



# Protocols for Laboratory Verification of Performance of the BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) and BIOFIRE® SPOTFIRE® Respiratory/Sore Throat *plus* (R/ST*plus*) Panels

## Laboratory Protocols for Use with ZeptoMetrix NATtrol™ Control Materials

### For Non-US Customers

#### Purpose

This document provides examples of procedures to assist your laboratory in developing a protocol for the verification of the BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel (SPOTFIRE R/ST Panel) and the BIOFIRE® SPOTFIRE® Respiratory/Sore Throat *plus* (R/ST*plus*) Panel (SPOTFIRE R/ST*plus* Panel) performance on the BIOFIRE® SPOTFIRE® System. Multiple verification schemes have been designed using non-clinical specimens. The methods described provide positive and negative tests for each organism detected by the SPOTFIRE R/ST Panel and the SPOTFIRE R/ST*plus* Panel Respiratory and Sore Throat menus and may be easily modified or expanded to meet specific criteria. Day-to-day variation is evaluated by testing each sample on two separate days. To evaluate user-to-user variation, multiple laboratory technicians may test the same sample. In addition, testing patient samples for verification or to evaluate matrix effects on the performance of the Panels should be done under the guidance of the Laboratory Director, but is not described here.

The Laboratory Director is ultimately responsible for ensuring that verification procedures meet the appropriate standards for applicable laboratory accrediting agencies.

#### Intended Use

The BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel (SPOTFIRE R/ST Panel) test kit is a single product with **two intended uses**, where the intended use for a given test is based upon the sample type selected by the operator based on patient signs and symptoms and sample type collected.

The SPOTFIRE R/ST Panel is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from **individuals with signs**





and symptoms of respiratory tract infections, including COVID-19; (Respiratory) or from individuals with signs and/or symptoms of pharyngitis (using a throat swab (TS); Sore Throat).

The BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel (SPOTFIRE R/STplus Panel) test kit is a single product with **two intended uses**, where the intended use for a given test is based upon the sample type selected by the operator based on patient signs and symptoms and sample type collected.

The SPOTFIRE R/STplus Panel is an automated multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from **individuals with signs and symptoms of respiratory tract infections, including COVID-19; (Respiratory) or from individuals with signs and/or symptoms of pharyngitis (using a throat swab (TS); Sore Throat)**.

The following organism types and subtypes are identified and differentiated using the SPOTFIRE R/ST Panel and the SPOTFIRE R/STplus Panel:

**Table 1: SPOTFIRE R/ST Panel and SPOTFIRE R/STplus Panel Menu**

| <b>Viruses (Respiratory and Sore Throat)</b> | <b>Bacteria (Respiratory and Sore Throat)</b>       |
|--|---|
| Adenovirus                                   | <i>Chlamydia pneumoniae</i>                         |
| Coronavirus (seasonal)                       | <i>Mycoplasma pneumoniae</i>                        |
| Coronavirus SARS-CoV-2                       | <b>Bacteria (Respiratory only)</b>                  |
| Human metapneumovirus                        | <i>Bordetella parapertussis</i>                     |
| Human rhinovirus/enterovirus                 | <i>Bordetella pertussis</i>                         |
| Influenza A virus                            | <b>Bacteria (Sore Throat only)</b>                  |
| Influenza A virus A/ H1-2009                 | <i>Streptococcus dysgalactiae</i> (Group C/G Strep) |
| Influenza A virus A/ H3                      | <i>Streptococcus pyogenes</i> (Group A Strep)       |
| Influenza B virus                            |   |
| Parainfluenza virus                          |   |
| Respiratory syncytial virus                  |   |

Always refer to the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Instructions for Use* or the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use* for the complete intended use statement and additional information about the use of the SPOTFIRE System.

## Performance Verification: Overview

Three different examples of performance verification procedures are described: (1) a Respiratory Protocol for the verification of the SPOTFIRE R/ST and SPOTFIRE R/STplus Panel performance using the Respiratory Menu; (2) a Sore Throat Protocol for verification of the SPOTFIRE R/ST and SPOTFIRE R/STplus Panel performance using the Sore Throat Menu; and (3) a Combined Respiratory and Sore Throat Protocol for verification of both the Respiratory and Sore Throat Panel Menus. Each protocol can be used with a different media or expanded to test multiple media to evaluate matrix effects. Refer to the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Instructions for Use* or the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use* for a complete list of acceptable media types. These protocols are examples of procedures to assist your laboratory in developing



a protocol for the verification of SPOTFIRE R/ST and SPOTFIRE R/ST*plus* Panel performance on the SPOTFIRE System.

 **Note:** Transport media may contain non-viable organisms and/or nucleic acids at levels that can be detected by the SPOTFIRE R/ST and SPOTFIRE R/ST*plus* Panels and may lead to false positive results. Transport media may be screened using the Panel prior to starting the verification procedure. The optimal transport media will be negative for all analytes tested on the SPOTFIRE R/ST or the SPOTFIRE R/ST*plus* Panel.

The procedures have been designed to take advantage of the multiplex nature of the SPOTFIRE R/ST and SPOTFIRE R/ST*plus* Panels. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures described will generate multiple positive and negative detections for each of the Panel assays. The procedures were developed using the NATtrol™ Respiratory/Sore Throat Verification Panel (NATRST-BIO) available from ZeptoMetrix, Buffalo, NY.

A SPOTFIRE System is composed of one to four BIOFIRE® SPOTFIRE® Modules connected to a BIOFIRE® SPOTFIRE® Control Station running BIOFIRE® SPOTFIRE® Software. If the laboratory director chooses not to perform the entire verification protocol on each individual module of the SPOTFIRE System, it is advised that test replicates are evenly distributed among the modules. Examples of performance verification workflows using 1 to 4 modules is provided in Figures 1 through 4.

Clinical/patient samples may be used in place of, or in addition to the verification schemes described here to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the SPOTFIRE R/ST and SPOTFIRE R/ST*plus* Panels.

 **Note:** The laboratory should only perform the verification study with analytes that will be reported using the Panel, sample types and media that will be used in their laboratory setting.

**Table 2.** Overview of Verification Protocols

| Verification Protocol                                  | Organisms per Pool | Number of Sample Pools | Test Replicates per Sample Pool | Pouches Required <sup>a</sup> | Expected Positive Results <sup>b</sup> | Expected Negative Results | Approximate Days of Testing <sup>c</sup> |
|--|--------------------|------------------------|---------------------------------|-------------------------------|--|---------------------------|--|
| <b>Example 1: Respiratory</b>                          | 4, 6, or 7         | 4                      | 4                               | 16                            | ≥4 per organism                        | ≤12 per organism          | 2  |
| <b>Example 2: Sore Throat</b>                          | 4, 6, or 7         | 4                      | 4                               | 16                            | ≥4 per organism                        | ≤12 per organism          | 2  |
| <b>Example 3: Combined Respiratory and Sore Throat</b> | 6 or 7             | 4                      | 8                               | 32                            | ≥8 per organism                        | ≤24 per organism          | ≥2                                       |

<sup>a</sup> Pouches required does not include pouches that may be needed for screening transport media.

<sup>b</sup>The expected number of positives and negatives per organism is dependent upon the number strains of a particular organism used to complete the verification. The proposed verification procedure recommends multiple strains of adenovirus, coronavirus (seasonal) and parainfluenza virus; therefore, the number of expected positive and negative detections for adenovirus, coronavirus (seasonal) and parainfluenza virus will vary.

<sup>c</sup> Two days is shown to meet day-to day testing requirements; the number of days can be expanded or decreased, as needed.



