

Protocols for Laboratory Verification of Performance of the FilmArray® Respiratory Panel (RP)

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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.


This document provides examples of procedures to assist your laboratory in developing a protocol for the verification of FilmArray RP performance on FilmArray® 1.5, 2.0 and Torch Systems as required by CLIA. Two possible verification schemes, compatible with the FilmArray RP, have been designed. Each verification scheme provides positive and negative tests for each organism detected by the FilmArray RP using non-clinical specimens 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 FilmArray RP 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.

FilmArray Intended Use

The FilmArray RP is a multiplexed nucleic acid test intended for use with FilmArray Systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organisms and subtypes are identified using the FilmArray RP: adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, human metapneumovirus, influenza A, influenza A subtype H1, influenza A subtype H3, influenza A subtype 2009 H1, influenza B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, rhinovirus/enterovirus, respiratory syncytial virus, *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

The complete intended use statement and additional information about the use of the FilmArray System can be found in the *FilmArray Respiratory Panel Instruction Booklet*.

 **Note:** *Chlamydia pneumoniae* is sometimes referred to as *Chlamydophila pneumoniae*.

Performance Verification: Overview

Each procedure described below will generate multiple positive and negative results for each of the FilmArray® Respiratory Panel (RP) assays. The procedures were developed using a Respiratory Verification Panel available from ZeptoMetrix Corporation, Buffalo, NY (part number NATRVP-IDI).

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of the FilmArray RP performance on any FilmArray® System and (2) a Viral Transport Media (VTM) Protocol that evaluates FilmArray RP performance when organisms are in a VTM sample matrix.

A FilmArray System is defined as one or more FilmArray® Instruments or modules connected to and controlled by a single computer system. If the laboratory director chooses not to perform the verification protocol on each individual instrument, it is advised that test replicates are evenly distributed among the instruments or modules.

Performance verification protocols should be designed to take advantage of the multiplex nature of the FilmArray RP. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run.

Clinical/patient specimens may be used in place of or in addition to the verification protocol examples described here in order to assess clinical sensitivity and sample matrix effects in the performance verification of the FilmArray RP.

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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	Expected Negative Results	Approximate Days of Testing ^b
Example 1: Simple protocol	2 or 3	7	4	28	4 per organism	24 per organism	6
Example 2: Viral Transport Medium (VTM) protocol	1	19	4	76	4 per organism	72 per organism	14

^a Depending on the material used for verification, pooling of organisms may not be appropriate and the values in the table may need to be modified.

^b The approximate number of days for testing assumes a system configured with one instrument.

Performance Verification: Materials

The following materials may be used to perform verification procedures:

Table 2. Materials needed for recommended verification protocols


Material	Part Number
FilmArray RP Kit (30 tests)	BioFire Diagnostics, LLC RFIT-ASY-0124
Control Organism	ZeptoMetrix NATRVP-IDI ^a
Transport Medium (e.g. Remel M4 Viral Transport Media)	Various media are appropriate

^a Any appropriate source of organism may be used for verification of any or all of the assays in the FilmArray RP. However, when alternate organism sources are used (i.e. not the ZeptoMetrix NATRVP-IDI 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 utilizes samples prepared by pooling together either 2 or 3 different organisms (ZeptoMetrix NATRVP-IDI control organism). 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 Respiratory Verification Panel organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

The Simple Protocol can be followed to test a total of 28 pooled samples, providing 4 positive results and 24 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. The testing scheme can be modified to run more samples per day based on the number of instruments configured on the FilmArray® System.

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

Organism	Approximate volume	Approximate Final Volume of Pool
Pool 1		
Adenovirus	0.6 mL	1.2 mL
Influenza A subtype H1	0.6 mL	
Pool 2		
Influenza B	0.6 mL	1.8 mL
Parainfluenza virus 4	0.6 mL	
Coronavirus OC43	0.6 mL	
Pool 3		
Human rhinovirus/Enterovirus	0.6 mL	1.8 mL
Influenza A subtype H3	0.6 mL	
Coronavirus 229E	0.6 mL	
Pool 4		
Parainfluenza virus 1	0.6 mL	1.8 mL
Parainfluenza virus 2	0.6 mL	
<i>Mycoplasma pneumoniae</i>	0.6 mL	
Pool 5		
Influenza A subtype H1-2009	0.6 mL	1.2 mL
Parainfluenza virus 3	0.6 mL	
Pool 6		
Respiratory Syncytial Virus	0.6 mL	1.8 mL
Coronavirus NL63	0.6 mL	
Human Metapneumovirus	0.6 mL	
Pool 7		
<i>Bordetella pertussis</i>	0.6 mL	1.8 mL
<i>Chlamydia pneumoniae</i>	0.6 mL	
Coronavirus HKU1	0.6 mL	

An example Simple Protocol workflow is provided below.


