Protocols for Laboratory Verification of Performance of the BioFire® Respiratory Panel 2.1 (RP2.1)

Laboratory Protocols for Use with ZeptoMetrix NATtrol™ Control Materials

Purpose

The Clinical Laboratory Improvement Amendments (CLIA), passed in 1988, establishes quality standards for all laboratory testing to ensure the accuracy and reliability of patient test results, regardless of where the test is performed. The CLIA regulations include a requirement for verifying the performance specifications of unmodified, moderate complexity tests cleared or approved by the FDA.

Verification of an FDA Emergency Use Authorized (EUA) Test

Laboratories using an unmodified Emergency Use Authorization (EUA) diagnostic test must verify the test method performance specifications as applicable for their own laboratory prior to beginning patient testing. The laboratory may use information published in the manufacturer's package insert and other published literature for some aspects of the study (eg, interferences).

While the ultimate-objective is to fully verify the method performance of the assay, the pandemic crisis, the urgent need for patient testing, and the possible lack of reagents and supplies make it difficult to fully evaluate the accuracy, precision, and reportable range, as stated in COM.40300. A more limited approach may be acceptable. Each laboratory, in coordination with the laboratory director, should determine the depth of verification needed to begin testing and the laboratory director (or qualified alternate designee) must approve the verification study prior to testing (COM.40475). Applicable checklist requirements include: COM.40300, COM.40475, and COM.40500.

For Analytic Interferences (COM.40500) please refer to the *BioFire® Respiratory Panel 2.1 (RP2.1) Instructions for Use* for a list of interfering substances.

Note: EUA guidelines are frequently revised. Confirm that current regulatory guidelines are being followed prior to establishing performance verification for the BioFire RP2.1.

This document provides examples of procedures to assist your laboratory in developing a protocol for the verification of BioFire® Respiratory Panel 2.1 (RP2.1) performance on

Technical Note
BioFire Diagnostics, LLC
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BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems. Two possible verification schemes, compatible with the BioFire RP2.1, have been designed. Each verification scheme provides positive and negative tests for each organism detected by the BioFire RP2.1 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 BioFire RP2.1 should be done under the guidance of the Laboratory Director, but is not described here.

As per the CLIA regulation, the Laboratory Director is ultimately responsible for ensuring that verification procedures meet the appropriate standards for CLIA and applicable laboratory accrediting agencies.

Intended Use

The BioFire RP2.1 is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and differentiation of nucleic acids from multiple viral and bacterial respiratory organisms, including nucleic acids from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), in nasopharyngeal swabs (NPS) obtained from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity or moderate complexity tests.

The BioFire RP2.1 is intended for the detection and differentiation of nucleic acid from SARSCoV-2 and the following organism types and subtypes identified using the BioFire RP2.1.

Viruses	Bacteria
Adenovirus	
Coronavirus 229E	*Bordetella parapertussis*
Coronavirus HKU1	*Bordetella pertussis*
Coronavirus NL63	*Chlamydia pneumoniae*
Coronavirus OC43	*Mycoplasma pneumoniae*
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	
Human Metapneumovirus	
Human Rhinovirus/Enterovirus	
Influenza A, including subtypes	
H1, H3 and H1-2009	
Influenza B	
Parainfluenza Virus 1	
Parainfluenza Virus 2	
Parainfluenza Virus 3	
Parainfluenza Virus 4	
Respiratory Syncytial Virus	

The complete intended use statement and additional information about the use of the BioFire System can be found in the *BioFire® Respiratory Panel 2.1 Instructions for Use*.