## Performance Verification: Materials

The following materials may be used to perform the verification procedure:

**Table 3.** Recommended materials for the verification protocols

| Material  | SPOTFIRE R/ST (OUS) Part Number       | SPOTFIRE R/STplus Panel Part Number   |
|---|---------------------------------------|---|
| BIOFIRE® SPOTFIRE® Respiratory/Sore Throat Panel Test Kit (30 tests)  | BioFire Diagnostics, LLC 423485       | BioFire Diagnostics, LLC 425090   |
| BIOFIRE® SPOTFIRE® Respiratory/Sore Throat Panel Instructions for Use | BioFire Diagnostics, LLC BFR0001-7220 | BioFire Diagnostics, LLC BFR0002-9365                                       |
| BIOFIRE® SPOTFIRE® Respiratory/Sore Throat Panel Quick Guide          | BioFire Diagnostics, LLC BFR0000-2786 | BioFire Diagnostics, LLC BFR0002-9367                                       |
| BIOFIRE® SPOTFIRE® System Operator Manual                             |                                       | BioFire Diagnostics, LLC BFR0001-1641                                       |
| Control Organisms <sup>a</sup>  |                                       | ZeptoMetrix NATtrol™ Respiratory/Sore Throat Verification Panel: NATRST-BIO |
| Universal Transport Media (NPS) or Amies Media <sup>b</sup> (TS)      |                                       | Various manufacturers   |
| 5 mL Sample Tubes   |                                       | Various manufacturers   |
| Disposable Transfer Pipets, graduated                                 |                                       | Avantor (VWR), 414004-024 (or equivalent)                                   |

<sup>a</sup> Any appropriate source of organism may be used for verification of any or all of the assays in the SPOTFIRE R/ST Panel or SPOTFIRE R/STplus Panel. However, when alternate organism sources are used (i.e. not the ZeptoMetrix control material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.

<sup>b</sup> Compatibility of the SPOTFIRE R/ST Panel and the SPOTFIRE R/STplus Panel with multiple transport media has been demonstrated analytically. Refer to the Interference section of the Instructions for Use for more details.

## Performance Verification: Respiratory Protocol

The Respiratory Protocol evaluates the SPOTFIRE R/ST and SPOTFIRE R/STplus Panel performance when sample material (ZeptoMetrix NATRST-BIO) is pooled and combined with an equal volume of transport media or synthetic matrix/negative (provided in the control panel) and tested with the Respiratory Menu. The proposed organism pooling scheme (Table 4) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

Verification sample tests should be run by following on-screen instructions for patient sample testing. Refer to the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Quick Guide*, or the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Quick Guide* for detailed instructions.

 **Note:** Dilution of ZeptoMetrix control organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

 **Note:** Transport media may contain non-viable organisms and/or nucleic acids at levels that can be detected by the SPOTFIRE R/ST Panel or the SPOTFIRE R/STplus Panel and may lead to false positive results. Transport media may be screened using the Panel (Respiratory Menu) prior to starting the verification procedure. The optimal transport media will be negative for all analytes tested on the SPOTFIRE R/ST or SPOTFIRE R/STplus Panel Respiratory Menu.



Figures 1 and 2 (below) illustrate workflow schemes for testing 4 replicates per pool for 4 different pools over multiple days. This produces a total of 16 verification sample test runs and provides at least 4 positive results and as many as 12 negative results per assay. Some organisms, such as adenovirus, are represented multiple times. This is done to ensure all adenovirus assays are represented in the verification protocol. The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run fewer samples per day based on the number of modules in the SPOTFIRE System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples may be stored overnight (or up to 14 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation. To evaluate user-to-user variation, multiple laboratory operators may perform testing.

**Table 4.** Proposed Organism Pooling Scheme for the Verification of the SPOTFIRE R/ST Panel and the SPOTFIRE R/STplus Panel Respiratory Menu

| NATRST-BIO Control Organisms<br>(Respiratory Menu) | Approximate<br>Organism Volume | Approximate<br>Volume Transport<br>Media or Negative | Approximate Pool<br>Volume |
|--|--------------------------------|--|----------------------------|
| <b>Pool 1- Viruses</b>                             |                                |  |                            |
| Adenovirus Type 3                                  | 0.3 mL                         | 1.8 mL   | 3.6 mL                     |
| SARS-CoV-2 USA-WA1/2020)                           | 0.3 mL                         |  |                            |
| Influenza A H1N1pdm (A/NY/02/09)-subtype H1-2009   | 0.3 mL                         |  |                            |
| Influenza B (B/Florida/02/06)                      | 0.3 mL                         |  |                            |
| Parainfluenza Type 4                               | 0.3 mL                         |  |                            |
| Rhinovirus 1A                                      | 0.3 mL                         |  |                            |
| <b>Pool 2- Viruses</b>                             |                                |  |                            |
| Adenovirus Type 1                                  | 0.3 mL                         | 2.1 mL   | 4.2 mL                     |
| Coronavirus 229E                                   | 0.3 mL                         |  |                            |
| Coronavirus HKU-1 (recombinant)                    | 0.3 mL                         |  |                            |
| Metapneumovirus 8 (Peru6-2003)                     | 0.3 mL                         |  |                            |
| Influenza AH3 (A/Brisbane/10/07)-subtype H3        | 0.3 mL                         |  |                            |
| Parainfluenza Type 1                               | 0.3 mL                         |  |                            |
| Parainfluenza Type 2                               | 0.3 mL                         |  |                            |
| <b>Pool 3- Viruses</b>                             |                                |  |                            |
| Adenovirus Type 31                                 | 0.3 mL                         | 1.8 mL   | 3.6 mL                     |
| Coronavirus OC43                                   | 0.3 mL                         |  |                            |
| Coronavirus NL63                                   | 0.3 mL                         |  |                            |
| Influenza AH1 (A/New Caledonia/20/99)-no subtype   | 0.3 mL                         |  |                            |
| Parainfluenza Type 3                               | 0.3 mL                         |  |                            |
| Respiratory Syncytial Virus A (RSV A)              | 0.3 mL                         |  |                            |
| <b>Pool 4- Bacteria</b>                            |                                |  |                            |
| <i>Bordetella parapertussis</i> (A747)             | 0.3 mL                         | 1.2 mL   | 2.4 mL                     |
| <i>Bordetella pertussis</i> (A639)                 | 0.3 mL                         |  |                            |
| <i>Chlamydia pneumoniae</i> (IOL-207)              | 0.3 mL                         |  |                            |
| <i>Mycoplasma pneumoniae</i> (M129)                | 0.3 mL                         |  |                            |



## Example of Protocol for Respiratory Verification

This verification protocol example can be completed in 1 or more days depending on the number of modules in the SPOTFIRE System configuration and the laboratory's work schedule. Testing over multiple days provides day-to-day variation data; testing with multiple operators provides user-to-user variation data; testing multiple replicates of pooled verification material verifies the precision of the test system.

 **Note:** It is important to prepare only the number of sample pools that will be tested within 14 days of preparation. The number of samples prepared may be modified based on the laboratory's work schedule and number of modules connected within a SPOTFIRE System.

