culture, in your 510(k), you should provide the viral identification, e.g., staining with viral specific monoclonal antibodies, or PCR followed by sequencing of the amplicons as an alternative method for identification of the virus, in addition to cytopathic effect (CPE). CPE alone may not provide accurate viral identification. You should describe the performing laboratories' procedure(s) for virus isolation in your submission, as well as specific data or literature to show that a particular cell line is validated to isolate a specific virus. You should not use previously frozen specimens for culture, as freeze-thawing results in loss of viral infectivity. We recommend that the viral culture method used in your study follow the CLSI document M41-A, *Viral Culture; Approved Guideline*; 2006, and World Health

[13] In this guidance, *pre-selected banked specimens* refers to banked or archived specimens that are selected by the sponsor for testing because they are known to contain a certain analyte. Because these specimens may **not** have been sequentially collected between two pre-determined dates and do not adequately represent the analyte prevalence in the intended use population, their use can result in biased test performance results.

Organization (WHO) Manual on Animal Influenza Diagnosis and Surveillance[14]. For DFA testing, you should provide the procedure description and data or literature to show that a particular antibody/fluorescent pattern and procedure are appropriate for a specific virus. If the DFA antibody used for virus detection in cultured cells is FDA-cleared, no validation information is needed in the submission, as long as the laboratory performing the test follows the package insert instructions. If the antibody used in the DFA is a preamendments device[15], then you should provide published literature or laboratory data in your premarket submission in support of the antibody validation for detection of a specific virus. If public health authorities recommend against culturing a novel virus, we recommend that you use nucleic acid amplification (targeted to the different genomic regions from the ones probed by your assay) followed by sequencing of the amplicons to confirm the identity of the novel virus.

6.D.iii Sample Size/ Data Analysis

The total number of samples you should include in your study for substantiating a claim for detection of each respiratory virus in your panel will depend on the prevalence of the virus and on assay performance. For the prospectively collected samples, the performance for each respiratory virus is described by the clinical sensitivity and specificity. Sensitivity for a respiratory virus is the ability of the test to obtain positive results for this respiratory virus in the samples with positive results obtained by the comparator method (reference method or composite reference method) for this respiratory virus. Specificity for a respiratory virus is the ability of the test to obtain negative results for this respiratory virus in the samples with negative results obtained by the comparator method for this respiratory virus. For each respiratory virus in the panel, sensitivity is calculated by dividing the number of true positive results by the sum of true positive and false negative results; and specificity is calculated by dividing the number of true negative results by the sum of true negative and false positive results (for additional details, see CLSI document MM17-A, *Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline*; 2008). The estimation of sensitivity and specificity should be provided along with 95% two-sided confidence intervals (for more details about confidence intervals, see CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline.* CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008).

As a general rule, we recommend that you include a sufficient number of prospectively collected samples for each specimen type you intend to claim to generate a result with at least 90% sensitivity with a lower bound of the two-sided 95% confidence interval (CI) greater than 80%. We recommend your device demonstrate specificity with a lower bound of the two-sided 95% CI greater than 90%.

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[14] WHO Manual on Animal Influenza Diagnosis and Surveillance. 2002, Geneva, (World Health Organization). Complete document WHO/CDS/CSR/NCS/2002.5, available at: http://www.who.int/csr/resources/publications/influenza/en/whocdscsrncs20025rev.pdf

[15] Preamendments devices are those devices that were introduced or delivered for introduction into interstate commerce for commercial distribution prior to May 28, 1976 (the date of enactment of the Medical Device Amendments of 1976).

6.D.iv Data Presentation

You should present sensitivity and specificity (with 95% CI) separately for each virus and subtype your device identifies. Also, you should present (1) the results of your test for the samples which have co-infections as obtained by the reference method, and (2) the results of the reference method for the samples which have co-infections as obtained by your RVP assay.

