

**510(k) DENOVO
DECISION SUMMARY**

A. 510(k) Number: k060159

B. Purpose for Submission: Marketing authorization of new device

C. Measurand: Novel influenza A/H5 ribonucleic acid (RNA)

D. Type of Test: Qualitative real-time RT-PCR

E. Applicant: Centers for Disease Control and Prevention

F. Proprietary and Established Names:

Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set
Reagents for Detection of Specific Novel Influenza A Virus Reagents

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3332 Reagents for Detection of Specific Novel Influenza A viruses
2. Classification: Class II (de novo)
3. Product code: NXD, (nucleic acid amplification, novel influenza A virus, A/H5 RNA)
4. Panel: 83 (Microbiology)

H. Intended Use:

1. Intended use(s):

The Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set is intended for the in vitro qualitative detection of Influenza A/H5 (Asian lineage) virus RNA either directly in patient respiratory specimens or in viral cultures for the presumptive laboratory identification of Influenza A/H5 (Asian lineage) virus.

Testing with the Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set should be used in conjunction with other laboratory testing and clinical observations for the following indications:

1. Providing epidemiological information for the surveillance of human infection with Influenza A/H5 (Asian lineage) virus

2. Identifying patients who may be infected with Influenza A/H5 (Asian lineage) virus based on clinical and epidemiological risk factors

The definitive identification of influenza A/H5 (Asian lineage) either directly from patient specimens or from viral cultures requires additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza surveillance experts.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

Use of this assay is limited to Laboratory Response Network (LRN) designated laboratories.

2. Indication(s) for use: Same as intended use.
3. Special conditions for use statement(s):
Restricted Device
4. Special instrument requirements:

Real-time PCR instrumentation (Roche LightCycler[®], Cepheid SmartCycler[®], Applied Biosystems ABI 7000 Sequence Detection System or ABI Prism[®] 7700 Sequence Detection) along with accessory supplies and lab equipment recommended for each instrument system.

I. Device Description:

The Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set is composed of 2 primer pairs (4 unlabeled oligonucleotides) and 3 labeled probes, along with an inactivated virus control. These reagents are for use in single-tube real-time RT-PCR testing to directly detect novel Influenza A virus specific RNA in human respiratory specimens, or in viral cultures. The real-time PCR process simultaneously amplifies and detects nucleic acid targets in the same reaction.

The primer and probe sets (FluA2 and FluA3) target two distinct RNA regions that are both present in the influenza A/H5 (Asian lineage) hemagglutinin (HA) gene of highly pathogenic H5N1 viruses from the Asian lineage. These target regions were chosen to allow specific detection of Asian lineage influenza A/H5 viruses without detection of other influenza virus subtypes, including the North American lineage influenza A/H5 viruses (e.g., avian H5N2 strains).

Note: There are two lineages of avian influenza A/H5 viruses: the Eurasian (Asian) and North American (American) lineages. Viruses from these two lineages are genetically different. All known human influenza H5 infections have been caused by

highly pathogenic viruses of the Asian lineage.

- **Primers/Probes:** The 2 primer and probe sets (FluA2 and FluA3), each target a different region within the HA gene. The FluA2 target is in the HA2 region of the HA gene and the FluA3 target spans the cleavage site of the HA gene. These sets were selected from multiple candidates based on preliminary assessment of reaction efficiency and primer-dimer effects. FluA2 contains an equal mixture of two probes, to ensure optimum homology with viruses within both clades. The probes are labeled with FAM (6-carboxyfluorescein) and Blackhole Quencher™ 1 (BHQ™1). The BHQ chemistry is designed to minimize non-specific fluorescence. Experimental efficiency values of 100% +/- 5% are considered optimal. Taqman reaction efficiencies of FluA2 and FluA3 sets were demonstrated to be 100.6% (R2=0.996) and 100.3% (R2=0.996).
- **Influenza A/H5N1 positive control** (500 µL of virus suspended in 0.01 M PBS): a reverse-engineered vaccine candidate virus that may safely be handled in BSL-2. Inactivated with beta- propiolactone (0.05%). The reassortant virus is non-infectious in chickens and USDA has removed it from the select agent list, classifying it as a BSL2 infectious agent. Additionally, the inactivated virus preparation is non-infectious in embryonated chicken eggs.