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Simple Protocol Example


The estimated total time for completion for this Simple Protocol verification example is 6 days for a FilmArray® System configured with 1 instrument. A proposed organism pooling scheme is presented above in Table 3.

Day 1


1. Prepare three sample pools (i.e. pools #1, #2, and #3 in Table 3 above) from ZeptoMetrix NATRVP-IDI control material.

 **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 6 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of instruments connected within a FilmArray System.

- a. Use a transfer pipette to remove the entire contents of the ZeptoMetrix organism vial (approximately 0.6 mL) and transfer to a new vial or tube.
- b. Repeat with the second (and third) organism to combine the appropriate organisms into a single vial or tube (approximately 1.2 mL total volume for two organisms or 1.8 mL for three organisms).

 **Note:** Pools 1 and 5 contain 1.2 mL and will be used to test four pouches (approximately 0.3 mL for each pouch). If the volume of the sample is not sufficient to reach the 3rd line of the Transfer Pipette when loading sample into your pouch, note the volume shortage and proceed with testing.

- c. Ensure the pooled sample is effectively mixed by vortexing prior to testing.
2. Test two samples from a single sample pool. The duplicate samples should be tested in a single day by different users.

 **Note:** For each sample, follow instructions in the *FilmArray® Respiratory Panel Instruction Booklet* and *FilmArray Respiratory Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

3. Repeat Step 2 for the remaining sample pools (i.e. pools #2 and #3) to be tested that day.
4. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

Day 2

To evaluate day-to-day variation, test the remaining volume of the sample pools prepared on Day 1 by repeating Step 2 above.

Day 3

Prepare 3 new sample pools (i.e. pools #4, #5, and #6) as described in Step 1. Test samples according to Step 2.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Step 2.

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Day 5-6

Repeat Steps 1–2 as above for any remaining sample pools (e.g. pool #7).