### Day 1- Respiratory Protocol

1. Organize materials needed (Table 3); refer to Table 4 for the pooling scheme. Negativity of transport media may be confirmed by screening on the SPOTFIRE R/ST or the SPOTFIRE R/ST<sup>plus</sup> Panel (Respiratory Menu) prior to starting the verification procedure. Negative vials included in the control panel contain 1.8 mL of synthetic matrix; the control panel contains sufficient volume to complete the protocol described. More than one vial of negative may be needed for preparing some pools (i.e., Pool 2).
2. Prepare one sample pool (i.e., Pool 1) using the ZeptoMetrix NATRST-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool.
  - a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5 mL tube.
  - b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The combined volume of organisms for each pool will be between 1.2 to 2.1 mL, depending upon the pool.
  - c. Add transport media or synthetic matrix/negative (as described in Table 4) to the tube containing the organism pool (step b). The volume of transport media/ negative should be the same as the organism pool volume. For example: for Pools 1 and 3, 1.8 mL of transport media/negative is added to 1.8 mL of pooled organism. The final volume of Pools 1 and 3 will be approximately 3.6 mL.

 **Note:** If the laboratory verification study will include multiple types of transport media, see the section *Expanding or Modifying the Protocol* below.

3. Repeat Step 2 for the remaining sample pools (i.e., Pools 2, 3, and 4) to be prepared on Day 1.
4. Test 2 replicates from a single sample pool (Figure 1: Pool 1 replicates A and B) using the Respiratory Menu. Ensure the pooled sample is well mixed prior to removing a sample for testing. Replicate samples A and B should be tested in a single day by different operators to evaluate user-to-user variance. Refer to Figure 2 for suggested workflows depending upon the module configuration in the verification study.

 **Note:** For each sample, follow instructions in the *BIOFIRE<sup>®</sup> SPOTFIRE<sup>®</sup> Respiratory/Sore Throat (R/ST) Panel Instructions for Use*, the *BIOFIRE<sup>®</sup> SPOTFIRE<sup>®</sup> Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use*, the *BIOFIRE<sup>®</sup> SPOTFIRE<sup>®</sup> Respiratory/Sore Throat (R/ST) Panel Quick Guide*, or the *BIOFIRE<sup>®</sup> SPOTFIRE<sup>®</sup> Respiratory/Sore Throat plus (R/STplus) Panel Quick Guide* for pouch preparation,



pouch hydration, sample loading, and sample testing. Verification sample tests should be run by following on-screen instructions for patient sample pouch testing.

5. Repeat Step 4 for the remaining sample replicates to be tested that day (Figure 1: replicates A and B for Pools 2, 3 and 4).
6. Refrigerate samples (2–8°C) for up to 14 days for the evaluation of day-to-day variation.



**Note:** The proposed organism pooling scheme, described in Table 4, provides sufficient material for running samples as described in Figure 1. The volume is sufficient for testing more samples if desired.

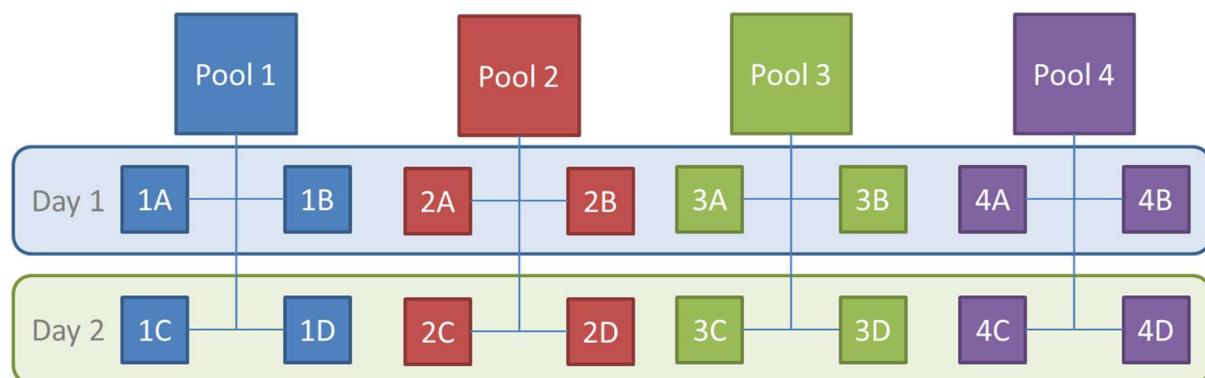
## Day 2 - Respiratory Protocol

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 by repeating Steps 4 and 5 above (Figure 1: test replicates C and D from Pools 1-4).



**Note:** A Verification Record for the SPOTFIRE Respiratory Menu protocol is provided and may serve as a template for recording your results.

**Figure 1.** Verification Protocol Workflow for Testing One Menu (Respiratory or Sore Throat) over two Days





**Figure 2.** Examples of the Verification Workflow for Testing One Menu (Respiratory or Sore Throat) over two days with different SPOTFIRE System Module configurations.

| Respiratory or Sore Throat Protocol | Module 1             |                      |                      |                      |
|-------------------------------------|----------------------|----------------------|----------------------|----------------------|
| Day 1                               | Pool 1A / Operator 1 | Pool 2A / Operator 1 | Pool 3A / Operator 1 | Pool 4A / Operator 1 |
|                                     | Pool 1B / Operator 2 | Pool 2B / Operator 2 | Pool 3B / Operator 2 | Pool 4B / Operator 2 |
| Day 2                               | Pool 1C / Operator 1 | Pool 2C / Operator 1 | Pool 3C / Operator 1 | Pool 4C / Operator 1 |
|                                     | Pool 1D / Operator 2 | Pool 2D / Operator 2 | Pool 3D / Operator 2 | Pool 4D / Operator 2 |

| Respiratory or Sore Throat Protocol | Module 1             |                      | Module 2             |                      |
|-------------------------------------|----------------------|----------------------|----------------------|----------------------|
| Day 1                               | Pool 1A / Operator 1 | Pool 2A / Operator 1 | Pool 1B / Operator 2 | Pool 2B / Operator 2 |
|                                     | Pool 3A / Operator 1 | Pool 4A / Operator 1 | Pool 3B / Operator 2 | Pool 4B / Operator 2 |
| Day 2                               | Pool 1D / Operator 2 | Pool 2D / Operator 2 | Pool 1C / Operator 1 | Pool 2C / Operator 1 |
|                                     | Pool 3D / Operator 2 | Pool 4D / Operator 2 | Pool 3C / Operator 1 | Pool 4C / Operator 1 |

| Respiratory or Sore Throat Protocol | Module 1             |                      | Module 2             |                      | Module 3             |                      |
|-------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Day 1                               | Pool 1A / Operator 1 | Pool 2B / Operator 2 | Pool 2A / Operator 1 | Pool 3B / Operator 2 | Pool 1B / Operator 2 | Pool 3A / Operator 1 |
|                                     |                      |                      | Pool 4B / Operator 2 |                      | Pool 4A / Operator 1 |                      |
| Day 2                               | Pool 1D / Operator 2 | Pool 3C / Operator 1 | Pool 1C / Operator 1 | Pool 2D / Operator 2 | Pool 3D / Operator 2 | Pool 2C / Operator 1 |
|                                     | Pool 4C / Operator 1 |                      |                      |                      | Pool 4D / Operator 2 |                      |

| Respiratory or Sore Throat Protocol | Module 1             | Module 2             | Module 3             | Module 4             |
|-------------------------------------|----------------------|----------------------|----------------------|----------------------|
| Day 1                               | Pool 1A / Operator 1 | Pool 1B / Operator 2 | Pool 2A / Operator 1 | Pool 2B / Operator 2 |
|                                     | Pool 3B / Operator 2 | Pool 3A / Operator 1 | Pool 4B / Operator 2 | Pool 4A / Operator 1 |
| Day 2                               | Pool 2D / Operator 2 | Pool 2C / Operator 1 | Pool 1D / Operator 2 | Pool 1C / Operator 1 |
|                                     | Pool 4C / Operator 1 | Pool 4D / Operator 2 | Pool 3C / Operator 1 | Pool 3D / Operator 2 |



## Performance Verification: Sore Throat Protocol

The Sore Throat Protocol evaluates the SPOTFIRE R/ST and SPOTFIRE R/ST<sup>plus</sup> Panel performance when sample material (ZeptoMetrix NATRST-BIO) is pooled and combined with an equal volume of transport media, such as Amies, or negative/synthetic matrix (provided in the control panel) and tested with the Sore Throat Menu. The proposed organism pooling scheme (Table 5) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

Verification sample tests should be run by following on-screen instructions for patient sample testing. Refer to the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Quick Guide*, or the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Quick Guide* for detailed instructions.

