

SPECIAL 510(k): Device Modification OIR Decision Summary

To: Centers for Disease Control and Prevention **RE:** K153148

This 510(k) submission contains information/data on modifications made to the SUBMITTER'S own Class II devices requiring 510(k). The following items are present and acceptable:

1. The name and 510(k) number of the SUBMITTER'S previously cleared device.
CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit (K141859)
2. Submitter's statement that the **INDICATION/INTENDED USE** of the modified device as described in its labeling **HAS NOT CHANGED** along with the proposed labeling which includes instructions for use, package labeling.
3. A description of the device **MODIFICATION(S)**, including to demonstrate that the **FUNDAMENTAL SCIENTIFIC TECHNOLOGY** of the modified device **has not changed**.
This device modification involved small changes to the primers and probes of the assay. The gene target and location of the primers has not changed from the predicated device. The diagnostic panel includes two assays H5a and H5b. Three primers and 1 probe were added to the H5a assay in addition to minor nucleotide changes to adapt the assay to the evolution of the highly pathogenic avian influenza A/H5. One primer was added to the H5b assay and minor nucleotide changes were made. Additionally the fluorescent probe quencher chemistry was changed to use the ZEN™ and Iowa Black® FQ quenchers on the probes included in the H5a and H5b assays.

To show that there was no impact on the performance of the assay with the modifications listed above a limit of detection study, analytical inclusivity study, analytical cross-reactivity study with non H5 influenza A viruses and microorganisms, and a small mock clinical study were conducted.

Limit of Detection Study:

Analytical sensitivity of the Influenza A/H5 Subtyping Kit was demonstrated by determining the LoD using Quanta qScript™ and Invitrogen SuperScript™ enzyme kits. The LoD for each primer and probe set was confirmed by testing extraction replicates (n=20) of the highest virus dilution where ≥95% of all replicates tested positive. Virus dilutions were prepared in virus transport medium containing human A549 cells to emulate clinical specimen matrix. The lowest concentration where the InfA and both H5a and H5b primer and probe sets demonstrate uniform detection was reported as the LoD. The results are summarized in the table below.

Influenza Virus Tested	Influenza Strain Designation	LOD (EID50/mL)	
		Invitrogen SuperScript™	Quanta qScript™
A/H5N1	A/Vietnam/1203/2004×A/Puerto Rico/8/34 reassortant (A/Vietnam/1203/2004 PR8-VNH5N1- PR8/CDC-RG)	10 ^{3.8}	10 ^{2.4}
	A/duck/Vietnam/NCVD-1544/2012	10 ^{3.1}	10 ^{3.1}
A/H5N8	A/gyrfalcon/Washington/41088-6/2014	10 ^{3.35}	10 ^{3.35}

Analytical Sensitivity-Inclusivity Testing:

Inclusivity testing was conducted to demonstrate the capability of the modified oligonucleotide primers and probes in the Influenza A/H5 Subtyping Kit to detect strains of influenza A/H5 viruses (Asian lineage) representative of different geographic locations and phylogenetic clades at or near the established LoD. Inclusivity testing was performed with sixteen representative H5 viruses (Asian lineage). A virus of the phylogenetic clade 2.2.2.1 was unavailable for testing therefore reactivity of the probes with A/Bangladesh/3222/2011 was performed *in silico*. The remaining fifteen viruses were grown to high titer, harvested, and serially diluted to near the LoD of the assays. The diluted influenza A/H5 viruses were extracted and tested in triplicate with the InfA, H5a, and H5b assays to demonstrate reactivity. Inclusivity of the Influenza A/H5 Subtyping Kit was evaluated with both enzyme systems (i.e. Invitrogen SuperScript™ and Quanta qScript™) and one cleared extraction method.

The Influenza A/H5 Subtyping Kit was reactive with all H5 (Asian lineage) isolates that were tested and predicted to be reactive with the influenza A/Bangladesh/3222/2011 based on the *in silico* analyses.

Analytical Specificity –Cross-Reactivity

Cross-reactivity of the Influenza A/H5 Subtyping Kit was evaluated by testing influenza A viruses of different types and subtypes that include viruses representing diverse geographic locations and different sources. Samples were tested in triplicate using RNA extracted from high titer preparations of viruses ($\geq 10^6$ EID50/mL). Cross-reactivity testing of the Influenza A/H5 Subtyping Kit was evaluated with the Invitrogen Superscript™ enzyme system and one cleared extraction method. The results showed no cross-reactivity with any non-influenza A H5 virus strains.

