Draft Guidance for Industry and FDA Staff

Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Document issued on: February 15, 2008

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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

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Draft Guidance for Industry and FDA Staff

Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic.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

I. Introduction

FDA is issuing this draft guidance to provide industry and agency staff with recommendations for studies to establish the analytical and clinical performance of in vitro diagnostic devices (IVDs) intended for the detection, or detection and differentiation, of influenza viruses. These devices are used to aid in the diagnosis of influenza infection. They include devices that detect one specific type or subtype, as well as devices that detect more than one type or subtype of influenza virus and further differentiate among them, to indicate whether the specimen contains influenza A virus versus influenza B virus, or which subtype of influenza A is present.[1]

This guidance provides detailed information on the types of studies FDA recommends to support Class I and Class II premarket submissions for these devices. The guidance

[1] There are three types of influenza viruses: A, B, and C. Influenza A viruses are further classified by subtype on the basis of the two main surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Influenza A subtypes and B viruses are further classified by strains (http://www.cdc.gov/flu/avian/gen-info/flu-viruses.htm). Strains are sub-groups of a given virus species that differ slightly in only some of their features.

includes a list of influenza virus strains recommended for analytical sensitivity studies, a list of microorganisms recommended for analytical specificity studies, and an example of a suggested format for presenting data from cross-reactivity studies.

This document is limited to studies intended to establish the performance characteristics of devices that detect either influenza viral antigens or influenza viral genome (protein or nucleic acid). It includes rapid detection devices and nucleic acid-based devices. It does not address detection of serological response from the host to the viral antigen, nor does it address establishing performance of non-influenza components of multi-analyte or multiplex devices.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance documents describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance documents means that something is suggested or recommended, but not required.

The Least Burdensome Approach

This draft guidance document reflects our careful review of what we believe are the relevant issues related to establishing performance characteristics of in vitro diagnostic devices for the detection, or detection and differentiation, of influenza viruses and what we believe would be the least burdensome way of addressing these issues. If you have comments on whether there is a less burdensome approach, however, please submit your comments as indicated on the cover of this document.

II. Background

This document recommends studies for establishing the performance characteristics of in vitro diagnostic devices for the detection, or detection and differentiation, of influenza viruses, including those for the detection or detection and differentiation of novel influenza viruses[2] in either human specimens or culture isolates. FDA believes that these recommended studies will be relevant for Class I and Class II premarket submissions (e.g., 510(k) or de novo classification petition) that may be required for a particular test.

A manufacturer who intends to market an in vitro diagnostic device for detection, or detection and differentiation, of influenza viruses must conform to the general controls of the Federal Food, Drug, and Cosmetic Act (the Act) and, unless exempt, obtain

[2] Novel influenza viruses are new or re-emergent human strains of influenza that cause cases or clusters of human disease, as opposed to those human strains commonly circulating that cause seasonal influenza and to which human populations have residual or limited immunity (either by vaccination or previous infection).

premarket clearance or approval prior to marketing the device (sections 510(k), 513, 515 of the Act; 21 U.S.C. 360(k), 360c, 360e)

This document is intended to supplement 21 CFR 807.87 (information required in a premarket notification) and other FDA resources such as "Premarket Notification: 510(k)", http://www.fda.gov/cdrh/devadvice/314.html. Guidance on the content and format for abbreviated and traditional 510(k)s is available at http://www.fda.gov/cdrh/ode/guidance/1567.html. Information regarding the use of standards can be found in section 514(c)(1)(B) of the Act (21 U.S.C. 360d(c)(1)(B)), and in the FDA guidance, "Use of Standards in Substantial Equivalence Determinations," http://www.fda.gov/cdrh/ode/guidance/1131.pdf. The Special 510(k) is an option for manufacturers considering modifications to their own cleared devices. Information on how to prepare a Special 510(k) is available at http://www.fda.gov/cdrh/devadvice/3144.html.

In addition, this document complements two FDA guidance documents that specifically address influenza IVDs: "In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path," (http://www.fda.gov/cdrh/oivd/guidance/1594.html), and "Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses," (http://www.fda.gov/cdrh/oivd/guidance/1596.pdf).

