Protocols for Laboratory Verification of Performance of the BioFire® FilmArray® Pneumonia Panel *plus*

Laboratory Protocols for Use with a ZeptoMetrix NATtrol™ Verification Panel

For use outside US

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, and high complexity tests cleared or approved by the FDA. The BioFire® FilmArray® Pneumonia Panel *plus* has been categorized by the FDA as a CLIA high complexity test.

This document provides examples of verification procedures to assist your laboratory in developing a protocol for the verification of the BioFire Pneumonia Panel *plus* performance on BioFire® FilmArray® Systems as required by CLIA. Verification schemes, compatible with the BioFire Pneumonia Panel *plus*, have been designed using non-clinical specimens. Each scheme provides positive and negative tests for each organism detected by the BioFire Pneumonia Panel *plus*, as well as semi-quantitative bin results for bacterial interpretations to demonstrate the ability to distinguish between relative abundance levels in a single test. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is provided as two inactive recombinants, each targeting a unique assay on the BioFire Pneumonia Panel *plus*. The pooling scheme provided will give equivocal detections for MERS-CoV and is designed to reduce false positive detections. The schemes 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 representative of the different types expected to be tested by the laboratory (ex. Sputum, ETA, BAL) for verification of the performance of the BioFire® Pneumonia Panel *plus* 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.

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
FLM1-PRT-0264-04

QS-339H-01

BioFire® Pneumonia Panel *plus* Intended Use (Outside of the USA)

The BioFire Pneumonia Panel *plus* is a multiplexed nucleic acid test intended for use with FilmArray® 2.0, or FilmArray® Torch systems for the simultaneous detection and identification of multiple respiratory viral and bacterial nucleic acids, as well as select antimicrobial resistance genes, in sputum-like specimens (induced or expectorated sputum, or endotracheal aspirates) or bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL) obtained from individuals suspected of lower respiratory tract infection.

The following bacteria are reported semi-quantitatively with bins representing approximately 10^4, 10^5, 10^6, or ≥10^7 genomic copies of bacterial nucleic acid per milliliter (copies/mL) of specimen, to aid in estimating relative abundance of nucleic acid from these common bacteria within a specimen:

Bacteria reported with bins of 10^4, 10^5, 10^6, or ≥107 copies/mL		
Acinetobacter calcoaceticus-baumanni complex	*Klebsiella oxytoca*	*Serratia marcescens*
Enterobacter cloacae complex	*Klebsiella pneumoniae* group	*Staphylococcus aureus*
Escherichia coli	*Moraxella catarrhalis*	*Streptococcus agalactiae*
Haemophilus influenzae	*Proteus* spp.	*Streptococcus pneumoniae*
Klebsiella aerogenes	*Pseudomonas aeruginosa*	*Streptococcus pyogenes*

The following atypical bacteria, viruses, and antimicrobial resistance genes are reported qualitatively:

Atypical Bacteria		
Chlamydia pneumoniae	*Legionella pneumophila*	*Mycoplasma pneumoniae*
Viruses		
Adenovirus	Human Rhinovirus/Enterovirus	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
Coronavirus	Influenza A	Parainfluenza virus
Human Metapneumovirus	Influenza B	Respiratory Syncytial Virus
Antimicrobial Resistance Genes		
CTX-M	NDM	*mecA/C* and MREJ
IMP	OXA-48-like	
KPC	VIM	

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

Note: The intended use listed above is not cleared in the USA.

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
FLM1-PRT-0264-04

QS-339H-01

Performance Verification: Overview

Two different examples of performance verification procedures are described. Both procedures evaluate the performance of each assay on the BioFire® Pneumonia Panel *plus* using samples in the presence of synthetic or clinical matrix background. A Synthetic Matrix Protocol describes the verification of the BioFire Pneumonia Panel *plus* performance in either a synthetic background (Negative) provided with the ZeptoMetrix NATtrol control organisms or in Artificial Bronchoalveolar Lavage Matrix (aBAL). Preparation of aBAL is described in the Performance Verification: Protocols section below. A Clinical Matrix Protocol evaluates the performance of each assay on the BioFire Pneumonia Panel *plus* in a clinical specimen matrix (induced or expectorated sputum, endotracheal aspirates or bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL)). These protocols are examples of procedures to assist your laboratory in developing a protocol for the verification of BioFire Pneumonia Panel *plus* performance on BioFire® Systems.

