

## **Model Practices for Verification of an Alternate Enzyme for Influenza RT-PCR Diagnostic Panel**

### **New Hampshire Public Health Laboratory**

- Perform side by side testing of a total of 21 specimens consisting of AH1 (N=1), AH3 (N=5), pdmH1 (N=5), Flu B(N=5) and Negative(N=5) samples, using both the Invitrogen and the Quanta enzyme kits.
- Specimens were tested in duplicate for the influenza screen and subtypes.
- Training for staff was provided, and 5 specimen panels were then assembled as a training panel for each tech performing flu testing.
- Upon successful completion of the panels, each tech will be considered competent to perform testing.

### **New York: Wadsworth Center - Virology Laboratory**

- Perform a dilution series, five points to extinction, running influenza A, seasonal H1, H1pdm09, H3, H5, H7, influenza B and RP assays. The positive controls may be used.
  - Acceptable Criteria – ideally, extinction should be observed at the same dilution with both assays. Extra replicates (near the LoD) could be beneficial if discrepant results are observed.
- It is also important to include previously tested specimens in the verification, encompassing a minimum of 10 influenza A-positives, with a mixture of A subtypes; 10 influenza B-positives; 10 influenza-negative specimens. Emphasis should be placed on including specimens near the LoD.
- Side-by-side experiments should be performed using both the Invitrogen Superscript III Platinum One-Step RT-PCR and the Quanta BioSciences qScript One-Step qRT-PCR kit.
- Acceptable criteria: Ct values less than 38 should be 90% concordant. Extra replicates, minimum of 10 near the LoD, could be beneficial if discrepant results are observed.
- Ensure worksheets, SOPs and 7500Dx templates are updated with the new enzyme.

### **Wisconsin State Laboratory of Hygiene**

- Run 50 positive specimens (mixture of high, medium, and low Ct's) and 50 negatives against the old enzyme (Invitrogen Superscript III Platinum One-Step RT-PCR) and new enzyme (Quanta BioSciences qScript One-Step qRT-PCR).
  - The number of specimens could be reduced: 50 total specimens (30 positives with 10 each of high, medium, and low CT values) and 20 negatives against both the old and new enzymes.
  - The positive specimens should include a variety different subtypes (A(H1N1)pdm09, A(H3N2), and B's.