An exclusivity study was performed to demonstrate the specificity of each primer and probe set of the Influenza A/H5 Subtyping Kit when tested with common non-influenza human respiratory viruses, respiratory bacteria, and commensal organisms of the human respiratory tract. Nucleic acids were purified from thirty-five (35) non-influenza organisms (16 viruses, 18 bacteria, and 1 yeast) representing common respiratory pathogens or flora commonly present in specimens collected from the human nasopharynx region. High titer preparations of bacteria and yeast, generally greater than or equal to 10^6 cfu/mL, and non-influenza respiratory virus preparations at concentrations greater than 10^6 TCID50/mL were tested (except in cases where production of high titer virus stock was not possible). The Influenza A/H5 Subtyping Kit was evaluated with the Invitrogen Superscript™ enzyme system and one cleared extraction method. No cross-reactivity was detected with any of the organisms tested.

Mock Clinical Study

The clinical performance of oligonucleotide primer and probe sets of the Influenza A/H5 Subtyping Kit were evaluated using contrived samples of grown virus added to an A549 cell suspension to simulate positive clinical samples. A total of fifty positive contrived samples at high, moderate, and low concentrations were evaluated. In addition, sixty-five specimens that tested negative for influenza A with the CDC Human Influenza rRT-PCR Diagnostic Panel that were obtained from a clinical study conducted during the 2011-2012 influenza season were evaluated. Testing was performed with both enzymes cleared for use with the kit. The results are summarized in the table below.

Clinical Performance Evaluation Results

Enzyme Utilized	# of Positives ¹	% Positive Agreement (95% CI)	# of Negatives ²	% Negative Agreement (95% CI)
Quanta BioSciences TM qScript	50/50	100 (92.9 – 100.00)	65/65	100 (94.4 – 100.00)
Invitrogen SuperScript TM	44/50	88.0 (76.2 - 94.4)	65/65	100 (94.4 – 100.00)

¹Proportion of contrived samples correctly identified as positive by both influenza A H5a and H5b primer and probe sets.

²Proportion of negative samples correctly identified versus the comparator.

4. **Comparison Information** (similarities and differences) to applicant’s legally marketed predicate device including, labeling, intended use, physical characteristics.

	<p>CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel, Influenza A/H5 Subtyping Kit (K141859)</p>	<p>CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel, Influenza A/H5 Subtyping Kit (modified)</p>
<p>Similarities</p>		
<p>Intended Use</p>	<p>The Influenza A/H5 Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument in conjunction with clinical and epidemiological information:</p> <ul style="list-style-type: none"> •For the presumptive identification of virus in patients who may be infected with influenza A subtype A/H5 (Asian lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors; •To provide epidemiologic information for surveillance of circulating influenza viruses. <p>Performance characteristics for influenza were established during a season when seasonal influenza viruses A/H1 and A/H3 were the predominant influenza A viruses in circulation and during a season when the A/H1pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.</p> <p>Testing with the influenza H5a and H5b primer and probe sets should not be performed unless the patient meets the most current U.S. Department of Health and Human Services (DHHS) clinical and epidemiologic criteria for testing suspect A/H5 specimens. The definitive identification of influenza A/H5 (Asian lineage) either directly from patient specimens or from virus cultures requires additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza surveillance experts.</p> <p>Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, 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.</p> <p>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 department for testing. Viral culture should not be attempted unless a BSL 3+ facility is available to receive and culture specimens.</p> <p>All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.</p>	<p>Same</p>

Organism Detected	Universal influenza A viruses (animal and human) and Influenza A/H5 Subtype (Asian lineage) viruses.	Same
Specimen Types	Human respiratory specimens and viral culture.	Same
Nucleic Acid Extraction	Yes	Same
Extraction Method	<ul style="list-style-type: none"> • QIAamp® DSP Viral RNA Mini Kit, Qiagen • MagNA Pure Compact –Nucleic Acid Isolation Kit I, Roche • MagNA Pure Compact – RNA Isolation Kit, Roche • MagNA Pure LC – Total Nucleic Acid Kit, Roche • Qiagen QIAcube – QIAamp® DSP Viral RNA Mini Kit, Qiagen • NucliSENS® easyMAG®, bioMerieux 	Same
Enzyme Master Mix	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT- PCR Kit (with or without ROX) OR Quanta BioSciences qScript™ One-Step qRT-PCR Kit, Low ROX	Same
PCR Technology	Real-Time RT-PCR	Same
Required Instrumentation	Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4	Same
Differences		
Probe Quenching Molecule	Black Hole Quencher Probe® (BHQ-1)	ZEN™ Double-Quenched Probe (InfA, H5a, H5b, and RP assays) OR Black Hole Quencher Probe® (InfA and RP assays)
Oligonucleotides	H5a assay-Targets a region of the HA gene H5b assay-Targets a region of the HA gene InfA assay-Targets a conserved region of the matrix gene in Influenza A viruses	Gene targets of the oligonucleotide assays are the same as the predicate; minor changes to the oligonucleotide sequences have been made