-  **Note:** Dilution of ZeptoMetrix control organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.
-  **Note:** Transport media (including Amies) may contain non-viable organisms and/or nucleic acids at levels that can be detected by the SPOTFIRE R/ST or the SPOTFIRE R/ST<sup>plus</sup> Panel and may lead to false positive results. Transport media may be screened using the Panel (Sore Throat Menu) prior to starting the verification procedure. The optimal transport media will be negative for all analytes tested on the SPOTFIRE R/ST or SPOTFIRE R/ST<sup>plus</sup> Panel Sore Throat Menu.

Figures 1 and 2 (above) illustrate workflow schemes for testing 4 replicates per pool for 4 different pools over multiple days. This produces a total of 16 verification sample test runs and provides at least 4 positive results and as many as 12 negative results per assay. Some organisms, such as adenovirus, are represented multiple times. This is done to ensure all adenovirus assays are represented in the verification protocol. The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run fewer samples per day based on the number of modules in the SPOTFIRE System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples may be stored overnight (or up to 14 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation. To evaluate user-to-user variation, multiple laboratory operators may perform testing.

**Table 5.** Proposed Organism Pooling Scheme for the Verification of the SPOTFIRE R/ST and SPOTFIRE R/ST<sup>plus</sup> Panel Sore Throat Menu

| NATRST-BIO Control Organisms<br>(Sore Throat Menu) | Approximate<br>Organism<br>Volume | Approximate<br>Volume Amies<br>or Negative | Approximate<br>Pool Volume |
|--|-----------------------------------|--|----------------------------|
| <b>Pool 1- Viruses</b>                             |                                   |  |                            |
| Adenovirus Type 3                                  | 0.3 mL                            | 1.8 mL                                     | 3.6 mL                     |
| SARS-CoV-2 (USA-WA1/2020)                          | 0.3 mL                            |  |                            |
| Influenza A H1N1pdm (A/NY/02/09)-subtype H1-2009   | 0.3 mL                            |  |                            |
| Influenza B (B/Florida/02/06)                      | 0.3 mL                            |  |                            |
| Parainfluenza Type 4                               | 0.3 mL                            |  |                            |
| Rhinovirus 1A                                      | 0.3 mL                            |  |                            |



| Pool 2- Viruses                                  |        |        |        |
|--|--------|--------|--------|
| Adenovirus Type 1                                | 0.3 mL | 2.1 mL | 4.2 mL |
| Coronavirus 229E                                 | 0.3 mL |        |        |
| Coronavirus HKU-1 (recombinant)                  | 0.3 mL |        |        |
| Metapneumovirus 8 (Peru6-2003)                   | 0.3 mL |        |        |
| Influenza AH3 (A/Brisbane/10/07)- (subtype H3)   | 0.3 mL |        |        |
| Parainfluenza Type 1                             | 0.3 mL |        |        |
| Parainfluenza Type 2                             | 0.3 mL |        |        |
| Pool 3- Viruses                                  |        |        |        |
| Adenovirus Type 31                               | 0.3 mL | 1.8 mL | 3.6 mL |
| Coronavirus OC43                                 | 0.3 mL |        |        |
| Coronavirus NL63                                 | 0.3 mL |        |        |
| Influenza AH1 (A/New Caledonia/20/99)-no subtype | 0.3 mL |        |        |
| Parainfluenza Type 3                             | 0.3 mL |        |        |
| Respiratory Syncytial Virus A (RSV A)            | 0.3 mL |        |        |
| Pool 4- Bacteria                                 |        |        |        |
| <i>Chlamydia pneumoniae</i> (IOL-207)            | 0.3 mL | 1.2 mL | 2.4 mL |
| <i>Mycoplasma pneumoniae</i> (M129)              | 0.3 mL |        |        |
| <i>Streptococcus pyogenes</i>                    | 0.3 mL |        |        |
| <i>Streptococcus dysgalactiae</i>                | 0.3 mL |        |        |

## Example of Protocol for Sore Throat Verification

This verification protocol example can be completed in 1 or more days depending on the number of modules in the SPOTFIRE System configuration and the laboratory's work schedule. Testing over multiple days provides day-to-day variation data; testing with multiple operators provides user-to-user variation data; testing multiple replicates of pooled verification material verifies precision of the test system.



**Note:** It is important to prepare only the number of sample pools that will be tested within 14 days of preparation. The number of samples prepared may be modified based on the laboratory's work schedule and number of modules connected within a SPOTFIRE System.

### Day 1-Sore Throat Protocol

1. Organize materials needed (Table 3); refer to Table 5 for the pooling scheme. Negativity of Amies may be confirmed by screening on the SPOTFIRE R/ST or the SPOTFIRE R/ST<sup>plus</sup> Panel prior to starting the verification procedure. Negative vials included in the control panel contain 1.8 mL of synthetic matrix; the control panel contains sufficient volume to complete the protocol described. More than one vial of negative may be needed for preparing some pools (i.e., Pool 2).
2. Prepare one sample pool (i.e., Pool 1) using the ZeptoMetrix NATRST-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool.
  - a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5 mL tube.
  - b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The combined volume of organisms for each pool will be between 1.2 to 2.1 mL, depending upon the pool.



c. Add Amies or synthetic matrix/negative (as described in Table 5) to the tube containing the organism pool (step b). The volume of Amies/ negative should be the same as the organism pool volume, for example: for Pools 1 and 3, 1.8 mL of transport media/negative is added to 1.8 mL of pooled organism. The final volume of Pools 1 and 3 will be approximately 3.6 mL.



**Note:** If the laboratory verification study will include multiple types of transport media, see the section *Expanding or Modifying the Protocol* below.

3. Repeat Step 2 for the remaining sample pools (i.e., Pools 2, 3, and 4) to be prepared on Day 1.
4. Test 2 replicates from a single sample pool (Figure 1: Pool 1 replicates A and B) using the Sore Throat Menu. Ensure the pooled sample is well mixed prior to removing a sample for testing. Replicate samples A and B should be tested in a single day by different operators to evaluate user-to-user variance. Refer to Figure 2 for suggested workflows depending upon the module configuration in the verification study.



**Note:** For each sample, follow instructions in the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Quick Guide*, or the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing. Verification sample tests should be run by following on-screen instructions for patient sample pouch testing.

5. Repeat Step 4 for the remaining sample replicates to be tested that day (Figure 1, replicates A and B for Pools 2, 3 and 4).
6. Refrigerate samples (2–8°C) for up to 14 days for the evaluation of day-to-day variation.



**Note:** The proposed organism pooling scheme, described in Table 5, provides sufficient material for running samples as described in Figure 1. The volume is sufficient for testing more samples if desired.