Other reagents or accessories required to perform testing with the device are:

- Qiagen QuantiTect™ Probe RT-PCR Kit (Qiagen), a master-mix for reverse-transcription and cDNA amplification.
- Materials for extraction and purification of specimens (three commercial extraction kits are suggested for use in isolating RNA in the test procedure based on specifications meeting the requirements for quality of RNA to be used in the FluA2 and FluA3 reactions). These extraction procedures were used during development and testing of the *Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set*.
- An RNase P (RP) Real-time PCR Primer and probe set that targets the human ribonuclease P (RP) sequence. Extracted clinical specimens should contain human RNA. The RP assay thus serves as a control to ensure RNA resulted from the extraction of clinical specimens.

J. Substantial Equivalence Information:

1. Predicate device name(s): None.
2. Predicate 510(k) number(s): None.
3. Comparison with predicate: Not applicable.

There are other devices that generally detect Influenza A viruses, however, there is no identifiable predicate that detects specific novel Influenza A viruses. There are other IVD devices that use probe/primer sets for the detection of other specific pathogenic

microorganisms, and that rely on real-time PCR methods for their principles of operation.

K. Standard/Guidance Document Referenced (if applicable):

1. A special controls guidance document will be promulgated.
2. CDRH Draft Guidance for Industry and FDA Staff titled: *Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens*, December 8, 2005.

L. Test Principle:

Human respiratory specimens (nasopharyngeal swabs or aspirates, throat swabs, nasal swabs, or liquefied lower respiratory samples) from symptomatic patients, and viral cultures must be processed initially to extract and purify RNA from the cellular specimen matrix, using general purpose reagents.

Each extracted RNA sample is reacted separately with each primer and probe set in a single-tube real-time RT-PCR reaction. After initial reverse transcription of RNA into cDNA, amplification cycles proceed during which the fluorogenic probes, (each dual-labeled with a reporter dye (FAM) attached to the 5' end and a quencher dye attached at or near the 3' end), anneal to specific target sequences located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of *Taq* polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by fluorimeter modules within specified real-time instruments.

If both of the H5 HA targets (FluA2 and FluA3) are detected, testing is presumptive positive for Influenza A/H5 (Asian lineage) virus. When only one or the other of the targets is detected, results are reported as equivocal for Influenza A/H5 (Asian lineage) virus. Testing is negative if neither target is detected. A positive RP target test assures that human cellular material was extracted and that the PCR chemistry is unaffected by inhibition. All presumptive positive and equivocal results are subjected to additional testing procedures to definitively identify an influenza A/H5 (Asian lineage) virus.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

At two different times, 6 simulated respiratory specimens (3 swabs and 3 liquid) that were spiked with both human cellular material and titered amounts of the virus control material were distributed to multiple laboratories. These samples were tested in 10 laboratories using the ABI 7000, 7 laboratories using ABI 7700, 9

laboratories using LightCycler[®], and 10 laboratories using SmartCycler[®] Real-time PCR instrument systems.

With these contrived samples that contained levels of virus at the lower end of the levels that have been recovered from respiratory specimens from human cases (10^1 - 10^6 TCID₅₀/mL),¹ all 36 samples (tested in different laboratories, using the 4 different real-time PCR instruments) containing 400 TCID₅₀/mL, and the majority of samples containing 40 TCID₅₀/mL were detected (47/55 or 85.5% of these samples were positive; 52/55 or 94.5% were positive or equivocal, with 3 negative for both targets). At the 4 TCID₅₀ level, 32/36 or 88.9% were positive (both targets), while 35/36, or 97.2% were positive in at least one target (positive or equivocal). Of the 72 negative control samples, 1 was equivocal for an agreement with expected results of 98.6%. Breakouts for each instrument by sample level are below.