Performance Verification Overview

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of the BioFire RP2.1and (2) a Transport Media Protocol that evaluates BioFire RP2.1performance in a transport media sample matrix. These protocols are examples of procedures to assist your laboratory in developing a protocol for the verification of BioFire RP2.1 performance on the BioFire Systems

The verification procedures described here may be used to evaluate the performance of each assay on the BioFire RP2.1. The performance verification protocols have been designed to take advantage of the multiplex nature of the BioFire RP2.1. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures described below will generate multiple positive and negative detections for each of the BioFire RP2.1 assays. The procedures were developed using a Respiratory Verification Panel 2 and a SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control available from ZeptoMetrix™ Corporation, Buffalo, NY (part numbers NATRVP2-BIO and NATSARS(COV2)-ERC.

A BioFire System is defined as all BioFire® FilmArray® Instruments or Modules that are connected to and controlled by a single computer system. If the laboratory director chooses not to perform the entire verification protocol on each individual instrument, it is advised that test replicates are evenly distributed among the instruments or modules. An example of a performance verification workflow using 2, 4, or 6 modules is provided in Figure 2.

Clinical/patient samples may be used in place of, or in addition to the verification schemes described here in order to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the BioFire RP2.1.

Table 1. Overview of Verification Protocols

Verification Protocol	Organisms per Pool[a]	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results[a]	Expected Negative Results	Approximate Days of Testing[b]
Example 1: Simple protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4
Example 2: Transport Media protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4

[a] 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 adenovirus strains; therefore the number of expected adenovirus positives would be 12 and the number of expected negatives would be 4.

[b] The approximate number of days for testing assumes a BioFire® system configured with one instrument/module.

Performance Verification Materials

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

Table 2. Recommended materials for the verification protocols

Material	Part Number
BioFire® Respiratory Panel 2.1 (RP2.1) Kit (30 tests)	BioFire Diagnostics, LLC 423738
BioFire® Respiratory Panel 2.1 (RP2.1) Instructions for Use	BioFire Diagnostics, LLC BFR0000-8303
BioFire® Respiratory Panel 2.1 (RP2.1) Quick Guide	BioFire Diagnostics, LLC BFR0000-8304
Control Organism[a]	ZeptoMetrix Respiratory Verification Panel 2 (NATRVP2-BIO)
	ZeptoMetrix NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control (NATSARS(COV2)-ERC)
Transport Media (e.g. Remel M4 Viral Transport Media)	Various media are appropriate
2 mL or 5 mL Sample Tubes	Various manufacturersA10:E32
Disposable Transfer pipets, graduated	VWR, 414004-024 (or equivalent)

[a]Any appropriate source of organism may be used for verification of any or all of the assays in the BioFire RP2.1 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.

Performance Verification Protocols

Simple Protocol

The Simple Protocol evaluates the BioFire RP2.1 performance when verification materials (ZeptoMetrix NATRVP2-BIO and NATSARS(COV2)-ERC) are pooled in the absence of clinical matrix. The proposed organism pooling scheme (Table 3) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

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

Figures 1 and 2 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 more samples per day based on the number of modules in the BioFire System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples can be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

Table 3. Proposed Organism Pooling Scheme for the Simple Protocol

Organism	Approximate Organism Volume	Approximate Pool Volume
Pool 1		
Adenovirus Type 3	0.3 mL	1.8 mL
Coronavirus OC43	0.3 mL	
Severe Acute Syndrome Coronavirus 2 (SARS-CoV-2)	0.3 mL	
Influenza A 2009 H1N1	0.3 mL	
Influenza B	0.3 mL	
Parainfluenza virus Type 4	0.3 mL	
Pool 2		
Coronavirus 229E	0.3 mL	1.5 mL
Influenza A H3	0.3 mL	
Parainfluenza virus Type 1	0.3 mL	
Parainfluenza virus Type 2	0.3 mL	
Rhinovirus 1A	0.3 mL	
Pool 3		
Adenovirus Type 1	0.3 mL	1.8 mL
Coronavirus NL63	0.3 mL	
Influenza A H1	0.3 mL	
Parainfluenza virus Type 3	0.3 mL	
Respiratory Syncytial Virus A	0.3 mL	
Bordetella parapertussis	0.3 mL	
Pool 4		
Adenovirus Type 31	0.3 mL	1.8 mL
Coronavirus HKU1	0.3 mL	
Human Metapneumovirus 8	0.3 mL	
Bordetella pertussis	03 mL	
Chlamydia pneumoniae	0.3 mL	
Mycoplasma pneumoniae	0.3 mL	