The samples in the clinical study should be tested with the RVP assay as described in the instructions for use of your device. For example, if the samples with initial equivocal or invalid results should be re-tested according to the instruction for use of the RVP assay, then these samples should be re-tested in the clinical study and the final results for these samples should be used in your statistical analysis. For the samples in your clinical study, you should provide the percent of re-tested samples because of initial equivocal results (if applicable) and the percent of re-tested samples because of initial invalid results (if applicable) for each respiratory virus separately and for all combined. In addition, you should present the percent of final invalid and final equivocal results (if applicable) for each respiratory virus separately and for all combined.

For the samples in the clinical study, you should provide signal (result) distributions (frequencies of signals) of the RVP assay for:

- all prospectively collected fresh, prospectively collected archived, and banked pre-selected samples shown separately, for each respiratory virus and for all viruses combined;
- the samples which are positive (i.e., virus detected) by the reference method for the prospectively collected fresh, prospectively collected archived, and banked pre-selected samples shown separately, for each virus and for all viruses combined; and
- the samples that do not contain any respiratory viruses according to the reference method results.

If the RVP assay has an equivocal zone, you should provide the following in support of the validation of the equivocal zone, for the prospectively collected, prospectively collected archived, and banked pre-selected samples separately for each respiratory virus:

- total number of samples with the initial values in the equivocal zone;
- number of samples with the initial values in the equivocal zone and positive results of the reference method; and
- number of samples with the initial values in the equivocal zone and negative results of the reference method.

If the equivocal zone values require re-testing, provide information about how the numbers described above were changed after re-testing of the samples.

6.D.v Study Samples/ Specimen Types

You should use clinical samples from all sample types and matrices you claim in your intended use (e.g., nasal swabs, nasopharyngeal swabs, nasal aspirates) to demonstrate that correct results can be obtained from clinical material. We recommend that you provide a justification using statistical methods to support your study sample size. For samples you use in your clinical studies, you should provide data demonstrating that storage and transport of any banked samples have not affected assay results. If you have questions regarding the choice of appropriate specimen type(s) and numbers, please contact the FDA.

7. Labeling

Respiratory viral panel multiplex nucleic acid assays, like other devices, are subject to statutory requirements for labeling (sections 502(a), 201(n) of the Act; 21 USC §§ 352(a), 321(n)). IVD devices for detection of respiratory viruses must also provide adequate directions for use and adequate warnings and precautions (section 502(f); 21 USC § 352(f)). Specific labeling requirements for IVD devices are set forth in 21 CFR 809.10. See also 21 CFR § 801.119 (IVDs labeled in accordance with 21 CFR 809.10 are deemed to satisfy section 502(f)(1).)

Although final labeling is not required for 510(k) clearance, final labeling for in vitro diagnostic devices must comply with the requirements of 21 CFR 809.10 before an in vitro diagnostic device is introduced into interstate commerce.

To ensure compliance with section 502 of the Act and 21 CFR 809.10, FDA recommends that labeling for these devices address the items identified below. These labeling recommendations also help to mitigate the risks identified previously in this guidance to help ensure safe and effective use of these devices.

Intended Use

The intended use should specify virus types or subtypes the test measures, the clinical indications for which the test is to be used, specimen type and the specific population for which the test is intended. Your intended use should specify that the device should be used in conjunction with other laboratory testing and clinical observations.

FDA recommends that the statement of intended use be clarified by statements such as:

> *The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and laboratory findings.*
>
> *Negative results do not preclude respiratory virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.*
>
> *Positive results do not rule out bacterial infection, or co-infection with other viruses.*

The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial culture, immunofluorescence, x-ray findings) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory viral infection.

If your device detects influenza viruses, the intended use should also include statements such as the following:

Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Device Description

In the device description, you should briefly describe the test methodology used in this type of device.

General Procedure

This section should include a general description of the analysis procedure, from physician sampling to result reporting.