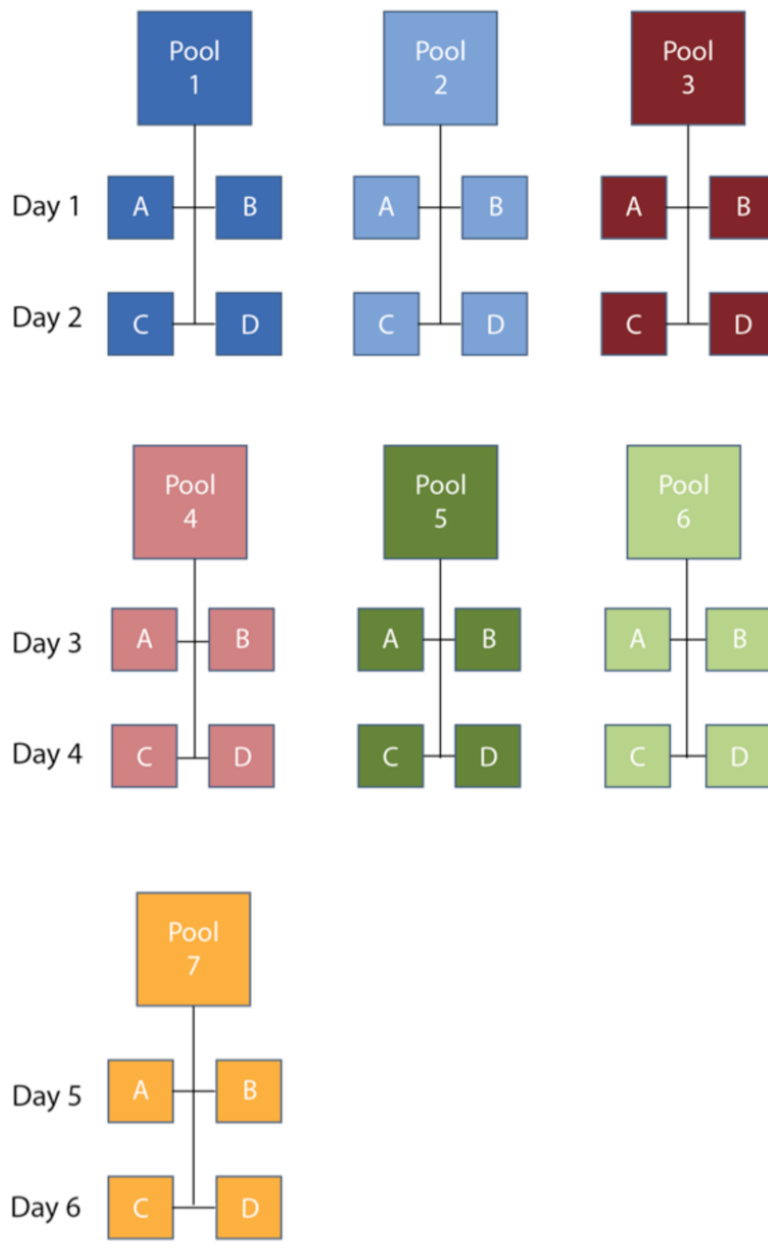


Figure 1. Workflow for Simple Protocol Example

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VTM Protocol

The VTM Protocol utilizes samples prepared by adding a single control organism (ZeptoMetrix NATRVP-IDI control organism) to an equal volume of Viral Transport Medium (VTM) sample matrix.

The VTM Protocol can be followed to test a total of 76 samples, providing 4 positive results and 72 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. The testing scheme can be modified to run more samples per day based on the number of instruments or modules configured on the FilmArray® System.

VTM 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. To evaluate user-to-user variation, multiple laboratory technicians may perform testing.

Table 4. Proposed VTM Sample Preparation Scheme

VTM Sample	
	Approximate volume
ZeptoMetrix NATRVP-IDI Control Organism	0.6 mL
VTM	0.6 mL


An example VTM Protocol workflow is provided below.

VTM Protocol Example


The estimated total time to completion for this VTM Protocol verification example is 14 days for a FilmArray System configured with 1 instrument.

Day 1

1. Prepare three VTM Samples (i.e. sample #1, #2, #3) by mixing ZeptoMetrix NATRVP-IDI control material with VTM as proposed in Table 4.

 **Note:** It is important to prepare only the number of samples that will be tested within 3 days of preparation. The suggestion to prepare 3 samples is based on testing 6 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of instruments configured on the FilmArray System.

- a. Pipet 0.6 mL of VTM into a sterile tube or vial.
 - b. Use a transfer pipette to remove the entire contents of the ZeptoMetrix organism vial (approximately 0.6 mL) and add organism to the tube or vial containing VTM (total sample volume for one organism in VTM will be approximately 1.2 mL).
 - c. Ensure the VTM sample is effectively mixed by vortexing prior to testing.
2. Test two samples (pouches) from a single VTM sample. The duplicate samples should be tested in a single day by different users.

 **Note:** For each sample, follow instructions in the *FilmArray® Respiratory Panel Instruction Booklet* and *FilmArray Respiratory Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

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3. Repeat Step 2 for the remaining samples to be tested that day.



Note: The approximate volume of VTM samples is 1.2 mL, which should be sufficient to test four pouches per sample (approximately 0.3 mL for each pouch). If the volume of the sample is not sufficient to reach the 3rd line of the Transfer Pipette when loading sample into your pouch, note the volume shortage and proceed with testing.

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

Day 2

To examine day-to-day variation, test the samples prepared on Day 1 by repeating Step 2.

Day 3–4

Repeat Steps 1–4 for 3 additional samples (i.e. #4, #5, and #6).

Day 5–6

Repeat Steps 1–4 for 3 additional samples (i.e. #7, #8, and #9).

Day 7–8

Repeat Steps 1–4 for 2 additional samples (i.e. #10, #11, and #12).

Day 9–10

Repeat steps 1–4 for the remaining samples (i.e. #13, #14 and #15).