Simple Protocol Example

The estimated total time for completion for this Simple Protocol verification example is 4 days for a BioFire System configured with 1 module. A proposed organism pooling scheme is presented above in Table 3. Figure 1 illustrates a simplified workflow schematic. The number of samples tested per day should be determined by the individual laboratory. The protocol can be modified to run more samples per day (or fewer) based upon the number of modules in the BioFire System. The proposed organism pooling scheme in Table 3 provides sufficient volume for testing more replicates, if desired. Figure 2 provides an examples of user-to-user, day-to-day, and module-to-module testing for labs with multiple BioFire Modules.

Note: It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The suggestion to prepare 3 sample pools is based on testing up to 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of modules connected within a BioFire System.

Day 1

1. Organize materials needed (Table 2).

2. Prepare two sample pools (i.e. Pools #1 and 2) from ZeptoMetrix NATRVP2-BIO, and NATSARS(COV2)-ERC control materials. Organism vials should be well mixed prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.
    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 2 mL tube. Alternatively, a 5mL tube may be used.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The total volume for each pool will be approximately 1.5 or 1.8 mL.
    c. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for the remaining sample pool (i.e. Pool #2) to be prepared on Day 1.

4. Test 2 replicates from a single sample pool (i.e. Figure 1: Pool # 1 replicates A and B). The replicate samples should be tested in a single day by different users.

Note: For each sample, follow instructions in the *BioFire® Respiratory Panel 2.1 (RP2.1) Instructions for Use* and the *BioFire® Respiratory Panel 2.1 (RP2.1) Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e. Pool # 2 replicates A and B)

6. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

Note: The proposed organism pooling scheme (Table 3) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more samples if desired.

Day 2

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 1 by repeating Step 4 and 5 above (i.e. Pool # 1 replicates C and D).

Day 3

Prepare 2 new sample pools (i.e. Pools #3 and 4) as described in Steps 2 and 3. Test replicates as described in Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 3 by repeating Step 4 and 5 above (i.e. Pool # 3 replicates C and D).

Note: A BioFire RP2.1 Verification Record is provided and may serve as a template for recording your results.

Figure 1. Workflow for the Simple Protocol and the Transport Media Protocol

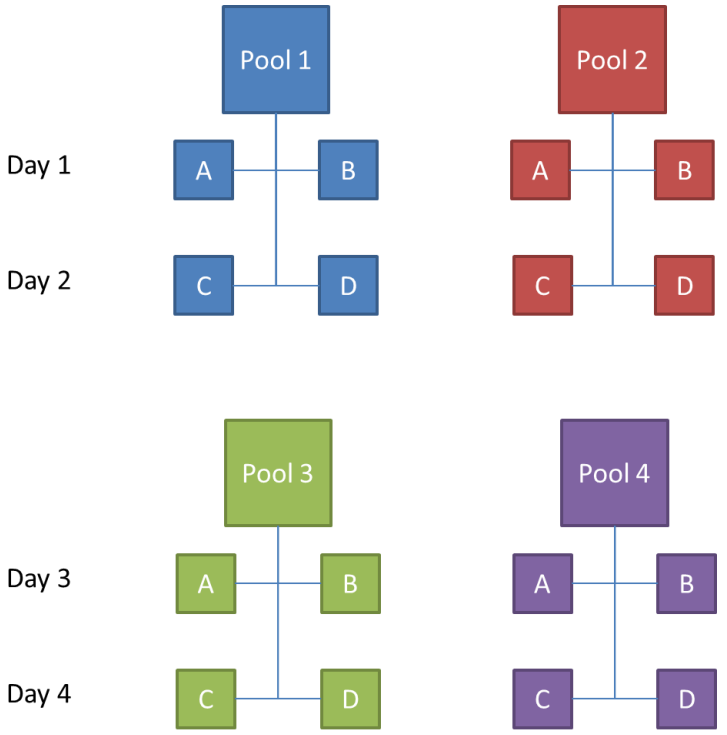

Figure 2. Example of a Verification workflow for use with multiple BioFire Modules