Directions for Use

You should present clear instructions that delineate the procedures for using the device, and types of controls that will minimize risks of inaccurate results. Instructions should encourage use of additional control measures and testing of control material to ensure use in a safe and effective manner. Instructions should encourage users to familiarize themselves with the features of the device and how to use it in a safe and effective manner.

The instructions for use provided with your test system should supply all instructions necessary to allow the test to achieve its claimed performance, as well as all limitations and warnings required for safe use of the test.

You should include handling and storage instructions. You should describe stability (i.e., expiration dating) under the opened and closed storage conditions that you recommend to users.

For test systems that call for ancillary reagents of concern (see Section 5.C.) you should:

- Emphasize through conspicuous labeling that proper product performance requires use of specific ancillary reagents as directed. This labeling may include warnings against use of the RVP device if specified ancillary reagents are not available.

- Assure that users can clearly identify which ancillary reagents are suitable for use with your test. For example, if only certain lots of a named ancillary reagent are appropriate for use, the labeling for your RVP should identify those lots by number. (See 21 CFR 809.10(b)(8)(ii).)
- When your labeling calls for ancillary reagents that are supplied with instructions for use or other warnings or limitations by the ancillary reagent manufacturer, you should ensure that users of your RVP will understand which instructions they should follow when using those ancillary reagents in your RVP system. If there is a conflict between the directions and warnings provided by the manufacturer of the ancillary reagent and the instructions for use that you supply with your RVP, you should assess and address the risk that users may mistakenly follow the labeling provided directly with the ancillary reagent and consequently obtain invalid results with your RVP. We note that in some circumstances, statements in the labeling of the RVP may not be sufficient to address the risks created by this conflict.

Quality Control

Quality control recommendations in the package insert should include a clear explanation of what controls are to be used in the assay and the expected results for the control material.

If controls are included with your device, the specifications for control materials, including level of virus, source of that virus, method of inactivation and method for determining non-infectiousness should be provided.

Precautions, Warnings, and Limitations

You should clearly describe any assay limitations in the labeling, including all 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.

Precautions

We recommend that you specify procedures for handling, storing, and disposing of specimens, including a reiteration and expansion of the procedures for working with specimens suspected to be infected with a novel influenza strain.

Limitations

In addition to any limitations and warnings that are relevant to your specific assay, we recommend providing statements such as the following under Limitations (as applicable):

- *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, nor are the causative agents for clinical symptoms.*
- *The detection of viral sequences is dependent upon proper specimen collection, handling, transportation, storage and preparation (including extraction). Failure*

to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.

- *There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.*
- *There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay.*
- *It is recommended that specimens found to be negative after examination using respiratory viral panel be confirmed by an alternate method (e.g. cell culture).* (Depending on the assay performance for specific analytes.)
- *The performance of the assay has not been established in individuals who received nasally administered Influenza A vaccine.*
- *RVP performance was not established in immunocompromised patients.*
- *Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the [e.g., 2006/2007] season. The performance for some viruses may vary depending on the prevalence and population tested.*
- *Additional testing is required to differentiate influenza type A and B viruses*. (We recommend providing this if your device detects both Influenza A and B viruses, without distinguishing the two.)
- *Additional testing is required to differentiate any specific Influenza A subtypes or strains, in consultation with state or local public health departments*. (We recommend providing this if your device detects influenza A and distinguishes it from influenza B viruses.)

If the pre-selected banked specimens were used for the estimation of the performance for any of the viruses or subtypes in the assay, there should be a limitation stating this, since the established performance of that specific virus or subtype does not reflect the performance or prevalence in the intended use population.

If positive or negative interference has been reported for any commonly used collection materials or substances that may be endogenously or exogenously introduced into a specimen prior to testing, you should advise users of the possibility of false negative or false positive results due to such interference.

Specimen Collection

We recommend that you state that inadequate or inappropriate specimen collection, storage, and transport are likely to yield false negative test results. We also recommend that you state that operator training in specimen collection is highly recommended because of the importance of specimen quality.