Combined laboratory results for the different instruments are shown below:

Summary of Reproducibility Study by 10 LRN Laboratories using the ABI7000					
Simulated specimen type	Virus concentration TCID ₅₀ /mL ¹	Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set Result			Agreement with expected results
		Presumptive positive	Equivocal	Negative	
Liquid, mod	400	10	-	-	100% (10/10)
Swab, mod	40	10	-	-	100% (10/10)
Liquid, low	4	10	-	-	100% (10/10)
Swab, low	0.4	1	2	7	10% (1/10)
Liquid, neg	none	-	-	10	100% (10/10)
Swab, neg	none	-	-	10	100% (10/10)
Overall agreement with expected result					85% (51/60)

¹ Virus concentration (TCID₅₀/mL) was extrapolated for each simulated specimen by comparing RT-PCR crossing threshold (Ct) values to a standard curve prepared by testing serial dilutions of an Influenza A/H5N1 virus of a known concentration (TCID₅₀/mL). TCID₅₀/mL was determined using Madin-Darby canine kidney (MDCK) cells.

Summary of Reproducibility Study by 7 LRN Laboratories using the ABI7700					
Simulated specimen type	Virus concentration TCID ₅₀ /mL ¹	Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set Result			Agreement with expected results
		Presumptive positive	Equivocal	Negative	
Liquid, mod	400	7	-	-	100% (7/7)
Swab, mod	40	7	-	-	100% (7/7)
Liquid, low	4	7	-	-	100% (7/7)
Swab, low	0.4	-	2	5	0% (0/7)
Liquid, neg	none	-	-	7	100% (7/7)
Swab, neg	none	-	-	7	100% (7/7)
Overall agreement with expected result					83% (35/42)

¹ Virus concentration (TCID₅₀/mL) was extrapolated for each simulated specimen by comparing RT-PCR crossing threshold (Ct) values to a standard curve prepared by testing serial dilutions of an Influenza A/H5N1 virus of a known concentration (TCID₅₀/mL). TCID₅₀/mL was determined using Madin-Darby canine kidney (MDCK) cells.

¹ Ng EKO, Cheng PKC, Ng AYY, Hoang TL, Lim WWL., Influenza A H5N1 Detection Emerg Infect Dis. 2005 Aug 25, 2005

Summary of Multicenter Reproducibility Study by 9 LRN Laboratories using the LightCycler®					
Simulated specimen type	Virus concentration TCID ₅₀ /mL ¹	Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set Result			Agreement with expected results
		Presumptive positive	Equivocal	Negative	
Liquid, mod	400	9	-	-	100% (9/9)
Swab, mod	40	7	1	1	78% (7/9)
Liquid, low	40	8	-	1	89% (8/9)
Swab, low	4	6	2	1	67% (6/9)
Liquid, neg	none	-	-	9	100% (9/9)
Swab, neg	none	-	1	8	89% (8/9)
Overall agreement with expected result					87% (47/54)

¹ Virus concentration (TCID₅₀/mL) was extrapolated for each simulated specimen by comparing RT-PCR crossing threshold (Ct) values to a standard curve prepared by testing serial dilutions of an Influenza A/H5N1 virus of a known concentration (TCID₅₀/mL). TCID₅₀/mL was determined using Madin-Darby canine kidney (MDCK) cells

Summary of Multicenter Reproducibility Study by 10 LRN Laboratories using the SmartCycler®					
Simulated specimen type	Virus concentration TCID ₅₀ /mL ¹	Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set Result			Agreement with expected results
		Presumptive positive	Equivocal	Negative	
Liquid, mod	400	10	-	-	100% (10/10)
Swab, mod	40	6	3	1	67% (6/10)
Liquid, low	40	9	1	-	100% (9/10)
Swab, low	4	9	1	-	90% (9/10)
Liquid, neg	None	-	-	10	100% (10/10)
Swab, neg	None	-	-	10	100% (10/10)
Overall agreement with expected result					90% (54/60)