The guidance document entitled, "In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path," addresses recommendations for fulfilling labeling requirements applicable to all in vitro diagnostic devices intended to detect influenza A (or A/B) virus directly from human specimens, with a particular emphasis on ensuring appropriate labeling for legally marketed influenza A (or A/B) test devices whose clearances are not based on data addressing performance with regard to novel influenza A viruses infecting humans (including H5N1). It also discusses the FDA's thinking on premarket pathways for new or modified products intended to detect influenza A viruses, including a novel influenza A virus, or to detect and differentiate a specific influenza A virus.

The guidance document entitled "Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses," is one of two special controls for reagents for detection of specific novel influenza A viruses, classified into class II under 21 CFR 866.3332. This special controls guidance document includes recommendations for establishing device performance, as well as recommendations for labeling and postmarket measures. Devices classified under 21 CFR 866.3332 are subject to an additional special control limiting distribution of these devices to laboratories with experienced personnel having training in standardized molecular testing procedures and expertise in viral diagnosis and appropriate biosafety equipment and containment.

This draft guidance is intended to complement the two preceding guidance documents by describing the types of studies FDA recommends for establishing the analytical and clinical performance of in vitro diagnostic devices (IVDs) intended for the detection, or detection and differentiation, of influenza viruses. FDA recommends that when this draft

guidance is finalized, sponsors of influenza diagnostic devices use this guidance, in combination with the two existing guidances regarding influenza diagnostics, for information on FDA's current thinking about the regulation of these devices.

III. Scope

As previously described, this document recommends studies for establishing the performance characteristics of in vitro diagnostic devices for the detection or detection and differentiation of influenza viruses, including those for the detection of novel influenza viruses in either human specimens or culture isolates. This document is limited to studies intended to establish the performance characteristics of devices that either detect influenza viral antigens or influenza viral genome (protein or nucleic acid). These include rapid detection devices and nucleic acid-based devices. This guidance does not address detection of serological response from the host to the viral antigen. Nor does it address establishing performance of non-influenza components of multi-analyte or multiplex devices.

The scope of this document includes the devices described in existing classifications, as indicated below, and may also be applicable to future influenza diagnostic devices that may not fall within these existing classifications. Those future devices may include devices that will be subject to requests for initial classification under section 513(f)(2) of the act ("de novo classification"), as well as subsequent devices that seek determinations of substantial equivalence to future de novo cleared devices.

The following are existing influenza IVD classification regulations:

> **21 CFR 866.3330 Influenza virus serological reagents:**
>
> (a) Identification. Influenza virus serological reagents are devices that consist of antigens and antisera used in serological tests to identify antibodies to influenza in serum. The identification aids in the diagnosis of influenza (flu) and provides epidemiological information on influenza. Influenza is an acute respiratory tract disease, which is often epidemic.
>
> (b) Classification. Class I (general controls). The device is exempt from the premarket notification procedures in subpart E of part 807 of this chapter subject to the limitations in § 866.9.

Although devices within the classification described in 21 CFR 866.3330 are Class I devices, which are generally exempt from premarket notification, under FDA regulations a premarket notification may be required for some tests purported to fall within this type of device. Specifically, an IVD for detection of influenza is not exempt from 510(k) to the extent that it meets the limitations on exemption defined in 21 CFR 866.9:

- Under 21 CFR 866.9(c)(6), an IVD that is intended for use in *identifying or inferring the identity of a microorganism directly from clinical material* is not exempt from premarket notification requirements. An IVD that is intended to

detect an influenza virus directly from a human specimen falls within this provision.

- In addition, an IVD to detect influenza may trip the limitations in 21 CFR 866.9(a) if the new device is *intended for a use different from the intended use of a legally marketed device* classified under 21 CFR 866.3330; or may trip the limitations in 21 CFR 866.9(b), if it *operates using a different fundamental scientific technology* from existing influenza tests in that classification.

The following are the product codes for devices cleared under 21 CFR 866.3330:

GNX – Antigens, Cf, including Cf control, Influenza virus A, B, C
GNS – Antisera, Hai, Influenza virus A, B, C
GNT – Antigens, HA (including HA control), Influenza virus A, B, C
GNW – Antisera, Cf, Influenza Virus A, B, C

21 CFR 866.3332 Reagents for detection of specific novel influenza A viruses

(a) Identification. Reagents for detection of specific novel influenza A viruses are devices that are intended for use in a nucleic acid amplification test to directly detect specific virus RNA in human respiratory specimens or viral cultures. Detection of specific virus RNA aids in the diagnosis of influenza caused by specific novel influenza A viruses in patients with clinical risk of infection with these viruses, and also aids in the presumptive laboratory identification of specific novel influenza A viruses to provide epidemiological information on influenza. These reagents include primers, probes, and specific influenza A virus controls.