## Day 2- Sore Throat Protocol

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 by repeating Steps 4 and 5 above (Figure 1: test replicates C and D from Pools 1-4).



**Note:** A SPOTFIRE Sore Throat Menu Verification Record is provided and may serve as a template for recording your results.

## Performance Verification: Combined Protocol for Respiratory and Sore Throat

The combined verification protocol for Respiratory and Sore Throat evaluates the SPOTFIRE R/ST and SPOTFIRE R/STplus Panel performance when sample material (ZeptoMetrix NATRST-BIO) is pooled and combined with an equal volume of transport media or negative/synthetic matrix (provided in the control panel) and tested with the both the Respiratory and Sore Throat Menus. The proposed organism pooling scheme (Table 6) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.



Verification sample tests should be run by following on-screen instructions for patient sample testing. Refer to the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Quick Guide*, or the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Quick Guide* for detailed instructions.

 **Note:** Dilution of ZeptoMetrix control organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

 **Note:** Transport media may contain non-viable organisms and/or nucleic acids at levels that can be detected by the SPOTFIRE R/ST or the SPOTFIRE R/STplus Panel and may lead to false positive results. Transport media may be screened using the Panel (Respiratory and Sore Throat Menus) prior to starting the verification procedure. The optimal transport media will be negative for all analytes tested on the SPOTFIRE R/ST or the SPOTFIRE R/STplus Panels.

Figures 3 and 4 (below) illustrate workflow schemes for testing 8 replicates per pool for 4 different pools over multiple days. This produces a total of 32 verification sample test runs and provides at least 8 positive results and as many as 24 negative results per assay. Some organisms, such as adenovirus, are represented multiple times. This is done to ensure all adenovirus assays are represented in the verification protocol. The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run fewer samples per day based on the number of modules in the SPOTFIRE System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples may be stored overnight (or up to 14 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation. To evaluate user-to-user variation, multiple laboratory operators may perform testing.

**Table 6.** Proposed Organism Pooling Scheme for the Combined Verification of the SPOTFIRE R/ST and SPOTFIRE R/STplus Panels

| NATRST-BIO Control Organisms<br>(Respiratory and Sore Throat Menus) | Approximate<br>Organism Volume | Approximate<br>Volume<br>Transport<br>Media or<br>Negative | Approximate<br>Pool Volume |
|---|--------------------------------|--|----------------------------|
| <b>Pool 1- Viruses</b>  |                                |  |                            |
| Adenovirus Type 3   | 0.3 mL                         | 1.8 mL   | 3.6 mL                     |
| SARS-CoV-2 USA-WA1/2020)  | 0.3 mL                         |  |                            |
| Influenza A H1N1pdm (A/NY/02/09)-subtype H1-2009                    | 0.3 mL                         |  |                            |
| Influenza B (B/Florida/02/06)                                       | 0.3 mL                         |  |                            |
| Parainfluenza Type 4  | 0.3 mL                         |  |                            |
| Rhinovirus 1A   | 0.3 mL                         |  |                            |
| <b>Pool 2- Viruses</b>  |                                |  |                            |
| Adenovirus Type 1   | 0.3 mL                         | 2.1 mL   | 4.2 mL                     |
| Coronavirus 229E  | 0.3 mL                         |  |                            |
| Coronavirus HKU-1 (recombinant)                                     | 0.3 mL                         |  |                            |
| Metapneumovirus 8 (Peru6-2003)                                      | 0.3 mL                         |  |                            |
| Influenza AH3 (A/Brisbane/10/07)-subtype H3                         | 0.3 mL                         |  |                            |
| Parainfluenza Type 1  | 0.3 mL                         |  |                            |
| Parainfluenza Type 2  | 0.3 mL                         |  |                            |



| Pool 3- Viruses  |        | 1.8 mL | 3.6 mL |
|--|--------|--------|--------|
| Adenovirus Type 31   | 0.3 mL |        |        |
| Coronavirus OC43   | 0.3 mL |        |        |
| Coronavirus NL63   | 0.3 mL |        |        |
| Influenza AH1 (A/New Caledonia/20/99)-no subtype                     | 0.3 mL |        |        |
| Parainfluenza Type 3   | 0.3 mL |        |        |
| Respiratory Syncytial Virus A (RSV A)                                | 0.3 mL |        |        |
| Pool 4- Bacteria   |        |        |        |
| <i>Bordetella parapertussis</i> (A747) (Respiratory)                 | 0.3 mL | 1.8 mL | 3.6 mL |
| <i>Bordetella pertussis</i> (A639) (Respiratory)                     | 0.3 mL |        |        |
| <i>Chlamydia pneumoniae</i> (IOL-20-7) (Respiratory and Sore Throat) | 0.3 mL |        |        |
| <i>Mycoplasma pneumoniae</i> (M129) (Respiratory and Sore Throat)    | 0.3 mL |        |        |
| <i>Streptococcus pyogenes</i> (Sore Throat)                          | 0.3 mL |        |        |
| <i>Streptococcus dysgalactiae</i> (Sore Throat)                      | 0.3 mL |        |        |

## Example of a Combined Protocol for Respiratory and Sore Throat Verification

This verification protocol example can be completed in 2 or more days depending on the number of modules in the SPOTFIRE System configuration and the laboratory's work schedule. Testing over multiple days provides day-to-day variation data; testing with multiple operators provides user-to-user variation data; testing multiple replicates of pooled verification material verifies precision of the test system.

 **Note:** It is important to prepare only the number of sample pools that will be tested within 14 days of preparation. The number of samples prepared may be modified based on the laboratory's work schedule and number of modules connected within a SPOTFIRE System.

### Day 1- Combined Respiratory and Sore Throat Protocol

1. Organize materials needed (Table 3); refer to Table 6 for the pooling scheme. Negativity of transport media may be confirmed by screening on the SPOTFIRE R/ST or SPOTFIRE R/STplus Panel prior to starting the verification procedure. Negative vials included in the control panel contain 1.8 mL of synthetic matrix; the control panel contains sufficient volume to complete the protocol described. More than one vial of negative may be needed for preparing some pools (i.e., Pool 2).
2. Prepare one sample pool (i.e., Pool 1) using the ZeptoMetrix NATRST-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool.
  - a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5 mL tube.
  - b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The combined volume of organisms for each pool will be between 1.8 to 2.1 mL, depending upon the pool.
  - c. Add transport media or synthetic matrix/negative (as described in Table 6) to the tube containing the organism pool (step b). The volume of transport media/ negative should be the same as the organism pool volume, for example: for Pools 1, 3, and 4, 1.8 mL of transport media/negative is added to 1.8 mL of pooled organism. The final volume of Pools 1, 3 and 4 will be approximately 3.6 mL.





**Note:** If the laboratory verification study will include multiple types of transport media, see the section *Expanding or Modifying the Protocol* below.

3. Repeat Step 2 for the remaining sample pools (Figure 3: Pools 2, 3, and 4) to be prepared on Day 1.
4. Test 2 replicates from a single sample pool (Figure 3: Pool 1 replicates A and B) using the Respiratory Menu and 2 replicates (Figure 3: Pool 1 replicates E and F) using the Sore Throat Menu. Ensure the pooled sample is well mixed prior to removing a sample for testing. The replicate samples should be tested in a single day by different operators to evaluate user-to-user variance. Refer to Figure 4 for suggested workflows depending upon the module configuration in the verification study.



**Note:** For each sample, follow instructions in the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Instructions for Use*, the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Quick Guide*, or the *BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing. Verification sample tests should be run by following on-screen instructions for patient sample pouch testing.