¹ Virus concentration (TCID₅₀/ml) was extrapolated for each simulated specimen by comparing RT-PCR crossing threshold (Ct) values to a standard curve prepared by testing serial dilutions of an Influenza A/H5N1 virus of a known concentration (TCID₅₀/mL). TCID₅₀/mL was determined using Madin-Darby canine kidney (MDCK) cells

² RP reaction was not performed on one of the ten samples. Probe reactions for this sample were negative

b. *Linearity/assay reportable range:* NA

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The Influenza A/H5N1 (Asian lineage) Virus Real-time RT-PCR Positive Control is expected to yield a crossing threshold within 25-35 cycles.

d. *Detection limit:*

The detection level using the ABI 7000 and ABI 7700 instrument system is approximated to be 4 TCID₅₀/mL in MDCK cells for each target (with 10-fold dilutions and up to 5 replicates). The detection level using the LightCycler®, or SmartCycler® instrument system is approximated to be 40 TCID₅₀/mL in MDCK cells for each target.

e. *Analytical specificity:*

Cross-reactivity with non-H5 Asian HA types (17 stock strains from freshly grown culture lysates including representative non-H5s, two H7s and one H9). All subtypes that are known to infect humans, either sporadically or commonly are represented except for H7N3. Non-reactivity with H7N3 is presumed because of similarity of this H7 with H7N7 that was tested.

Note: high viral titers would be expected with confluent tissue cultures (>10E7 TCID₅₀ per the WHO manual).

Additional cross-reactivity with non-influenza respiratory viruses and bacteria (8 viruses tested are broadly representative of other known respiratory viruses; 11 broadly representative bacterial species). Sequence mining including updated BLAST searches reduces the potential cross-reactivity for known bacteria and viruses.

No cross-reactivity with the tested viral strains or bacterial species was observed.

f. *Assay cut-off:*

PCR reactions are run for 45 cycles. Growth curves with crossing thresholds within 45 cycles with either the FluA2 or FluA3 targets (when controls are acceptable) are considered reactive. The FluA2 and FluA3 reactions should both have crossing thresholds when Asian H5 (Asian lineage) hemagglutinin (HA) viral RNA is present. An equivocal result (only one of the two target reactions positive) may be due to contamination, failure of one primer/probe set to react, partial or incomplete inhibition, or non-specific reactivity. Both presumptive positive and equivocal results are subject to additional testing for definitive identification. The approach with 2 targets optimizes detection capability for influenza A/H5 (Asian lineage) viruses.

2. Comparison studies: NA

3. Clinical studies:

a. *Clinical Sensitivity:* Sensitivity could not be estimated due to rarity of Asian H5 (Asian lineage) virus infections.

b. *Clinical specificity:* Specificity could not be estimated due to rarity of Asian H5 (Asian lineage) virus infections.

c. Other clinical supportive data (when a. and b. are not applicable):

Testing with the device on respiratory specimens from laboratory-confirmed patient cases (from Indonesia) of influenza H5N1 virus is shown below.

Clinical Case Results

Specimen Type	Laboratory-confirmed cases ¹ (n= 10 patients ² ; 27 specimens ³)			Contact patient (Not laboratory confirmed) (1 patient; 2 specimens)	
	Influenza A/H5 (Asian lineage) Primer/probe Result			Influenza A/H5 (Asian lineage) Primer/probe Result	
	Presumptive Positive (FluA2 and FluA3)	Equivocal (FluA2 or FluA3)	Negative	Presumptive Positive or Equivocal	Negative
Throat Swab	7	1	0	0	1
Nasal Swab	1	5	2	0	1
Other ⁴	2	3	1	0	0
Total	10	9	3	0	2

¹ Confirmation by independent (WHO or Indonesian) laboratory or sequence analysis

² Patient ages: ≤ 4 years (2); 5-18 years (2); 19-40 years (7)

³ Lung biopsy (1), pleural fluid (2), rectal swab (1) and serum (1) specimen results excluded

⁴ Includes broncho suction (3), lung aspirate (1), pharyngeal aspirate (1) and endotracheal wash (1)

No influenza A/H5 (Asian lineage) virus was detected in the samples from the one contact case by a WHO laboratory or by additional testing done at CDC.

