CDC/APHL Teleconference on real-time RT-PCR Influenza Assay changes/updates

Background:

On August 11th, 2011 APHL and CDC held a joint teleconference for State and Local Public Health Laboratories (PHLs) to provide updates on the harmonization and reconfiguration of both the CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel and CDC Influenza 2009 (H1N1) pdm Real-Time RT-PCR Panel. The intent of the call was to review updates and changes to the new package insert, explain the new kit configuration, including ordering reagents, provide an update on the Influenza Reagent Resource (IRR), and provide an opportunity for public health laboratories to ask questions.

Teleconference Minutes and Important Points:

Harmonization and Reconfiguration of CDC Influenza Real-Time RT-PCR Panels

Dr. Stephen Lindstrom provided an overview of the major changes to the kit configuration and package insert. The key reasons for the changes to the current format is to 1) harmonize the two currently FDA-cleared kits to a single package insert and 2) to eliminate discrepancies between CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel and CDC Influenza 2009 (H1N1) pdm Real-Time RT-PCR Panel package inserts.

The new configuration consists of dividing the panels into three modular kits. The kits will each provide 1000 reactions per kit. The new modular configuration will allow PHLs to order kits more efficiently by minimizing the amount of reagents wasted. Laboratories will be able to order kits in the following formats:

1. Influenza A/B Typing Kit (InfA, InfB, RP, PIPC, HSC)
2. Influenza A Subtyping Kit (Inf A, H1, H3, pdm Inf A, pdm H1, RP, PIPC)
3. H5N1 kit (InfA, H5a, H5b, RP, H5VC, HSC)

This reconfiguration and changes to the package insert require a new FDA submission for clearance. The reconfiguration has not been cleared by FDA, CDC anticipates FDA clearance in the near future. CDC is currently coordinating the reagent manufacturing with the FDA clearance and anticipates a September release of the reagents. **Once the kits are cleared by FDA, CDC will automatically ship an initial set of kits (Influenza A/B Typing, and Influenza A Subtyping kit) to all Influenza qualified laboratories. Since the kits will be automatically shipped please do not order your laboratories first set of kits through the Influenza Reagent Resource (IRR).**

With this new package insert there are no changes to the primers and probes, however there is a new positive control for the influenza A/B typing kit. It is a pooled influenza positive control (PIPC) that combines the previous two controls from the CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel and the CDC Influenza 2009 (H1N1) pdm Real-Time RT-PCR Panel into one positive influenza control. Also the Influenza A subtyping kit will not contain an extraction control. The extraction control will only be provided with the influenza A/B kit. The procedure for diluting and aliquoting the control is the exact same as the past package inserts.

CDC and APHL will conduct a technical teleconference to answer questions once laboratories have received the new influenza kits and had the opportunity to review the new package insert. APHL will send out communication regarding the time and date for this teleconference.

Expiration Date Notification

The expiration date for the current kits is August 17, 2011; however, CDC has demonstrated the performance and quality of the reagents and has extended the expiration date 60 days. **The new expiration date for Influenza Lot KT0096; Lot #908171 is October 17, 2011.** CDC has provided a letter of extension via CDC Flu Support to all registered qualified laboratory contacts. The letter of extension has also been linked to APHL Influenza News #15 email. This letter of extension will allow the public health laboratories to meet CLIA qualifications. Due to the extended expiration date, laboratories should not destroy kits with an August 17, 2011 expiration date. If a laboratory is in need of reagents prior to the new kits being shipped, you can still order the current kit (Cat# KT0096; Lot # 908171) until the end of September through the Influenza Reagent Resource.

Package Insert Changes

Dr. Julie Villanueva discussed the major changes to the package insert. Listed below are the major changes to the new package insert.

Intended Use – Specimen Types:
The intended use statement in the new package insert was extended to included all upper and lower respiratory specimens. For qualitative detection of influenza virus, viral RNA in upper respiratory tract specimens (nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (broncheoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture can be tested.

Extraction Chemistries:

CDC was able to harmonize the extraction chemistries in the new package insert. The extraction chemistries have stayed the same as listed in the current package inserts. The extraction chemistries include:

Roche MagNA Pure LC 2.0 TNA Kit
Roche MagNA Pure Compact NA Isolation Kit and RNA Isolation Kit
Qiagen Qiacube DSP Viral RNA Mini Kit
Qiagen Manual DSP Viral RNA Mini Kit
BioMerieux NucliSENS easyMAG (reagents and buffers sold individually)

Specimen Storage Prior to Testing:

CDC has shown equivalence between fresh and frozen specimens. Although optimal performance is met when testing fresh specimens within 72 hours of collection, performance has been demonstrated with frozen specimens. If testing of a fresh specimen is not possible within 72 hours storage at 2–8ºC, the specimen may be frozen at ≤ -70ºC and tested at a later time.

Electronic Laboratory Reporting:

The new package insert has a brief background on uniform coding and vocabulary for this assay and includes a URL for more information. The URL will provide the most up-to-date information as it occurs.

Influenza Reagent Resource Update

Dr. Joe Miller provided an update on the status of ancillary reagents for the RT-PCR influenza assay. CDC foresees having the capability to provide ancillary influenza reagents for the upcoming flu season (through Spring 2012). However, there will be close monitoring of the reagent utilization this season for proper usage. For example, CDC will be looking to prevent ordering of ancillary reagents for non-influenza purposes or studies that are not sponsored by CDC. Ancillary reagents will not go out with the RT-PCR reagents in the initial automatic shipment of the new kits to qualified laboratories; therefore laboratories will need to order the necessary ancillary reagents through the IRR website.

For the following season (2012-2013) it is unlikely that ancillary reagents will be available due to a 30% IRR budget cut. Ancillary reagent kits were never envisioned to be provided during a normal flu season; however, it is an option that can still be exercised in the event of a pandemic or other public health emergency.

The budget cuts will not affect the real-time RT-PCR influenza kit availability.

Question and Answers:

- Questions about the availability of pyrosequencing reagents in the IRR were asked. CDC does have reagents available for the public health laboratories, and we will send out clear communication in the near future with regards to this matter.
- A CDC pyrosequencing panel is available to public health laboratories and can be provide upon request for assay validation.
- CDC recommends freezing all specimens at -70 degrees C, if your laboratory needs to test specimens kept at -20 degree C you laboratory will need to be qualified by your laboratory.
- Lung tissue is part of the intended use of the new package insert and can be tested by your laboratory.
- CDC is planning to provide a voluntary quality assessment panel for rRT-PCR in the fall 2011 following the deployment of the reagents.