Note: It is important to characterize aBAL or clinical matrix specimens for BioFire Pneumonia Panel *plus* targets by screening the specimen on the BioFire Pneumonia Panel *plus* prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes tested on the BioFire Pneumonia Panel *plus*. If a negative specimen is not available, note the positives and take that into account when reviewing results.

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 or module, 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.

The procedures have been designed to take advantage of the multiplex nature of the BioFire Pneumonia Panel *plus*. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures will generate multiple positive and negative detections for each of the BioFire Pneumonia Panel *plus* assays. In addition, semi-quantitative bacteria are reported with relative bin values of 10^4, 10^5, 10^6, or ≥10^7 copies/mL. The protocols described will generate detections in high bins (10^6-≥10^7) and low bins (10^4-10^5) to demonstrate the ability to differentiate organism levels in a single test run. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is provided as two inactive recombinants, each targeting a unique assay on the BioFire Pneumonia Panel *plus*. The pooling scheme provided will give equivocal detections for MERS-CoV and is designed to reduce false positive detections. The procedures were developed using a Pneumonia Verification Panel available from ZeptoMetrix LLC, Buffalo, NY (NATPPA-BIO + NATPPQ-BIO + NATMR-BIO).

Note: The verification schemes described here are designed to reduce possible false positive MERS-CoV detections as a result of control material. MERS-CoV is provided as 2 unique recombinants that target specific assays on the BioFire® Pneumonia Panel *plus.* The pooling scheme described in Table 3 will result in Equivocal detections for MERS-CoV in pool 4 and 5. Documentation of MERS-CoV equivocal detections in both pool 4 and pool 5 infers a MERS-CoV positive detection.

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 Pneumonia Panel *plus*.

Note: The laboratory should only perform the verification study with analytes that will be reported using the BioFire Pneumonia Panel *plus* in their laboratory setting.

Table 1. Overview of Verification Protocols

Verification Protocol	Organisms per Pool	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results[a]	Expected Negative Results	Targeted Low Bin Results[b]	Targeted High Bin Results[c]	Approximate Days of Testing[d]
Synthetic Matrix Protocol	5, 6, or 7	5	4	20	≥4 per organism	8-16 per organism	32	36	4
Clinical Matrix Protocol	5, 6, or 7	5	4	20	≥4 per organism	8-16 per organism	32	36	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 *K. pneumoniae* strains; therefore the number of expected *K. pneumoniae* group positives would be 12 and the number of expected negatives would be 8.

[b] Targeted Low Bin Results represents bacterial detections in the semi-quantitative bins of 10^4-10^5 copies/mL

[c] Targeted High Bin results represent bacterial detections in the semi-quantitative bins of 10^6-≥10^7 copies/mL

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

Performance Verification: Materials

The following materials may be used to perform verification procedures:

Table 2. Recommended materials for verification protocol

Material	Part Number
BioFire® FilmArray Pneumonia Panel *plus* Kit (30 tests per kit)	BioFire Diagnostics, LLC (RFIT-ASY-0143)
BioFire® FilmArray Pneumonia *plus* Instruction for Use	BioFire Diagnostics, LLC (RFIT-PRT-0895)
BioFire® FilmArray Pneumonia Panel *plus* Quick Guide	BioFire Diagnostics, LLC (RFIT-PRT-0896)
Control Organism[a]	ZeptoMetrix NATPPA-BIO, NATPPQ-BIO, and NATMR-BIO
Transfer pipettes	VWR Part # 13-711-43 (or similar)
Bronchoalveolar lavage (BAL)-like specimens or Sputum-like specimens[b]	Sputum available from Discovery Life Science and Lee BioSolutions
Human genomic DNA (1mL of 0.2mg/mL) or equivalent[c]	Roche 11691112001
Sterile normal saline (0.85 - 0.9% sodium chloride)[c]	Medline PCS1650 or equivalent
5 mL or 10 mL[c] sample tubes	Various manufacturers

[a]Any appropriate source of organism may be used for verification of any or all of the assays in the BioFire Pneumonia Panel. However, when alternate organism sources are used (i.e. not the ZeptoMetrix material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.