5. Repeat Step 4 for the remaining sample replicates to be tested that day (Figure 3: replicates A and B for Pools 2, 3 and 4 using the Respiratory Menu and replicates E and F for Pools 2, 3 and 4 using the Sore Throat Menu).
6. Refrigerate samples (2–8°C) for up to 14 days for the evaluation of day-to-day variation.



**Note:** The proposed organism pooling scheme, described in Table 6, provides sufficient material for running samples as described in Figure 3. The volume is sufficient for testing more samples if desired.

## Day 2- Combined Respiratory and Sore Throat Protocol

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 by repeating Steps 4 and 5 above (Figure 3: test replicates C, D, G, and H from Pools 1-4).



**Note:** A SPOTFIRE Respiratory and Sore Throat Menu Verification Record is provided and may serve as a template for recording your results.



Figure 3. Verification Protocol Workflow for Testing Respiratory and Sore Throat Menus over Two Days

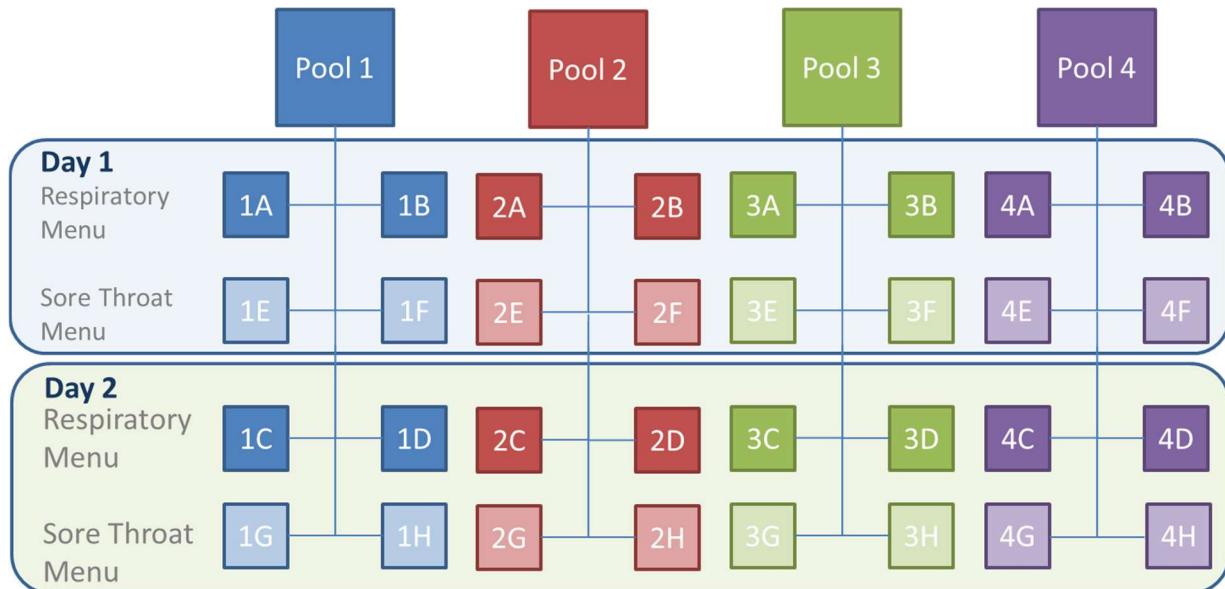


Figure 4. Examples of Verification Workflow testing over two days with different SPOTFIRE module configurations

| Combined Protocol with 1 module |                  | Module 1           |                    |                    |                    |                    |                    |                    |                    |
|---------------------------------|------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Day 1                           | Respiratory Menu | Pool 1A/Operator 1 | Pool 1B/Operator 2 | Pool 2A/Operator 1 | Pool 2B/Operator 2 | Pool 3A/Operator 1 | Pool 3B/Operator 2 | Pool 4A/Operator 1 | Pool 4B/Operator 2 |
|                                 | Sore Throat Menu | Pool 1E/Operator 1 | Pool 1F/Operator 2 | Pool 2E/Operator 1 | Pool 2F/Operator 2 | Pool 3E/Operator 1 | Pool 3F/Operator 2 | Pool 4E/Operator 1 | Pool 4F/Operator 2 |
| Day 2                           | Respiratory Menu | Pool 1C/Operator 1 | Pool 1D/Operator 2 | Pool 2C/Operator 1 | Pool 2D/Operator 2 | Pool 3C/Operator 1 | Pool 3D/Operator 2 | Pool 4C/Operator 1 | Pool 4D/Operator 2 |
|                                 | Sore Throat Menu | Pool 1G/Operator 1 | Pool 1H/Operator 2 | Pool 2G/Operator 1 | Pool 2H/Operator 2 | Pool 3G/Operator 1 | Pool 3H/Operator 2 | Pool 4G/Operator 1 | Pool 4H/Operator 2 |

| Combined Protocol with 2 modules |                  | Module 1           |                    |                    |                    | Module 2           |                    |                    |                    |
|----------------------------------|------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Day 1                            | Respiratory Menu | Pool 1A/Operator 1 | Pool 2A/Operator 1 | Pool 3A/Operator 1 | Pool 4A/Operator 1 | Pool 1B/Operator 2 | Pool 2B/Operator 2 | Pool 3B/Operator 2 | Pool 4B/Operator 2 |
|                                  | Sore Throat Menu | Pool 1E/Operator 1 | Pool 2E/Operator 1 | Pool 3E/Operator 1 | Pool 4E/Operator 1 | Pool 1F/Operator 2 | Pool 2F/Operator 2 | Pool 3F/Operator 2 | Pool 4F/Operator 2 |
| Day 2                            | Respiratory Menu | Pool 1D/Operator 2 | Pool 2D/Operator 2 | Pool 3D/Operator 2 | Pool 4D/Operator 2 | Pool 1C/Operator 1 | Pool 2C/Operator 1 | Pool 3C/Operator 1 | Pool 4C/Operator 1 |
|                                  | Sore Throat Menu | Pool 1H/Operator 2 | Pool 2H/Operator 2 | Pool 3H/Operator 2 | Pool 4H/Operator 2 | Pool 1G/Operator 1 | Pool 2G/Operator 1 | Pool 3G/Operator 1 | Pool 4G/Operator 1 |



| Combined Verification with 3 modules |                  | Module 1             |                      |                      | Module 2             |                      |                      | Module 3             |                      |                      |
|--------------------------------------|------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Day 1                                | Respiratory Menu | Pool 1A / Operator 1 | Pool 2A / Operator 1 | Pool 3A / Operator 1 | Pool 1B / Operator 2 | Pool 2B / Operator 2 | Pool 4B / Operator 2 | Pool 3B / Operator 2 | Pool 4A / Operator 1 |                      |
|                                      | Sore Throat Menu | Pool 1E / Operator 1 | Pool 2E / Operator 1 | Pool 4E / Operator 1 |                      | Pool 3F / Operator 2 | Pool 4F / Operator 2 | Pool 1F / Operator 2 | Pool 2F / Operator 2 | Pool 3E / Operator 1 |
| Day 2                                | Respiratory Menu | Pool 1D / Operator 2 | Pool 2D / Operator 2 | Pool 4D / Operator 2 | Pool 1C / Operator 1 | Pool 3C / Operator 1 | Pool 4C / Operator 1 | Pool 2C / Operator 1 | Pool 3D / Operator 2 |                      |
|                                      | Sore Throat Menu |                      |                      | Pool 3H / Operator 2 | Pool 4H / Operator 2 | Pool 2G / Operator 1 | Pool 1H / Operator 2 | Pool 3G / Operator 1 | Pool 1G / Operator 1 | Pool 2H / Operator 2 |