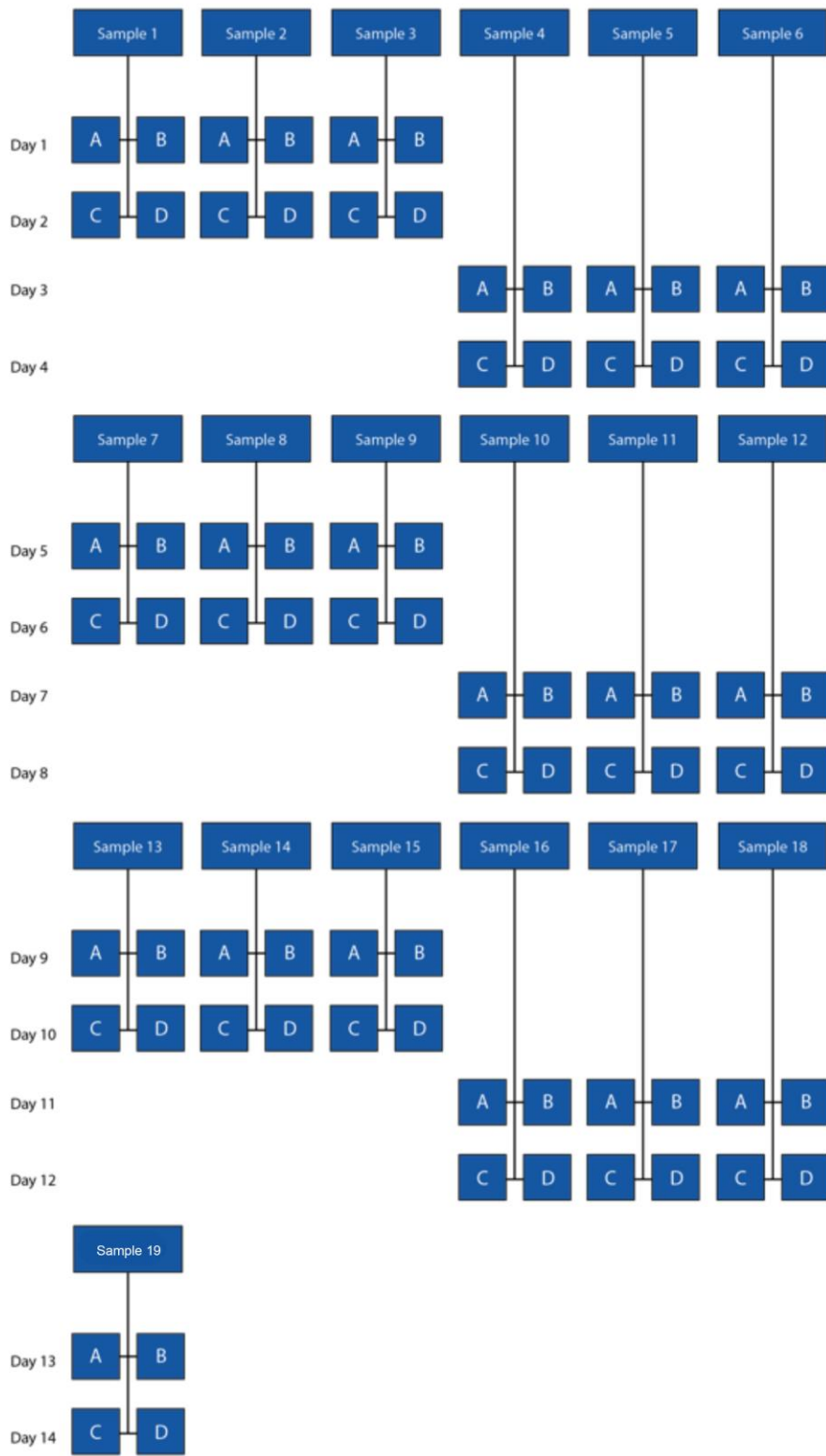
Day 11–12

Repeat steps 1–4 for the remaining samples (i.e. #16, #17, and #18).

Day 13–14

Repeat steps 1–4 for the remaining samples (i.e. #19).

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Figure 2. Workflow for VTM Protocol Example

Verification of loaner and repaired instruments

If it becomes necessary to verify the performance of a loaner or repaired 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 FilmArray® Respiratory Panel (RP). The Laboratory Director should determine the appropriate number of samples to test. Two to six samples may be sufficient. Proficiency samples should not be pooled or diluted.
2. Test the selected specimens/samples on the loaner or repaired 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 FilmArray Technical Support team for assistance.

BioFire Technical Support

Email: support@biofiredx.com

Phone: +1-801-736-6354, select Option 5



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FilmArray® Respiratory Panel (RP) Verification Record

Computer System Serial Number #: _____

FilmArray RP Kit Part #: _____ Lot #: _____

Organism/Sample Source and Lot #: _____

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Organism	Instrument Serial #	Was the Organism Detected?	No. Positive	No. Negative	No. Days Tested	No. Users	Patient Samples Tested?
Adenovirus		<input type="checkbox"/> Yes <input type="checkbox"/> No					
<i>Bordetella pertussis</i>		<input type="checkbox"/> Yes <input type="checkbox"/> No					
<i>Chlamydia pneumoniae</i>		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Coronavirus 229E		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Coronavirus NL63		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Coronavirus OC43		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Coronavirus HKU1		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Human Metapneumovirus		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Human Rhinovirus/Enterovirus		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Influenza A subtype H1		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Influenza A subtype H1-2009		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Influenza A subtype H3		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Influenza B		<input type="checkbox"/> Yes <input type="checkbox"/> No					
<i>Mycoplasma pneumoniae</i>		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Parainfluenza virus 1		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Parainfluenza virus 2		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Parainfluenza virus 3		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Parainfluenza virus 4		<input type="checkbox"/> Yes <input type="checkbox"/> No					
Respiratory Syncytial Virus		<input type="checkbox"/> Yes <input type="checkbox"/> No					

Reviewed by: _____
Signature Date