(b) Classification. Class II (special controls). The special controls are:

(1) FDA's guidance document entitled "Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses." See § 866.1(e) for information on obtaining this document.
(2) The distribution of these devices is limited to laboratories with experienced personnel who have training in standardized molecular testing procedures and expertise in viral diagnosis, and appropriate biosafety equipment and containment.

The product code for this device is NXD.

IV. Risks to Health

Illness caused by commonly circulating influenza viruses can cause high morbidity and mortality, particularly in special populations such as the elderly and the very young. The development of acquired immunity to seasonal influenza viruses is limited because influenza viruses mutate in small but important ways from year to year (a process known as antigenic drift). In addition to the risks posed by seasonal influenza viruses, novel influenza viruses have the potential to cause widespread disease and/or disease of

unusually high severity because few, if any, people have prior exposure to these viruses. This lack of immunity, as well as additional pathogenic factors that may also increase virulence, results in a greater likelihood of morbidity and mortality among those infected.

In vitro diagnostic devices for the detection, or detection and differentiation, of influenza viruses are important for establishing the diagnosis of influenza, for differentiating seasonal from novel influenza virus strains, and for obtaining epidemiologic information on influenza outbreaks. Public health officials have emphasized the need for reliable influenza diagnostic devices that can differentiate seasonal from emerging viral strains and provide rapid test results.

Failure of devices for detection of influenza viruses to perform as expected or failure to correctly interpret results may lead to incorrect patient management decisions and inappropriate public health responses. In the context of individual patient management, a false negative report could lead to delays in providing (or failure to provide) definitive diagnosis and appropriate treatment and infection control and prevention measures. A false positive report could lead to unnecessary or inappropriate treatment or unnecessary control and prevention actions. Therefore, establishing the performance of these devices and understanding the risks that might be associated with the use of these devices is critical to their safe and effective use.

The studies conducted by manufacturers to establish the performance of influenza detection devices are the basis for determining the safety and effectiveness or substantial equivalence of these devices.

V. Establishing Performance Characteristics

A. Controls

When conducting the performance studies described below, we recommend that you run appropriate external controls every day of testing for the duration of the analytical and clinical studies. Examples of appropriate external controls include vaccine or prototypic vaccine strains, low pathogenic viruses, and inactivated viruses. Specific information about controls for nucleic acid based devices is provided in the section "Controls for nucleic-acid based influenza assays" of this guidance document. You may contact the Division of Microbiology Devices within the Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD) at FDA for further information regarding controls.

B. Performance Studies

We recommend you perform the following studies:

1. Analytical sensitivity for known Influenza A and B strains.

Limit of Detection

We recommend that you determine the limit of detection (LoD) using limited dilutions of regrown and retitered viral stocks. The study should include serial dilutions of at least two strains representative of types or subtypes for each claimed influenza virus (please see Table 1 for suggested viral strains) and 3-5 replicates for each dilution. You should report the LoD as the level of virus that gives a 95% detection rate. The LoD may be confirmed by preparing at least 20 additional replicates at the LoD concentration and demonstrating that the virus was detected 95% of the time. The reference methods we recommend for LoD determination are the tissue culture infectious dose$_{50}$ ($TCID_{50}$) and plaque assay.

We recommend that you determine the LoD for each analyte and each specimen type tested by the device. We suggest that you refer to Clinical Laboratory Standards Institute (CLSI) document EP17-A [1], when designing your studies.

Analytical Reactivity (Inclusivity)

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. For subtypes for which is it difficult to obtain sufficient samples to demonstrate detection of ten strains, we recommend that you contact the Division of Microbiology Devices to discuss your study. All virus identities and titers should be confirmed.

We suggest that the strains for LoD and analytical reactivity studies be selected from those shown in Table 1. 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 www.cdc.gov/flu/professionals/vaccination/composition0607.htm [2]. Vaccine strains may vary from one influenza season to another.

Table 1. Influenza strains recommended for analytical sensitivity (LoD) studies.