For the 10 laboratory-confirmed H5N1 patient cases: 10/22 (45%, 24.4-67.8%) specimens were presumptive positive for the virus, while another 9 were equivocal. Thus 19/22 specimens (86%, 65.1-97.1%) had A/H5 (Asian lineage) virus detected in one or both of the probe-set reactions, agreeing with the patient case diagnosis. All lab-confirmed cases had at least one positive test when multiple specimens were tested (all patients contributed at least 2 samples, usually at least a throat and a nasal swab). Multiple WHO methods (culture, sequencing, RT-PCR testing for multiple HA targets) were used to characterize H5N1 in these patients.

Testing with the device on banked respiratory specimens from individuals with influenza-like illness is shown below.

Banked¹ Human Respiratory² Specimen Results

BRRAT ³ Lab Influenza A/H5 Primer/ Probe Result	Influenza Branch ⁴ Lab Real-time RT-PCR Panel Result			
	Influenza A/H1 Positive	Influenza A/H3 Positive	Influenza A/H5 Positive	No Influenza detected
Presumptive Positive	0	0	1	0
Equivocal	0	2	0	0
Negative	5	75	0	121

¹ Specimens received by CDC Influenza Branch Laboratory from Vietnam (168), United States (21), Thailand (11), Kuwait (4)

² Throat swab (171), nasopharyngeal swab (26), pharyngeal swab (2), oropharyngeal aspirate (2), nasal swab (1), nasal wash (1), and endotracheal aspirate (1)

³ Bioterrorism Rapid Response and Advanced Technology Laboratory at CDC

⁴ Influenza Branch Laboratory at CDC serves as the World Health Organization (WHO) Collaborating Center for Surveillance, Epidemiology and Control of Influenza and is one of four regional WHO collaborating centers and reference laboratories involved in surveillance and characterization of circulating influenza viruses around the world.

Overall agreement with an expected negative result was 98.5% (201/204), 95.8-99.7% (95% CI). One sample positive in both FluA2 and FluA3 was characterized by a panel of WHO-recognized RT-PCR assays as H5N1. The other non-negative samples were equivocal (reactive in only one of the primer/probe assays). Additional testing showed these samples contained A/H3 virus. Testing with the device on banked respiratory specimens from tissue culture samples is shown below.

Banked¹ Tissue Culture Sample Results

BRRAT ² Lab Influenza A/H5 Primer/ Probe Result	Influenza Branch ³ Lab Real-time RT-PCR Panel Result			
	Influenza A/H3 Positive	Influenza A/H5 Positive	Influenza B Positive	No Influenza detected
Presumptive Positive	0	0	0	0
Equivocal	1	0	0	0
Negative	7	0	1	12

¹ Tissue cultures received by CDC Influenza Branch Laboratory from international locations (18), and United States (3)

² Bioterrorism Rapid Response and Advanced Technology Laboratory at CDC

³ Influenza Branch Laboratory at CDC serves as the World Health Organization (WHO) Collaborating Center for Surveillance, Epidemiology and Control of Influenza

Agreement with the expected negative result was 95.2% (20/21), 76.2-99.9% (95% CI).

4. Clinical cut-off: NA

5. Expected values/Reference range:

Currently, influenza A/H5 (Asian lineage) virus has not been detected in poultry or humans in the United States. False-positive results are more likely to occur when disease prevalence in the community is low. Testing of patient specimens is indicated for symptomatic patients meeting the clinical criteria and epidemiologic risk for infection with influenza A/H5 (Asian lineage) virus.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.3332 with special controls. The special control guidance document "Reagents for Detection of Specific Novel Influenza A viruses" will be available shortly.