Performance Characteristics

You should include in the package insert a summary of the study designs and the results from the studies (described in Section 6) that would aid users in interpreting test results. This section should include a description of the clinical (i.e., medical) and analytical (i.e., technical) performance characteristics. Clinical performance characteristics typically comprise prospective clinical study results summarizing performance (sensitivity, specificity or positive and negative percent agreement, 95% confidence intervals) for each virus identified by your assay. In cases where some retrospective clinical samples were also used, these results should be presented separately from the prospective clinical study results, as positive and negative agreement for each virus tested by the device. Analytical performance characteristics contain descriptions of the results and methodology used for the studies outlined in Section 6. In addition, analytical sensitivity levels (limits of detection) should be described in this section.

We recommend that the Performance Characteristics section describe the population(s) (i.e., geographical location, specimen types, and age groups) used to establish the performance characteristics of the device and provide the season (e.g., calendar years of influenza season) when this evaluation took place, along with the predominant virus subtype(s) observed during that time.

We recommend that you stratify positive and negative test results from your submitted clinical study by specimen source(s) and age. We also recommend that you separate results for children <5 years of age, older children, and adults. If this information is not available, you should add a Warning statement such as "Differences in performance are expected when this test is used on specimens from adults versus children, but specific differences are not known."

If you represent results using standardized viral quantitation methods (such as WHO and CLSI) for various virus subtypes in the labeling, we also recommend qualifying the information with a statement such as "NOTE: Although the assay has been shown to detect cultured avian influenza viruses, including avian Influenza A subtype H5N1 virus, the performance characteristics of this test with specimens from humans infected with H5N1 or other avian influenza viruses are unknown." Such a statement may help avoid misleading users into thinking that this analytical information on the detection of specific viruses applies to detection of these specific viruses in human clinical specimens.

Interpretation of Results

Your interpretation of the results section in the package insert should list all possible assay outputs and determinations of the presence or absence of each individual pathogen and assay control.

If internal controls are part of the determination of valid positive and negative results, you should provide the interpretation of each possible control result and recommendation how to follow up any invalid (i.e., no-call) result.

If your assay has an equivocal zone, you should provide the interpretation and the recommendation for how to follow up the equivocal result for each pathogen on your panel

(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 invalid or equivocal result, you should provide the recommendation whether testing should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen for each of these outputs.
If interpretation of the assay results involves combining the outputs of several viruses and viral targets to get a final result, (as would be the case in the respiratory viral panel assay that both detects Influenza A and differentiates Influenza A subtypes) there should be a clear interpretation of valid and invalid output combinations, and recommendations for any required follow up or retesting e.g. detecting Influenza A, but not any of the tested subtypes.

If your assay performance (i.e. sensitivity) for a specific analyte(s) demonstrated a lower bound of the two-sided 95% CI as less than 90%, negative results for this analyte may need to be interpreted as presumptive prompting a recommendation for confirmation by an alternate method (e.g. cell culture).

If your assay both detects Influenza A and differentiates between Influenza A subtypes, the interpretation of the results should direct the user that if a specimen yields a positive test result for Influenza A, but produces negative test results for all specific influenza A subtypes intended to be differentiated (i.e., H1 or H3), this result necessitates notification of appropriate local, state or federal public health authorities to determine necessary measures for verification of in accordance with the MMWR notice (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5613a4.htm and http://www.cste.org/ps/2007pdfs/novelfluanndssjan10final23.pdf), to determine whether the specimen represents a novel strain of Influenza A.

We recommend that you incorporate into the Results section directions for reporting results that include statements such as the following, as applicable:

> *Report negative test results as e.g. Influenza A virus not detected. This result does not exclude influenza viral infection.*
>
> *Report positive test results as 'Positive for e.g. Influenza A virus. This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype*.

Expected Values

This section should include the expected values using your test and the explanation of the result. It should also include the number of samples, age, gender, and demographics of the population used to determine the expected values.