| Combined Verification with 4 modules |                  | Module 1             |                      | Module 2             |                      | Module 3             |                      | Module 4             |                      |
|--------------------------------------|------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Day 1                                | Respiratory Menu | Pool 1A / Operator 1 | Pool 3A / Operator 1 | Pool 1B / Operator 2 | Pool 3B / Operator 2 | Pool 2A / Operator 1 | Pool 4A / Operator 1 | Pool 2B / Operator 2 | Pool 4B / Operator 2 |
|                                      | Sore Throat Menu | Pool 1F / Operator 2 | Pool 3F / Operator 2 | Pool 1E / Operator 1 | Pool 3E / Operator 1 | Pool 2F / Operator 2 | Pool 4F / Operator 2 | Pool 2E / Operator 1 | Pool 4E / Operator 1 |
| Day 2                                | Respiratory Menu | Pool 2D / Operator 2 | Pool 4D / Operator 2 | Pool 2C / Operator 1 | Pool 4C / Operator 1 | Pool 1D / Operator 2 | Pool 3D / Operator 2 | Pool 1C / Operator 1 | Pool 3C / Operator 1 |
|                                      | Sore Throat Menu | Pool 2G / Operator 1 | Pool 3G / Operator 1 | Pool 2H / Operator 2 | Pool 3H / Operator 2 | Pool 1G / Operator 1 | Pool 4G / Operator 1 | Pool 1H / Operator 2 | Pool 4H / Operator 2 |

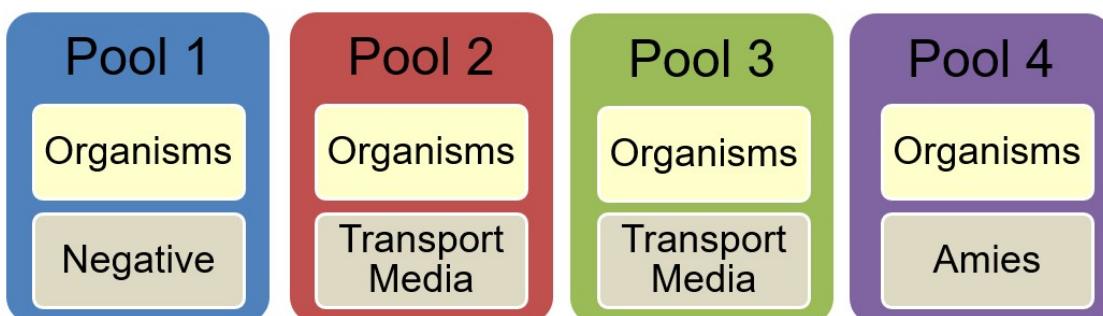
## Expanding or Modifying the Protocol

The protocols described above can be expanded by increasing the number of tests from each of the organism pools. Each organism pool contains sufficient volume for testing additional replicates. Some examples of expanding the verification study to include multiple media types are described below, but these should be done under the guidance of the Laboratory Director.

 **Note:** The laboratory should perform the verification study with specimen types and media that will be used with the SPOTFIRE R/ST or the SPOTFIRE R/ST*plus* Panel in their laboratory setting.

- 1) The verification study may use multiple types of transport media in the pools, as needed. Each organism pool can be prepared using a different media type; an example is shown in Figure 5.

**Figure 5.** Example Workflow for Testing Multiple Types of Media.





2) To perform a more extensive verification study, the volumes listed in Table 4, 5 and 6 can be increased proportionally. Using Pool 1 as an example, 0.5 mL of each organism can be combined and added to 3 mL of transport media/negative. Alternatively, additional organism pools may be prepared using the control material (NATRST-BIO) and following Steps 1-6 in the protocols above.



**Note:** Expanding the pool volume may require larger sample tubes to accommodate the increased volume.

## Verification of Loaner, Repaired, and Permanent Replacement Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement module, the following protocol may serve as a guideline but should be verified by the Laboratory Director.

1. Select an appropriate number of specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the SPOTFIRE R/ST or the SPOTFIRE R/ST<sup>plus</sup> Panel . The Laboratory Director should determine the appropriate number of samples to test. Proficiency samples should not be pooled or diluted.
2. Select a set of controls that verify detection of all targets on the SPOTFIRE R/ST or SPOTFIRE R/ST<sup>plus</sup> Panel .
3. Test the selected samples on the loaner, repaired, or permanent replacement module and document the results.

## Technical Support Contact Information

bioMérieux is dedicated to providing the best customer support available. If you have questions or concerns about this process, please contact your local bioMérieux representative or your authorized distributor.

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## SPOTFIRE R/ST and SPOTFIRE R/STplus Panel: Respiratory Menu Verification Record

BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel and BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Verification Record- Respiratory

|                             |                 |                 |
|-----------------------------|-----------------|-----------------|
| SPOTFIRE Kit Part and Lot # | Module Serial # | Module Serial # |
| SPOTFIRE Kit Part and Lot # | Module Serial # | Module Serial # |
| Media Type                  | Media Lot #     |                 |

| Organism and Representative Strain |   | Replicate Testing- Record Organism Detections |     |     |     |     |     |     |     |     |     |     |     | Respiratory Summary |     |     |     |             |             |             |        |           |
|------------------------------------|---|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------------|-----|-----|-----|-------------|-------------|-------------|--------|-----------|
|                                    |   | 1-A   | 1-B | 1-C | 1-D | 2-A | 2-B | 2-C | 2-D | 3-A | 3-B | 3-C | 3-D | 4-A                 | 4-B | 4-C | 4-D | # Positives | # Negatives | # Operators | # Days | # Modules |
| Pool 1                             | Adenovirus                                | Type 3  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Coronavirus SARS-CoV-2                    |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Influenza A virus A/H1-2009               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Influenza B virus                         |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Parainfluenza virus                       | PIV4  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
| Pool 2                             | Human rhinovirus/enterovirus              | Rhinovirus 1A                                 |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Adenovirus                                | Type 1  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Coronavirus (seasonal)                    | 229E  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    |   | HKU1  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Human metapneumovirus                     |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
| Pool 3                             | Influenza A virus A/H3                    |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Parainfluenza virus                       | PIV 1   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    |   | PIV2  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Adenovirus                                | Type 31                                       |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Coronavirus (seasonal)                    | NL63  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    |   | OC43  |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
| Pool 4                             | Influenza A virus (no subtype identified) |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Parainfluenza virus                       | PIV 3   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Respiratory syncytial virus               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Bordetella parapertussis                  |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Bordetella pertussis                      |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Chlamydia pneumoniae                      |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Mycoplasma pneumoniae                     |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |

Reviewed by:

Signature

Date



## SPOTFIRE R/ST and SPOTFIRE R/STplus Panel: Sore Throat Menu Verification Record

**BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel and BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Verification Record: Sore Throat**