Verification with 2 modules	Module 1		Module 2	
Day 1	Pool 1/ User 1	Pool 2/ User 2	Pool 1/ User 2	Pool 2/ User 1
Day 2	Pool 1/ User 2	Pool 2/ User 1	Pool 1/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1	Pool 4 / User 1	Pool 3/ User 2	Pool 4 / User 2
Day 4	Pool 3/ User 2	Pool 4 / User 2	Pool 3 / User1	Pool 4 / User 1

Verification with 4 modules	Module 1	Module 2	Module 3	Module 4
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 2	Pool 2/ User 2	Pool 2/ User 1	Pool 1/ User 2	Pool 1/ User 1
Day 3	Pool 3 / User 1	Pool 3/ User 2	Pool 4 / User 1	Pool 4 / User 2
Day 4	Pool 4 / User 2	Pool 4 / User 1	Pool 3/ User 2	Pool 3 / User1

Verification with 6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2		
Day 2			Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1	Pool 3/ User 2			Pool 4 / User 1	Pool 4 / User 2
Day 4	Pool 4 / User 2	Pool 4 / User 1	Pool 3 / User1	Pool 3/ User 2		

Transport Media Protocol

The Transport Media Protocol evaluates the BioFire RP2.1 performance when verification materials (ZeptoMetrix NATRVP2-BIO and NATSARS(COV2)-ERC) are tested in the presence of a transport media sample matrix. 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.

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

The protocol and workflow schemes (Figures 1 and 2) illustrate 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 more samples per day based on the number of modules in the BioFire System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples can be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

Table 4. Proposed Organism Pooling Scheme for the Transport Media Protocol

Organism	Approximate Organism Volume	Volume Transport Media	Approximate Pool Volume
Pool 1			
Adenovirus Type 3	0.3 mL	1.8 mL	3.6 mL
Coronavirus OC43	0.3 mL		
Severe Acute Syndrome Coronavirus 2 (SARS-CoV-2)	0.3 mL		
Influenza A 2009 H1N1	0.3 mL		
Influenza B	0.3 mL		
Parainfluenza virus Type 4	0.3 mL		
Pool 2			
Coronavirus 229E	0.3 mL	1.5 mL	3.0 mL
Influenza A H3	0.3 mL		
Parainfluenza virus Type 1	0.3 mL		
Parainfluenza virus Type 2	0.3 mL		
Rhinovirus 1A	0.3 mL		
Pool 3			
Adenovirus Type 1	0.3 mL	1.8 mL	3.6 mL
Coronavirus NL63	0.3 mL		
Influenza A H1	0.3 mL		
Parainfluenza virus Type 3	0.3 mL		
Respiratory Syncytial Virus A	0.3 mL		
Bordetella parapertussis	0.3 mL		
Pool 4			
Adenovirus Type 31	0.3 mL	1.8 mL	3.6 mL
Coronavirus HKU1	0.3 mL		
Human Metapneumovirus 8	0.3 mL		
Bordetella pertussis	03 mL		
Chlamydia pneumoniae	0.3 mL		
Mycoplasma pneumoniae	0.3 mL		

Transport Media Protocol Example

The estimated total time for completion for this Transport Media Protocol verification example is 4 days for a BioFire System configured with 1 module. A proposed organism pooling scheme is presented above in Table 4. Figure 1 illustrates a simplified workflow schematic. The number of samples tested per day should be determined by the individual laboratory. The protocol can be modified to run more samples per day (or fewer) based upon the number of modules in the BioFire System. The proposed organism pooling scheme in Table 4 provides sufficient volume for testing more replicates, if desired. Figure 2 provides an examples of user-to-user, day-to-day, and module-to-module testing for labs with multiple BioFire Modules.