[b]For use with the Clinical Matrix Protocol. BAL and sputum from various clinical or commercial sources may be used; the vendors listed may not be inclusive.

[c]Optional; needed for preparation aBAL matrix

Performance Verification: Protocols

Synthetic Matrix Protocol

The Synthetic Matrix Protocol evaluates BioFire® Pneumonia Panel *plus* performance when control material (ZeptoMetrix NATPPA-BIO, NATPPQ-BIO, and NATMR-BIO) is pooled and combined with a synthetic matrix. Examples of a synthetic matrix include the negative control provided in the control organism panel or Artificial Bronchoalveolar Lavage Matrix (aBAL). The proposed organism pooling scheme in Table 3 should be followed to obtain the expected results for each assay in a time and resource-efficient manner.

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

Figures 1 and 2 (below) illustrate protocol and workflow schemes for testing 4 replicates per pool for 5 pools over multiple days. This produces a total of 20 verification sample test runs and provides at least 4 positive results and as many as 16 negative results per assay, as well as 32 low bin and 36 high bin detections. Sample distribution in the targeted low and high bins are approximate and bin variation may occur due to sampling differences and run variability. The pooling scheme provides sufficient volume for testing more samples if desired. 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 modules configured on the BioFire® FilmArray® System.

Note: Target bin levels are relative and may vary between test runs.

Sample pools containing negative control or aBAL may 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 3. Proposed Organism Pooling Scheme

Organism	Target Bin Level[a]	Approximate organism volume	Synthetic or Clinical Matrix	Approximate Final Volume of Pool
Pool 1				
Acinetobacter baumanni	Low (10^4-10^5)	0.2 mL	1.2 mL	2.4 mL
Enterobacter cloacae	High (10^6-≥10^7)	0.2 mL		
Escherichia coli (IMP)	Low (10^4-10^5)	0.2 mL		
Klebsiella pneumoniae Z138 (OXA-48-like, CTX-M)	High (10^6-≥10^7)	0.2 mL		
Proteus mirabilis	Low (10^4-10^5)	0.2 mL		
Serratia marcescens	High (10^6-≥10^7)	0.2 mL		
Pool 2				
Klebsiella aerogenes	High (10^6-≥10^7)	0.2 mL	1.0 mL	2.0 mL
Klebsiella oxytoca	Low (10^4-10^5)	0.2 mL		
Klebsiella pneumoniae KPC-2 (KPC)	High (10^6-≥10^7)	0.2 mL		
Pseudomonas aeruginosa (VIM)	Low (10^4-10^5)	0.2 mL		
Streptococcus pyogenes	High (10^6-≥10^7)	0.2 mL		
Pool 3				
Haemophilus influenzae	High (10^6-≥10^7)	0.2 mL	1.2 mL	2.4 mL
Klebsiella pneumoniae Z460 (NDM/ CTX-M)	High (10^6-≥10^7)	0.2 mL		
Moraxella catarrhalis	Low (10^4-10^5)	0.2 mL		
Staphylococcus aureus (*mecA/C* and MREJ)	High (10^6-≥10^7)	0.2 mL		
Streptococcus agalactiae	Low (10^4-10^5)	0.2 mL		
Streptococcus pneumoniae	Low (10^4-10^5)	0.2 mL		
Pool 4				
Adenovirus Type 31	N/A	0.2 mL	1.4 mL	2.8 mL
Coronavirus NL63	N/A	0.2 mL		
Influenza A H3	N/A	0.2 mL		
MERS1 Recombinant	N/A	0.2 mL		
Parainfluenza virus Type 1	N/A	0.2 mL		
Respiratory Syncytial Virus (RSV) A2	N/A	0.2 mL		
Legionella pneumophila	N/A	0.2 mL		
Pool 5				
Adenovirus Type 3	N/A	0.2 mL	1.4 mL	2.8 mL
Human Metapneumovirus 8	N/A	0.2 mL		
Human Rhinovirus 1 A	N/A	0.2 mL		
Influenza B	N/A	0.2 mL		
MERS2 Recombinant	N/A	0.2 mL		
Chlamydia pneumoniae	N/A	0.2 mL		
Mycoplasma pneumoniae	N/A	0.2 mL		