Type	Subtype	Influenza Viral Strain	Comments
A	H1N1-like	A/New Caledonia/20/1999	Vaccine strains for 2006-2007season
A	H3N2-like	A2/Wisconsin/67/2005 or Ag equiv A/Hiroshima/522005	Vaccine strains for 2006-2007season
B		B/Malaysia/2506/2004 or Ag equiv B/Ohio/1/2005	Vaccine strains for 2006-2007season
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	

2. Analytical Specificity

Cross-reactivity

We recommend that you test for potential cross-reactivity with non-influenza respiratory pathogens and other microorganisms with which 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. The microorganisms recommended for cross-reactivity studies are listed in Table 2. We encourage sponsors to present the results from the cross-reactivity studies for devices detecting multiple pathogens using the display format shown in Table 3.

Table 2. Microorganisms recommended for analytical specificity (cross-reactivity) studies.

Organism	Type
Adenovirus	Type 1
Adenovirus	Type 7
Human coronavirus*	
Cytomegalovirus	
Enterovirus	
Epstein Barr Virus	
Human parainfluenza	Type 1
Human parainfluenza	Type 2
Human parainfluenza	Type 3

Measles	
Human metapneumovirus	
Mumps virus	
Respiratory syncytial virus	Type B
Rhinovirus	Type 1A
Bordetella pertussis	
Chlamydia pneumoniae	
Corynebacterium sp.	
Escherichia coli	
Hemophilus influenzae	
Lactobacillus sp.	
Legionella spp	
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	

*** We recommend that you include the OC43 and 229E strains of Human coronavirus in your cross-reactivity study.**

Table 3. Data Presentation Example. (We encourage sponsors to present cross-reactivity testing data for devices detecting multiple pathogens in the following format.)

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	-	-	-	-	-	-	+

Interference

We recommend that you conduct a comprehensive interference study using medically relevant concentrations of the interferent and at least two strains for each influenza type to assess the potentially inhibitory effects of substances encountered in respiratory specimens.

Potentially interfering substances include, but are not limited to, the following: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms. Examples of potentially interfering substances are presented in Table 4. We recommend that you test interference at the assay cut-off determined for each influenza virus and for each of the interfering substances. 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. Please refer to the CLSI document EP7-A2 [3] for additional information.

Table 4. 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

3. Precision

Within-Laboratory Precision/Repeatability

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

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

- A "high negative" sample (C_5 concentration): a sample with an analyte concentration below the clinical cut-off such that results of repeated tests of this sample are negative approximately 95% of the time (and results are positive approximately 5% of the time).
- A "low positive" sample (C_{95} concentration): a sample with a concentration of analyte just above the clinical cut-off such that results of repeated tests of this sample are positive approximately 95% of the time.
- A "moderate positive" sample: a sample with a concentration at which one can anticipate positive results approximately 100% of the time (e.g., approximately two to three times the concentration of the clinical cut-off).

When the limit of blank (LoB) is used as a cutoff, then the concentration C_{95} is the same as the limit of detection (LoD) and the zero concentration (no analyte present in

sample) is C_5 (1). CLSI documents EP5-A2 [4] and EP12-A [5] contain further information about designing and performing precision studies.

Reproducibility

The protocol for the reproducibility study may vary slightly depending on the assay format. As a general guide, we recommend the following protocol:

- Evaluate the reproducibility of your test at 3 testing sites (for example, two external sites and one in-house site).
- Use a five day testing protocol, including a minimum of two runs per day, (unless the assay design precludes multiple runs per day) and three replicates of each panel member per run.
- Each day, have at least two operators at each facility perform the test. We recommend that, for rapid testing or point-of-care (POC)[3] devices, you include a larger number of devices in your evaluation, in order to best represent the settings in which the devices will be used.
- Use the same sample panel as described in the repeatability study above.

The CLSI document, EP15-A2 [6], contains additional information on reproducibility study design.

4. Specimen Storage and Shipping Conditions

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

5. Clinical Performance Studies

We recommend that you conduct prospective clinical studies to determine the performance of your device for all the specimen types you claim in your labeling. We recommend that you compare your assay's performance to the established gold standard reference methods of viral culture and direct fluorescent antibody (DFA) tests.[4]

We recommend that you assess the ability of your device to detect influenza viruses in fresh specimens from patients suspected of having an influenza infection. Frozen archived specimens may be useful for developing pre-clinical data but are not

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

[4] Comparing performance of a new assay against an established reference method creates a frame of reference for evaluating the device that is useful whether the data is to be considered in an initial classification action or to facilitate comparison with the performance of a predicate device, in the case of a premarket notification and evaluation of substantial equivalence.

recommended for studies to calculate clinical sensitivity or specificity. Freeze-thawing can change the characteristics of the specimen from those of fresh specimens with which the test is intended to be used, possibly affecting assay performance. However, for devices intended to detect and/or differentiate novel influenza viruses for which fresh specimens are difficult to obtain, you may use frozen archived clinical specimens from patients who are case-confirmed, in accordance with World Health Organization (WHO) criteria for laboratory-confirmed cases, to demonstrate the performance of your device [8].