Note: It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The suggestion to prepare 2 sample pools is based on testing up to 41.5 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of modules connected within a BioFire System.

Day 1

1. Organize materials needed (Table 2).

2. Prepare two sample pools (i.e. Pools #1 and 2) from ZeptoMetrix NATRVP2-BIO, NATSARS(COV2)-ERC, and NATCOV(MR)-BIO control material. Organism vials should be well mixed prior to preparing each pool. Refer to Table 4 for example organism pooling schemes and specific volumes for each pool.

    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5mL tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The total volume for each pool will be approximately 1.5 to 1.8 mL.
    c. Add 1.5 or 1.8 mL of transport media (as described in Table 4) to the tube containing the organism pool (step b). The total volume will be approximately 3.0 to 3.6 mL.
    d. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for the remaining sample pool (i.e. Pool #2) to be prepared on Day 1.

4. Test 2 replicates from a single sample pool (i.e. Figure 1: Pool # 1 replicates A and B). The replicate samples should be tested in a single day by different users.

Note: For each sample, follow instructions in the *BioFire® Respiratory Panel 2.1 (RP2.1) Instructions for Use* and the *BioFire® Respiratory Panel 2.1 (RP2.1) Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e. Pool # 2 replicates A and B)

6. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

Note: The proposed organism pooling scheme (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

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 1 by repeating Step 4 and 5 above (i.e. Pool # 1 replicates C and D).

Day 3

Prepare 2 new sample pools (i.e. Pools #3 and 4) as described in Steps 2 and 3. Test replicates as described in Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 3 by repeating Step 4 and 5 above (i.e. Pool # 3 replicates C and D).

Note: A BioFireRP2.1 Verification Record is provided and may serve as a template for recording your results.

Expanding the Protocols

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.

Verification of Loaner, Repaired, and Permanent Replacement Instruments

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

1. Select a few specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the BioFire RP2.1. 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 BioFire RP2.1.

3. Test the selected samples on the loaner, repaired, or permanent replacement instrument and document the results.

Technical Support Contact Information

BioFire is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the BioFire Technical Support team for assistance.

BioFire Technical Support
Email: support@biofiredx.com
Phone: +1-801-736-6354, select Option 5

BioFire Respiratory Panel 2.1 (RP2.1) Verification Record

BioFire® Respiratory Panel 2.1 (RP2.1) Verification Record _____ **Module Serial #** _____ **Module Serial #** _____

Kit Part # _____ **Module Serial #** _____ **Module Serial #** _____

Lot # _____

Organism and Representative Strain		Replicate Testing- Record Organism Detections																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	# Users	# Days	# Modules	Patient Samples?
Pool 1	Adenovirus Type 3																						
	Coronavirus OC43																						
	Severe Acute Syndrome Coronavirus 2 (SARS-CoV-2)																						
	Influenza A H1-2009																						
	Influenza B																						
	Parainfluenza Virus 4																						
Pool 2	Coronavirus 229E																						
	Influenza A H3																						
	Parainfluenza Virus 1																						
	Parainfluenza Virus 2																						
	Human Rhinovirus/Enterovirus Rhinovirus 1A																						
Pool 3	Adenovirus Type 1																						
	Coronavirus NL63																						
	Influenza A H1																						
	Parainfluenza Virus 3																						
	Respiratory Syncytial Virus																						
	Bordetella parapertussis (IS1001)																						
Pool 4	Adenovirus Type 31																						
	Coronavirus HKU1																						
	Human Metapneumovirus																						
	Bordetella pertussis (ptxP)																						
	Chlamydia pneumoniae																						
	Mycoplasma pneumoniae																						

Reviewed by: _____

Signature **Date**

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