

## Appendix C: Laboratory Methods

### Requirements for Influenza Detection and Identification Methods

The below chart provides several influenza testing methodologies, described in the Laboratory Testing Implementation Guidance section. The chart provides only high level equipment, and material and reagent needs, more detailed needs should be obtained from the standard operating procedure being implemented within the laboratory.

|  | rRT-PCR | Culture | Shell Vial | Direct Specimen Immunofluorescence | RIDT | Antiviral Resistance | Neuraminidase Inhibition | Hemagglutination Inhibition | Serology                        |
|--|---------|---------|------------|------------------------------------|------|----------------------|--------------------------|-----------------------------|---------------------------------|
| <b>Equipment</b>                           |         |         |            |                                    |      |                      |                          |                             |                                 |
| Biological safety cabinet                  | X       | X       | X          | X                                  | X    | X                    | X                        | X                           | DEPENDENT ON SPECIFIC PROCEDURE |
| Centrifuge with carriers and containment   | X       |         | X          |                                    |      |                      |                          |                             |                                 |
| Centrifuge, cytospin                       |         |         |            | X                                  |      |                      |                          |                             |                                 |
| Cold block                                 | X       |         |            |                                    |      |                      |                          |                             |                                 |
| Microcentrifuge, benchtop                  | X       |         |            |                                    |      | X                    |                          |                             |                                 |
| Microscope, light                          |         | X       |            |                                    |      |                      |                          |                             |                                 |
| Microscope, fluorescence                   |         |         | X          | X                                  |      |                      |                          |                             |                                 |
| Heating block                              |         |         |            |                                    |      | X                    |                          |                             |                                 |
| Incubator                                  |         | X       | X          | X                                  |      |                      | X                        |                             |                                 |
| Real Time PCR instrument                   | X       |         |            |                                    |      | X                    |                          |                             |                                 |
| Gel documentation system                   |         |         |            |                                    |      | X                    |                          |                             |                                 |
| PyroMark™ vacuum prep tool and workstation |         |         |            |                                    |      | X                    |                          |                             |                                 |
| Roller apparatus                           |         | X       |            |                                    |      |                      |                          |                             |                                 |
| Mirror stand to read plate reactions       |         |         |            |                                    |      |                      |                          | X                           |                                 |
| Liquid dispensers and diluters             | X       | X       | X          | X                                  |      | X                    | X                        | X                           |                                 |
| Pyrosequencing instrument                  |         |         |            |                                    |      | X                    |                          |                             |                                 |
| Thermocycler-conventional                  |         |         |            |                                    |      | X                    |                          |                             |                                 |
| Transilluminator                           |         |         |            |                                    |      | X                    |                          |                             |                                 |
| Victor X4 or Equivalent Fluorescent Reader |         |         |            |                                    |      |                      | X                        | X                           |                                 |
| CDC JASPR Software (IC50)                  |         |         |            |                                    |      |                      | X                        | X                           |                                 |

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Table Continued

|  | rRT-PCR | Culture | Shell Vial | Direct Specimen | RIDT | Antiviral Resistance | Neuraminidase Inhibition | Hemagglutination Inhibition | Serology |
|--|---------|---------|------------|-----------------|------|----------------------|--------------------------|-----------------------------|----------|
| <b>Materials &amp; Reagents</b>                      |         |         |            |                 |      |                      |                          |                             |          |
| Miscellaneous laboratory glassware                   | X       | X       | X          | X               |      | X                    | X                        | X                           |          |
| NA Drugs (e.g., oseltamivir)                         |         |         |            |                 |      |                      | X                        |                             |          |
| NA Sensitive & NA Resistant Influenza Virus Controls |         |         |            |                 |      | X                    | X                        |                             |          |
| Opaque flat bottom 96well plates                     |         |         |            |                 |      |                      | X                        |                             |          |
| 12 column & single reservoirs                        |         |         |            |                 |      |                      | X                        |                             |          |
| Cell Culture Flasks                                  |         | X       | X          |                 |      |                      |                          |                             |          |
| Cell culture sensitive to influenza viruses          |         | X       | X          |                 |      |                      |                          |                             |          |
| Cell culture medium                                  |         | X       | X          |                 |      |                      |                          |                             |          |
| Ethanol, 100%/molecular grade                        | X       |         |            |                 |      | X                    | X                        |                             |          |
| Microcentrifuge tubes, DNase/RNase free              | X       |         |            |                 |      | X                    |                          |                             |          |
| Molecular grade water                                | X       |         |            |                 |      | X                    | X                        |                             |          |
| Monoclonal antibodies to influenza A and B viruses   |         |         | X          | X               |      |                      |                          |                             |          |
| Fluorescent conjugate (if MAbs not conjugated)       |         |         | X          | X               |      |                      |                          |                             |          |
| Fixative (e.g., acetone) to prepare (“fix”) cells    |         |         | X          | X               |      |                      |                          |                             |          |
| Containers to rinse stained slides                   |         |         |            | X               |      |                      |                          |                             |          |
| Incubation chamber/container                         |         |         |            | X               |      |                      |                          |                             |          |
| Influenza primers and probes                         | X       |         |            |                 |      | X                    |                          |                             |          |
| Influenza positive and human specimen controls       | X       |         |            |                 |      |                      |                          |                             |          |
| Neuraminidase inhibition drug                        |         |         |            |                 |      |                      | X                        |                             |          |
| Neuraminidase assay kit                              |         |         |            |                 |      |                      | X                        |                             |          |
| Nucleic acid extraction/isolation kits, multiple     | X       |         |            |                 |      | X                    |                          |                             |          |
| rRT-PCR mastermix                                    | X       |         |            |                 |      | X                    |                          |                             |          |
| Pyrosequencing Reaction mix                          |         |         |            |                 |      | X                    |                          |                             |          |
| Red blood cell solution, standardized                |         | X       |            |                 |      |                      |                          | X                           |          |
| Phosphate buffered saline (PBS)                      |         | X       | X          | X               |      |                      | X                        | X                           |          |
| Pyrosequencing instrument                            |         |         |            |                 |      | X                    |                          |                             |          |
| Transilluminator                                     |         |         |            |                 |      | X                    |                          |                             |          |
| U-bottom microtiter plates                           |         |         |            |                 |      |                      |                          | X                           |          |
| Plate cover tapes                                    |         |         |            |                 |      |                      |                          | X                           |          |
| Antisera against influenza A and B, treated          |         |         |            |                 |      |                      |                          | X                           |          |
| Expertise Level*                                     | ****    | ****    | ***        | ****            | *    | ****                 | ****                     | ***                         | ****     |

\*Expertise level ranges from 1 to 4 stars with 4 stars identified as complex.