If both fresh samples and frozen archived samples are tested, we recommend that you analyze the data separately. For frozen archived samples, results should be represented as percent agreement.

In general, when the number of specimens available for clinical testing is very low (e.g., newly emerging strains), the available evidence for FDA's premarket review may, of necessity, be obtained from analytical rather than clinical studies. In this circumstance, it is particularly critical to have well designed analytical studies. Animal studies are optional and can be used to supplement analytical studies.

Study Protocol

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

We encourage sponsors to contact the Division of Microbiology Devices to request a review of their proposed studies and selection of specimen types. This is referred to as the pre-IDE process. We particularly encourage manufacturers to seek this type of discussion when samples are difficult to obtain.

Specimen Type(s)

The total number of samples you should include in your study for substantiating a claim for detection of influenza A, influenza B, or H/N subtypes of influenza A, will depend on the prevalence of the virus and on assay performance. We recommend that all influenza detecting devices demonstrate specificity with a lower 95% (two-sided) confidence bound exceeding 90%.

- For rapid devices detecting influenza A virus antigen, we recommend that you include a sufficient number of prospectively collected samples for each specimen type you claim to generate a sensitivity result with a lower bound of the two-sided 95% confidence interval (CI) greater than 60%. Generally, we recommend testing a minimum of 50 samples, determined to be positive using the reference method, for each specimen type..
- For rapid devices detecting influenza B virus antigen, we recommend that you include a sufficient number of samples for each claimed specimen type to

generate a result for sensitivity with a lower bound of the two-sided 95% CI greater than 55%. Generally we recommend a minimum of 30 positive samples for each specimen type. If a limited number of influenza B samples are available, we recommend that you contact the Division of Microbiology Devices to discuss alternative proposals.

- We recommend that alternative test formats such as nucleic acid-based tests demonstrate at least 90% sensitivity with a lower bound of the two-sided 95% CI greater than 80%, irrespective of the influenza virus type.

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

Study Sites

We recommend that you conduct your studies at a minimum of three separate facilities, one of which may be in-house. Clinical investigations of unapproved and uncleared in vitro diagnostic devices, including diagnostic devices for influenza, are subject to the investigational device exemption (IDE) provisions of Section 520(g) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360j) and the implementing regulations. You should consider how 21 CFR part 812 (IDEs) applies to your particular study and refer to 21 CFR part 50 (informed consent), and 21 CFR part 56 (institutional review board review) for other applicable requirements. Investigational devices that detect novel influenza viruses, such as influenza A/H5, may be particularly likely to meet the definition of "significant risk device" in 21 CFR 812.3(m). Clinical investigations of significant risk devices require the submission of an IDE application to FDA for review and approval, in accordance with 21 CFR part 812.[5]

We recommend that the performance evaluation for devices intended for point-of-care (POC) use or rapid testing include, at a minimum, one site at a clinical laboratory as well as sites representative of non-laboratory settings where the device is intended to be used (e.g., physician's office, emergency department). Conducting testing with the device in a clinical laboratory with more experienced and trained personnel, in addition to testing in non-laboratory sites where the device is intended to be used but operators are likely to have less laboratory training, will help to determine whether training of the person conducting the test is likely to affect the performance of the device.

Study Population

We recommend that you conduct your studies on individuals presenting with influenza-like symptoms (e.g., cough, nasal congestion, rhinorrhea, sore throat, fever,

[5] You may also refer to the "Information Sheet Guidance for IRBs, Clinical Investigators, and Sponsors, Significant risk and Nonsignificant Risk Medical Device Studies" at http://www.fda.gov/oc/ohrt/irbs/devrisk.pdf and "Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" at http://www.fda.gov/cdrh/oivd/guidance/1588.pdf.

headache, myalgia). Influenza virus concentration in nasal and tracheal secretions remains high for 24-48 hours after the onset of the symptoms and may last longer in children. If your device is intended for screening individuals for influenza infection, you should also include asymptomatic individuals in your study population.