[a]Target bin levels are relative and may vary between test runs

Synthetic Matrix Protocol Example

The estimated total time for completion for this verification example is 4 days for a BioFire® FilmArray® System configured with 1 module. A proposed organism pooling scheme is presented above in Table 3. Refer to Figure 1 for the suggested workflow.

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 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 BioFire® System.

Day 1: Synthetic Matrix Protocol

1. Organize materials needed (Table 2). If using Artificial Bronchoalveolar Lavage Matrix (aBAL), follow the optional protocol below to prepare the synthetic matrix before starting Step 2. aBAL should be screened with the BioFire Pneumonia Panel *plus* to characterize the sample prior to preparing pools.

2. Prepare one sample pool (i.e. Pool 1) from ZeptoMetrix NATPPQ-BIO and NATPPA-BIO control material. The MERS1 and MERS2 recombinants are contained in ZeptoMetrix NATMR-BIO and should be used to prepare Pools 4 and 5. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.

    a. Transfer 1.0, 1.2, or 1.4 mL of Synthetic Matrix (either ZeptoMetrix Negative or aBAL) into a 5 mL tube.
    b. Transfer the entire contents of the ZeptoMetrix organism vial (approximately 0.2 mL) into the larger tube containing Synthetic Matrix.
    c. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for the pool into a single tube. The total volume will be between 2.0 mL and 2.8 mL depending upon the pool.
    d. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for the remaining sample pools (i.e. Pools 2 and 3) to be prepared on Day 1.

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

Note: Follow instructions in the *BioFire® FilmArray® Pneumonia Panel plus Instructions for Use* and the *FilmArray® Pneumonia Panel plus Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for remaining sample replicates to be tested that day (i.e. replicates A and B for Pools 2 and 3).

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: Synthetic Matrix Protocol

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 (i.e. replicates C and D from Pools 1, 2, and 3) by repeating Step 4 above.

Day 3: Synthetic Matrix Protocol

Prepare 2 new sample pools (i.e. pools 4 and 5) as described in Day 1, Steps 2 and 3. Test replicates as described in Step 4 above.

Day 4: Synthetic Matrix Protocol

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

Optional Protocol: Preparation of Artificial Bronchoalveolar Lavage Matrix (aBAL)

Note: Approximately 13 mL of Artificial Bronchoalveolar Lavage (aBAL) are needed if used as synthetic matrix for all 5 pools as described in Table 3.

1. Thoroughly decontaminate the work area with 10% bleach followed by water wipes.

2. Prepare a 20 ng/uL solution of Human genomic DNA (hgDNA) by adding 1.0 mL hgDNA stock solution (0.2mg/mL) to 9 mL sterile saline in a 10 mL tube. Prepare a second tube if more than 10 mL is needed.

3. Mix by vortexing or inversion.

4. Characterize aBAL matrix by running a BioFire® Pneumonia Panel *plus* test prior to preparing organism pools. The BioFire Pneumonia Panel *plus* should test negative for all analytes on the panel.

5. Aliquots of aBAL matrix may be stored refrigerated at 4°C for up to 3 days or stored frozen at ≤ -70°C.

Note: It is important to characterize aBAL or clinical matrix specimens for BioFire Pneumonia Panel *plus* targets by screening the specimen on the BioFire Pneumonia Panel *plus* prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes tested on the BioFire Pneumonia Panel *plus*. If a negative specimen is not available, note the positives and take that into account when reviewing results.