5. A **Design Control Activities Summary** which includes:

- a) Identification of Risk Analysis method(s) used to assess the impact of the modification on the device and its components, and the results of the analysis.

The Risk Analysis was conducted and the results are provided in the table below.

Device Modification	Modification of Influenza A/H5 Subtyping Kit oligonucleotides to update and improve reactivity of assays with Influenza A H5 Asian lineage viruses	Updated Influenza A/H5 Subtyping oligonucleotide information in package labeling and product insert
Cause of Risk	Modified oligonucleotides may demonstrate variable reactivity among different H5 Asian lineage influenza viruses.	Differences exist between the package labeling of the currently cleared and the updated Influenza A/H5 Subtyping Kit; a brief overlap of the current and the updated kits may result in testing errors.
Hazardous Situation	H5 Asian lineage influenza viruses may not be detected by modified Influenza A/H5 Subtyping Kit oligonucleotides.	Short term risk of commingling and/or confusing components of currently cleared Influenza A/H5 Subtyping Kit and the updated Influenza A/H5 Subtyping kit.
Consequence	Influenza A/H5 infections in humans may not be identified in a timely fashion, leading to a delay in implementation of public health mitigation measures.	Commingling and/or confusing components from different Influenza A/H5 Subtyping Kit versions could impact ability to accurately detect Influenza A/H5 Asian lineage viruses.
Risk Control Measure	Characterization of the performance of the device with representative highly pathogenic avian influenza A/H5 Asian lineage viruses from different clades.	Product Update Communication will be distributed to the end users to communicate transition to using the updated Influenza A/H5 Subtyping Kit. Influenza A/H5 Subtyping Kit package labeling and product Insert will describe the part numbers and associated procedures required for performing testing with the kit. Currently distributed Influenza A/H5 Subtyping Kit will expire and inventory of this model will no longer be available to end users.
Risk Acceptability Criteria	Updated Influenza A/H5 Subtyping Kit oligonucleotides will detect Influenza A/H5 Asian lineage viruses from different clades and geographic regions.	Influenza A/H5 Subtyping Kit package labeling and package insert shall describe part numbers of critical components and correct methods for performing diagnostic testing.
Verification Method	Analytical and clinical performance testing were executed to verify reactivity of updated H5 oligonucleotides with Influenza A/H5 Asian lineage viruses.	Appropriate package labeling and product insert instructions will be verified against product components by the end user upon receipt per CLIA requirements.
Summary Conclusion	Test results demonstrate that the risk of not detecting Influenza A/H5 Asian lineage viruses is mitigated to an acceptable level. Based on the results of the analytical and clinical testing, the device is capable of detecting Influenza A/H5 Asian lineage viruses.	Risk of commingling and/or confusing components of currently cleared Influenza A/H5 Subtyping Kit is mitigated to an acceptable level.

- b) Based on the Risk Analysis, an identification of the verification and/or validation activities required, including methods or tests used and acceptance criteria to be applied.

The results of the analysis indicated hazardous situations could arise as a result of the modifications and risk control measures should be implemented. A protocol was developed to perform analytical and clinical performance testing (as detailed in section 3) to mitigate the risk of hazardous situations occurring. The results of the testing reduced the probability of occurrence and the risk to the negligible.

The labeling for this modified subject device has been reviewed to verify that the indication/intended use for the device is unaffected by the modification. In addition, the submitter's description of the particular modification(s) and the comparative information between the modified and unmodified devices demonstrate that the fundamental scientific technology has not changed. The submitter has provided the design control information as specified in The New 510(k) Paradigm and on this basis, I recommend the device be determined substantially equivalent to the previously cleared device.