We recommend that you include a meaningful number of samples from each age group. We recommend that you present the data stratified by age (e.g., less than 5, 6-21, 22-59, and greater than 60 years old) in addition to the overall data summary table.

Reference Methods

We recommend that you compare results obtained with your device to the results obtained by using one or more of the following established reference methods: (1) virus culture followed by DFA or other antigen detection system (e.g. ELISA) and/or (2) a direct specimen fluorescence assay (DSFA) that has been cleared by the FDA. Direct specimen testing using immunofluorescent methods (DSFA) provides a specific result that is available faster than culture. However, because DSFA is generally less sensitive than culture, we recommend that all DSFA-negative specimens be cultured.

We recommend that you verify that the virus culture method used in your study follows the CLSI document M41-A [7], and WHO Manual on Animal Influenza Diagnosis and Surveillance [8]. It is essential that the specimens be rapidly transported to the laboratory for optimal virus recovery or detection. Culture should not be performed on frozen specimens as freeze-thawing results in loss of virus infectivity. 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 pre-Amendments device,[6] then you should provide published literature or laboratory data in your premarket submission in support of the antibody validation for detection of influenza virus. We recommend that you use polymerase chain reaction (PCR) followed by sequencing of the amplicons as an alternative method for identification of the virus in cultured cells when FDA-cleared or well characterized antibodies are not available. If your test is based on nucleic acid amplification technologies, the primer sequences for the comparator PCR should be different from the primer sequences included in your device.

Additionally, if public health authorities recommend against culturing a novel virus, we recommend that you use PCR testing followed by sequencing of the amplicons to confirm the identity of the novel virus. You should provide published literature or laboratory data in your submission, in support of the PCR validation for detection of the novel virus. If you use sequencing as a comparative method, we recommend that you perform the sequencing reaction on both strands of the amplicon (bidirectional

[6] Pre-Amendments 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).

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 [9, 10].

C. CLIA Waiver

If you are seeking waiver for your device under the Clinical Laboratory Improvement Amendments of 1988 (CLIA),[7] we recommend that you consult with Division of Microbiology Devices staff regarding the design of specific studies to support the CLIA waiver application for your device. The draft guidance for industry and FDA staff, "Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications," is available at http://www.fda.gov/cdrh/oivd/guidance/1171.pdf.

D. Nucleic Acid-based Influenza Devices

The information described here is relevant to studies intended to determine the performance of nucleic acid-based influenza assays. This section complements the recommendations for performance studies described earlier in this document.

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

We recommend that you demonstrate that carry-over and cross-contamination do not occur with your device. In a carry-over and cross-contamination study, we recommend that high positive samples be used in series alternating with high negative samples in patterns dependent on the operational function of the device. At least 5 runs with alternating high positive and high negative samples should 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 carry-over 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%. For details, see [11].

2. Controls for Nucleic Acid-based Influenza Assays

We recommend that you use quality control material for verification of assay performance in analytical and clinical studies. We recommend that you consult with FDA when designing specific controls for your device. If your device is based on nucleic acid technology, we generally recommend that you include the following types of controls:

Negative Controls

[7] See 42 U.S.C. § 263a(d)(3).

Blanks or no template control

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

Negative sample control

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

- Patient specimen from a non-influenza infected individual
- Samples containing a non-target organism (e.g., cell line infected with non-influenza virus)
- Surrogate negative control, e.g., alien encapsidated RNA [12]

Positive Controls

Positive control for complete assay

The positive control contains target nucleic acids, and is used to control the entire assay process, including RNA extraction, amplification, and detection. It is designed to mimic a patient specimen and is run as a separate assay, concurrently with patient specimens, at a frequency determined by a laboratory's Quality System (QS). Examples of acceptable positive assay control materials include:

- Cell lines infected with a non-pathogenic strain of influenza virus
- Packaged influenza RNA

Positive control for amplification/detection

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

Internal Control

The internal control is a non-target nucleic acid sequence that is co-extracted and co-amplified with the target nucleic acid. It controls for integrity of the reagents (polymerase, primers, etc.), equipment function (thermal cycler), and the presence of inhibitors in the samples. Examples of acceptable internal control materials include human nucleic acid co-extracted with the 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 [10].

VI. References

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