Figure 1. Workflow for Synthetic Matrix Protocol

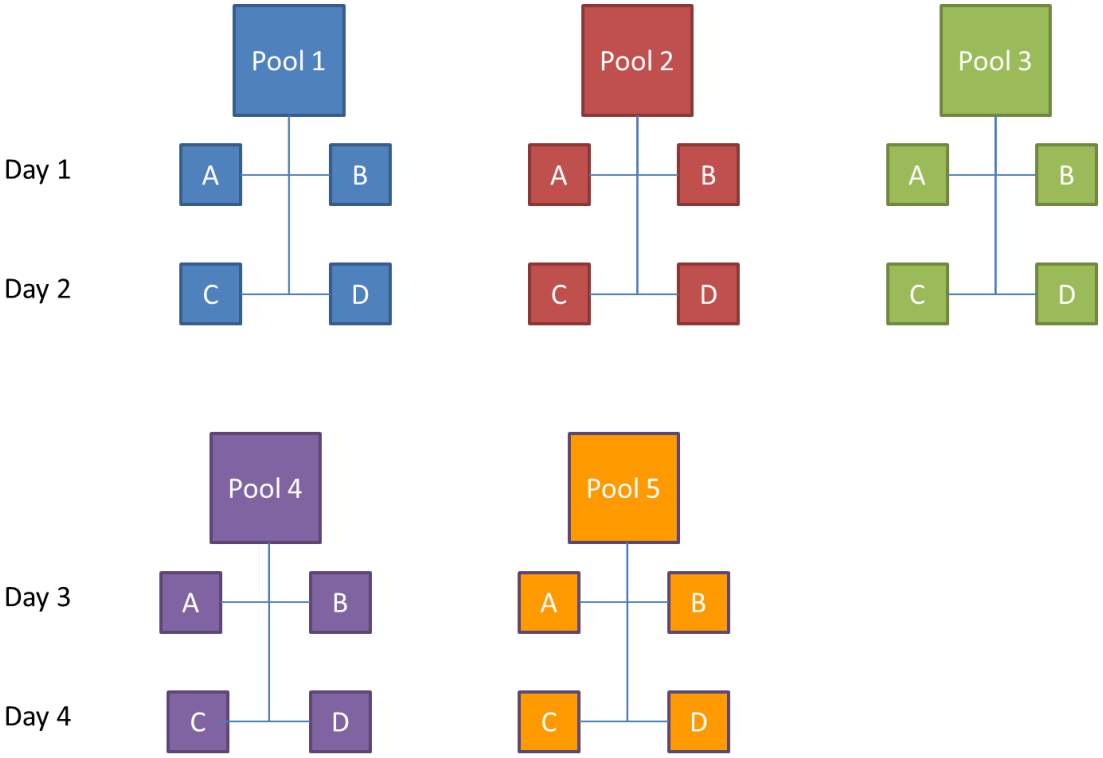

Figure 2. Example of Verification Workflows for use with Multiple BioFire modules.

2 modules	Module 1			Module 2		
Day 1	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 3 / Operator 1	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 3/ Operator 2
Day 2	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 3/ Operator 2	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 3 / Operator1
Day 3	Pool 4 / Operator 1	Pool 5 / Operator 2		Pool 4 / Operator 2	Pool 5 / Operator 1	
Day 4	Pool 4 / Operator 2	Pool 5 / Operator 1		Pool 4 / Operator 1	Pool 5 / Operator 2	

4 modules	Module 1		Module 2		Module 3		Module 4	
Day 1	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 3 / Operator 1		Pool 3/ Operator 2	
Day 2	Pool 3 / Operator1		Pool 3/ Operator 2		Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 1/ Operator 2	Pool 2/ Operator 1
Day 3	Pool 4 / Operator 1		Pool 4 / Operator 2		Pool 5 / Operator 2		Pool 5 / Operator 1	
Day 4	Pool 5 / Operator 1		Pool 5 / Operator 2		Pool 4 / Operator 1		Pool 4 / Operator 2	

6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1/ Operator 1	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 2/ Operator 2	Pool 3 / Operator 1	Pool 3/ Operator 2
Day 2	Pool 3/ Operator 2	Pool 3 / Operator1	Pool 1/ Operator 2	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 2/ Operator 1
Day 3			Pool 4 / Operator 1	Pool 4 / Operator 2	Pool 5 / Operator 1	Pool 5 / Operator 2
Day 4	Pool 5 / Operator 1	Pool 4 / Operator 2	Pool 5 / Operator 2		Pool 4 / Operator 1	