SPOTFIRE Kit Part and Lot # \_\_\_\_\_ Module Serial # \_\_\_\_\_ Module Serial # \_\_\_\_\_  
 SPOTFIRE Kit Part and Lot # \_\_\_\_\_ Module Serial # \_\_\_\_\_ Module Serial # \_\_\_\_\_  
 Media Type \_\_\_\_\_ Media Lot # \_\_\_\_\_

| Organism and Representative Strain |  |               | Replicate Testing- Record Organism Detections |     |     |     |     |     |     |     |     |     |     |     | Sore Throat Summary |     |     |     |             |             |             |        |           |
|------------------------------------|--|---------------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------------|-----|-----|-----|-------------|-------------|-------------|--------|-----------|
|                                    |  |               | 1-A   | 1-B | 1-C | 1-D | 2-A | 2-B | 2-C | 2-D | 3-A | 3-B | 3-C | 3-D | 4-A                 | 4-B | 4-C | 4-D | # Positives | # Negatives | # Operators | # Days | # Modules |
| Pool 1                             | Adenovirus                                   | Type 3        |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Coronavirus SARS-CoV-2                       |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Influenza A virus A/H1-2009                  |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Influenza B virus                            |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Parainfluenza virus                          | PIV4          |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Human rhinovirus/enterovirus                 | Rhinovirus 1A |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
| Pool 2                             | Adenovirus                                   | Type 1        |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Coronavirus (seasonal)                       | 229E          |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    |  | HKU1          |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Human metapneumovirus                        |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Influenza A virus A/H3                       |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Parainfluenza virus                          | PIV 1         |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    |  | PIV2          |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
| Pool 3                             | Adenovirus                                   | Type 31       |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Coronavirus (seasonal)                       | NL63          |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    |  | OC43          |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Influenza A virus (no subtype identified)    |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Parainfluenza virus                          | PIV 3         |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Respiratory syncytial virus                  |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
| Pool 4                             | Chlamydia pneumoniae                         |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Mycoplasma pneumoniae                        |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Streptococcus dysgalactiae (Group C/G Strep) |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |
|                                    | Streptococcus pyogenes (Group A Strep)       |               |   |     |     |     |     |     |     |     |     |     |     |     |                     |     |     |     |             |             |             |        |           |

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Date \_\_\_\_\_



## SPOTFIRE R/ST and SPOTFIRE R/STplus Panel: Combined Respiratory/Sore Throat Menu Verification Record- Respiratory Detections

**BIOFIRE® SPOTFIRE® Respiratory/Sore Throat and BIOFIRE® SPOTFIRE® Respiratory/Sore Throat plus (R/STplus) Panel Verification Record-Combined Respiratory and Sore Throat - Respiratory Detections**

|                             |                 |                 |
|-----------------------------|-----------------|-----------------|
| SPOTFIRE Kit Part and Lot # | Module Serial # | Module Serial # |
| SPOTFIRE Kit Part and Lot # | Module Serial # | Module Serial # |
| Media Type                  | Media Lot #     |                 |

| Organism and Representative Strain |   | Replicate Testing- Record Organism Detections |     |     |     |     |     |     |     |     |     |     |     |     |     | Respiratory Summary |     |             |             |             |        |           |
|------------------------------------|---|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------------|-----|-------------|-------------|-------------|--------|-----------|
|                                    |   | Respiratory                                   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    |   | 1-A   | 1-B | 1-C | 1-D | 2-A | 2-B | 2-C | 2-D | 3-A | 3-B | 3-C | 3-D | 4-A | 4-B | 4-C                 | 4-D | # Positives | # Negatives | # Operators | # Days | # Modules |
| Pool 1                             | Adenovirus  | Type 3  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Coronavirus SARS-CoV-2  |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Influenza A virus A/H1-2009                                       |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Influenza B virus   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Parainfluenza virus   | PIV4  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
| Pool 2                             | Human rhinovirus/enterovirus                                      | Rhinovirus 1A                                 |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Adenovirus  | Type 1  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Coronavirus (seasonal)  | 229E  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    |   | HKU1  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Human metapneumovirus   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
| Pool 3                             | Influenza A virus A/H3  |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Parainfluenza virus   | PIV 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    |   | PIV2  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Adenovirus  | Type 31                                       |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Coronavirus (seasonal)  | NL63  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    |   | OC43  |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
| Pool 4                             | Influenza A virus (no subtype identified)                         |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Parainfluenza virus   | PIV 3   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | Respiratory syncytial virus                                       |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | <i>Bordetella parapertussis</i> (Respiratory)                     |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | <i>Bordetella pertussis</i> (Respiratory)                         |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | <i>Chlamydia pneumoniae</i> (Respiratory and Sore Throat)         |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | <i>Mycoplasma pneumoniae</i> (Respiratory and Sore Throat)        |   |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |     |             |             |             |        |           |
|                                    | <i>Streptococcus dysgalactiae</i> (Group C/G Strep) (Sore Throat) |   | N/A                 | N/A | N/A         | N/A         | N/A         | N/A    |           |
|                                    | <i>Streptococcus pyogenes</i> (Group A Strep) (Sore Throat)       |   | N/A                 | N/A | N/A         | N/A         | N/A         | N/A    |           |

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## SPOTFIRE R/ST and SPOTFIRE R/STplus Panel: Combined Respiratory/Sore Throat Menu Verification Record- Sore Throat Detections

BIOFIRE® SPOTFIRE® Respiratory/Sore Throat and BIOFIRE® SPOTFIRE® Respiratory/Sore Throat *plus* (R/STplus) Panel Verification Record-Combined Respiratory and Sore Throat- Sore Throat Detections

|                             |                 |                 |
|-----------------------------|-----------------|-----------------|
| SPOTFIRE Kit Part and Lot # | Module Serial # | Module Serial # |
| SPOTFIRE Kit Part and Lot # | Module Serial # | Module Serial # |
| Media Type                  | Media Lot #     |                 |

| Organism and Representative Strain |   | Replicate Testing- Record Organism Detections |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Sore Throat Summary |             |             |        |           |                  |
|------------------------------------|---|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------------|-------------|-------------|--------|-----------|------------------|
|                                    |   | Sore Throat                                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    |   | 1-E   | 1-F | 1-G | 1-H | 2-E | 2-F | 2-G | 2-H | 3-E | 3-F | 3-G | 3-H | 4-E | 4-F | 4-G | 4-H | # Positives         | # Negatives | # Operators | # Days | # Modules | Patient Samples? |
| Pool 1                             | Adenovirus  | Type 3  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Coronavirus SARS-CoV-2  |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Influenza A virus A/H1-2009                                       |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Influenza B virus   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Parainfluenza virus   | PIV4  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
| Pool 2                             | Human rhinovirus/enterovirus                                      | Rhinovirus 1A                                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Adenovirus  | Type 1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Coronavirus (seasonal)  | 229E  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    |   | HKU1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Human metapneumovirus   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Influenza A virus A/H3  |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
| Pool 3                             | Parainfluenza virus   | PIV 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    |   | PIV2  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Adenovirus  | Type 31                                       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Coronavirus (seasonal)  | NL63  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    |   | OC43  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Influenza A virus (no subtype Identified)                         |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
| Pool 4                             | Parainfluenza virus   | PIV 3   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | Respiratory syncytial virus                                       |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | <i>Bordetella parapertussis</i> (Respiratory)                     |   | N/A                 | N/A         | N/A         | N/A    | N/A       | N/A              |
|                                    | <i>Bordetella pertussis</i> (Respiratory)                         |   | N/A                 | N/A         | N/A         | N/A    | N/A       | N/A              |
|                                    | <i>Chlamydia pneumoniae</i> (Respiratory and Sore Throat)         |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | <i>Mycoplasma pneumoniae</i> (Respiratory and Sore Throat)        |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | <i>Streptococcus dysgalactiae</i> (Group C/G Strep) (Sore Throat) |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |
|                                    | <i>Streptococcus pyogenes</i> (Group A Strep) (Sore Throat)       |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |                     |             |             |        |           |                  |

Reviewed by:

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Signature \_\_\_\_\_ Date \_\_\_\_\_