Clinical Matrix Protocol

The Clinical Matrix Protocol evaluates BioFire® FilmArray® Pneumonia Panel *plus* performance in a clinical specimen matrix. The laboratory will require a clinical specimen (induced or expectorated sputum, endotracheal aspirates or bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL)) appropriate for the laboratory's verification needs and, in a volume, sufficient for testing as described in Table 3. The number of different sample types to be tested is up to the discretion of the laboratory director; multiple specimens may be used, if necessary. Clinical specimens should be screened in the BioFire Pneumonia Panel *plus* in order to characterize the sample.

Note: It is important to characterize aBAL or clinical matrix specimens for BioFire Pneumonia Panel *plus* targets by screening the specimen on the BioFire Pneumonia Panel *plus* prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes tested on the BioFire Pneumonia Panel *plus*. If a negative specimen is not available, note the positives and take that into account when reviewing results.

Control material (ZeptoMetrix NATPPA-BIO, NATPPQ-BIO, and NATMR-BIO) is pooled and added to an equal volume of clinical matrix. The proposed organism pooling scheme (Table 3) should be followed to obtain the expected results for each assay in a time and resource-efficient manner. Specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated.

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

Figures 1 and 2 (above) illustrate protocol and workflow schemes for testing 4 replicates per pool for 5 pools over multiple days. This produces a total of 20 verification sample test runs and provides 4 positive results and 16 negative results per assay, as well as 32 low bin and 36 high bin detections. Sample distribution in the targeted low and high bins are approximate and bin variation may occur due to sampling differences and run variability. The pooling scheme provides sufficient volume for testing more samples if desired. 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 BioFire® System.

Note: Target bin levels are relative and may vary between test runs.

Pooled samples may be stored overnight at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation; but all tests should be completed within a day. To evaluate user-to-user variation, multiple laboratory technicians may perform testing.

Note: Clinical specimens may contain inhibitors and enzymes which may degrade the organism mixture and lead to unpredictable results. It is important to prepare the organism pools and test samples within a day to achieve expected results.

Clinical Matrix Protocol Example

The estimated total time for completion for the Clinical Matrix Protocol verification example is 4 days for a BioFire® FilmArray® System configured with 1 instrument/module.

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

Day 1: Clinical Matrix Protocol

1. Organize materials needed (Table 2.) Clinical specimens should be screened in the BioFire® Pneumonia Panel *plus* in order to characterize the sample prior to preparing pools.

2. Prepare one sample pool (i.e. Pool 1) from ZeptoMetrix NATPPQ-BIO and NATPPA-BIO control material. The MERS1 and MERS2 recombinants are contained in NATMR-BIO and should be used to prepare Pools 4 and 5. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.

    a. Transfer 1.0, 1.2, or 1.4 mL of Clinical Matrix into a 5 mL tube. Specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated. Organism to matrix ratios that differ from recommendations in Table 3 may affect detection results.
    b. Transfer the entire contents of the ZeptoMetrix organism vial (approximately 0.2 mL) into the larger tube containing clinical specimen matrix.
    c. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The total volume will be between 2.0 mL and 2.8 mL depending upon the pool.
    d. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for remaining sample pools (i.e. Pools 2 and 3) to be prepare on Day 1.

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

Note: Follow instructions in the *FilmArray® Pneumonia Panel plus Instructions for Use* and the *FilmArray® Pneumonia Panel plus Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for remaining sample replicates to be tested that day (i.e. replicates A and B for Pools 2 and 3).

Note: The proposed organism pooling scheme (Table 3) provides sufficient material for running samples as described in Figure. 2. The volume is sufficient for testing more samples if desired.

Day 2: Clinical Matrix Protocol

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

Day 3: Clinical Matrix Protocol

Prepare 2 new sample pools (i.e. Pools 4 and 5) as described in Day 1, Steps 2 and 3. Test replicates according to Step 4 above.

Day 4: Clinical Matrix Protocol

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

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 enough material to complete many tests for each pool. The verification study may use induced or expectorated sputum, endotracheal aspirates or bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL) as a clinical matrix in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960.

Verification of a Loaner, Repaired, and Permanent Replacement Instruments or Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument or module, 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® Pneumonia Panel *plus*. 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 Pneumonia Panel *plus*.
3. Test the selected specimens/samples/controls on the loaner, repaired, or permanent replacement instrument or module 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® FilmArray® Pneumonia *plus* Panel Verification Record Page 1

Bacteria Reported in Bins – Pools 1-3

BioFire® FilmArray® Pneumonia Panel *plus* Verification Record

Kit Part #	Module Serial #	Module Serial #	Module Serial #
Lot #	Module Serial #	Module Serial #	Module Serial #

Organism and Resistance Genes		Replicate Testing																				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	5-A	5-B	5-C	5-D	# Positives	# Negatives	Bin (copies/mL) Low Bin # 10^4	Low Bin # 10^5	High Bin # 10^6	High Bin # ≥10^7	# Operators	# Days	# Modules	Patient Samples?
Pool 1																															
Acinetobacter calcoaceticus-baumannii complex																															
Enterobacter cloacae complex																															
Escherichia coli	IMP																														
Klebsiella pneumoniae group Z138	CTX-M																														
	OXA-48 like																														
Proteus spp.																															
Serratia marcescens																															
Pool 2																															
Klebsiella aerogenes																															
Klebsiella oxytoca																															
Klebsiella pneumoniae group KPC-2	*KPC*																														
Pseudomonas aeruginosa	*VIM*																														
Streptococcus pyogenes																															
Pool 3																															
Haemophilus influenzae																															
Klebsiella pneumoniae group Z460	NDM																														
	CTX-M																														
Moraxella catarrhalis																															
Staphylococcus aureus	*mecA/C and MREJ*																														
Streptococcus agalactiae																															
Streptococcus pneumoniae																															

Summary of Detections for Bacteria Reported in Bins

Total number of Reported Detections: Positives, Negatives, and Individual Bins	# Positives	# Negatives	# 10^4	# 10^5	# 10^6	# ≥ 10^7

Total number of High (10^4-10^5) and Low (10^4-10^5) Bin Detections	Low Bin total	High Bin total

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
FLM1-PRT-0264-04

QS-339H-01

BioFire® FilmArray® Pneumonia Panel *plus* Verification Record- Page 2

Atypical Bacteria and Viruses– Pools 4-5

BioFire® FilmArray® Pneumonia Panel *plus* Verification Record

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

Lot # **Module Serial #** **Module Serial #** **Module Serial #**

Organism and Resistance Genes		Replicate Testing																				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	5-A	5-B	5-C	5-D	# Positives	# Negatives	Bin (copies/mL) Low Bin # 10^4	Bin (copies/mL) Low Bin # 10^5	Bin (copies/mL) High Bin # 10^6	Bin (copies/mL) High Bin # ≥10^7	# Operators	# Days	# Modules	Patient Samples?
Pool 4																														
Adenovirus	Type 31																							N/A						
Coronavirus	NL63																							N/A						
Influenza A	H3																							N/A						
Middle East Respiratory Syndrome Coronavirus*	MERS1 recombinant																													
Parainfluenza virus	Type 1																							N/A						
Respiratory Syncytial Virus	RSV A2																							N/A						
Legionella pneumophila																								N/A						
Pool 5																														
Adenovirus	Type 3																							N/A						
Human Metapneumovirus	8																							N/A						
Human Rhinovirus/Enterovirus	Rhinovirus 1 A																							N/A						
Influenza B																								N/A						
Middle East Respiratory Syndrome Coronavirus*	MERS2 recombinant																													
Chlamydia pneumoniae																								N/A						
Mycoplasma pneumoniae																								N/A						
Summary of Detections for Atypical Bacteria and Viruses: Total number of Positves and Negatives																														

*MERS 1 and MERS 2 tested in separate pools will each give an Equivocal detection. A MERS Equivocal detection in Pool 4 **and** Pool 5 infers a Positive detection.

Reviewed by: _____

Signature **Date**