Development of a Laboratory Verification Protocol for Qualitative and Semi-Quantitative Detections in a Multiplex Syndromic Pneumonia Panel

ASM Microbe 2019
Saturday-CPHM-940

M. Cronin[1], B. Hoelcle[2], R. Ellingson[1], K. Ortiz[1], A. Clark[1], K. Nielsen[1], R. Young[1], D. Phillips[1], L. Ogden[1], J. Green[1], S. Thatcher[1]

[1]BioFire Diagnostics, LLC, Salt Lake City, UT, USA; [2]ZeptoMetrix® Corporation, Buffalo, NY, USA

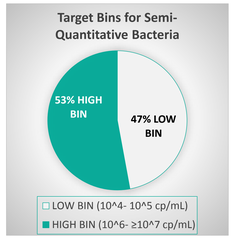
Monica Cronin
BioFire Diagnostics, LLC
Monica.Cronin@BioFireDx.com

Background

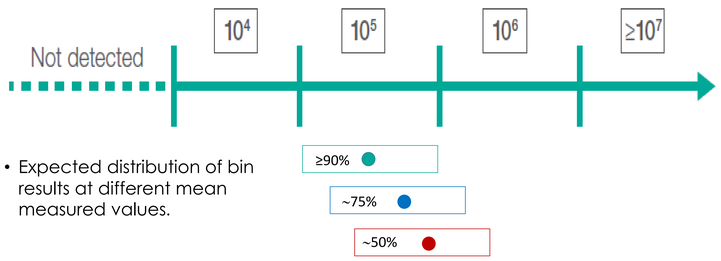
Verification and quality control (QC) are critical components of implementing a diagnostic test in a clinical laboratory and may be time-consuming and costly. The BioFire® FilmArray® Pneumonia Panel and the BioFire® FilmArray® Pneumonia Panel *plus* identify 33 or 34 (respectively) clinically relevant viral and bacterial targets and 7 antibiotic resistance markers from sputum or bronchoalveolar lavage samples. Each test includes a controlled Quantified Standard Material (QSM) that is co-processed with the sample allowing for accurate semi-quantitative reporting in log level bins representing approximately 10^4, 10^5, 10^6, or ≥10^7 copies/mL (cp/mL) of specimen for 15 bacteria. This allows determination of relative abundance and may aid in differentiating pathogens from colonizers. A protocol was developed using control material designed in collaboration with ZeptoMetrix® Corporation to verify both qualitative and semi-quantitative detections for efficient system verification and QC.

External Control Strategy

Verification	• All panel organisms • Positives and negatives • Semi-Quantitative bacterial detections: half high bin, half low bin
Internal control acceptability	• Repeated testing of high vs. low organism levels as part of IQCP to demonstrate the lab and the reagent Quantified Standard Material can repeatedly distinguish between high and low bins
Quality Control	• New lot or shipment • All targets positive or negative • If necessary for IQCP, rotate testing of high/low organisms



Model for Precision of the BioFire Pneumonia Panel *plus* Bin Results



- Expected distribution of bin results at different mean measured values.

- The precision of the bin result will vary based upon the bacterial nucleic acid concentration and its proximity to the bin boundary.
- Bin variability may also occur as a result of sample handling, matrix effects (homogeneity, inhibitors, enzymes), and normal variability in manufacturing of verification materials.

BioFire® FilmArray®
Pneumonia Panel *plus*

BACTERIA
Semi-Quantitative Bacteria
Acinetobacter calcoaceticus-baumannii complex
Enterobacter cloacae complex
Escherichia coli
Haemophilus influenzae
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae group
Moraxella catarrhalis
Proteus spp.
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

ATYPICAL BACTERIA
Qualitative Bacteria
Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

VIRUSES
Adenovirus
Coronavirus
Human Metapneumovirus
Human Rhinovirus/Enterovirus
Influenza A
Middle East Respiratory Syndrome Coronavirus (MERS-CoV) *
Influenza B
Parainfluenza Virus
Respiratory Syncytial Virus

ANTIMICROBIAL RESISTANCE GENES
Carbapenemases
IMP
KPC
NDM
OXA-48-like
VIM
ESBL
CTX-M
Methicillin Resistance
mecA/C and MREJ (MRSA)

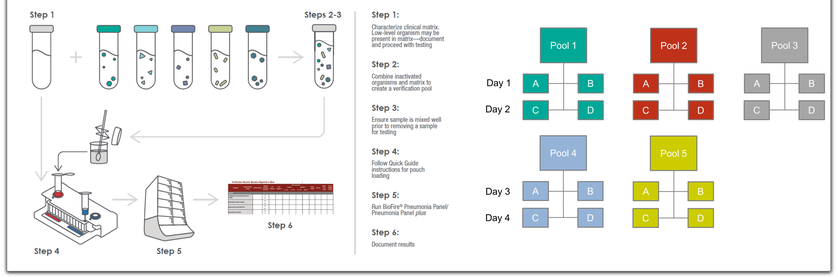
Sample type: Sputum, Endotracheal aspirate, Bronchoalveolar lavage, and mini-BAL

- **MERS-CoV Available on the BioFire Pneumonia Panel *plus* only**

The BioFire Pneumonia Panel *plus* is only available outside the United States

Laboratory Verification Workflow for the BioFire Pneumonia Panel *plus*

- Each assay is evaluated by testing the pooled material in a clinical or synthetic matrix background.
- Day-to-day variation is evaluated by testing samples on multiple days.
- User-to-user variation may be evaluated by having multiple technicians test the same samples.
- Test material should be evenly distributed among the instruments or modules.
- Patient samples (ex. Sputum, ETA, or BAL) should also be tested as part of the verification.



Methods

The protocol was optimized using prototype NATtrol™ controls from ZeptoMetrix® Corporation, bronchoalveolar lavage, sputa and the BioFire® FilmArray® 2.0 and the BioFire® FilmArray® Torch Systems. Control materials were tested in the presence of clinical matrix or negative control. The pooling scheme was designed to generate both qualitative and semi-quantitative detections of high and low levels to demonstrate that the system can quantify a range of target levels and provide multiple bin detections in the same test.

BioFire Pneumonia Panel *plus* Laboratory Verification Results

Qualitative Results for Viruses and Atypical Bacteria

NATPPA-BIO and NATMR-BIO	Positives	Negatives	Concordance
Adenovirus	44/44	86/86	100%
Chlamydia pneumoniae	22/22	108/108	100%
Coronavirus	22/22	108/108	100%
Human Metapneumovirus	22/22	108/108	100%
Human Rhinovirus/Enterovirus	22/22	108/108	100%
Influenza A	22/22	108/108	100%
Influenza B	22/22	108/108	100%
Legionella pneumophila	22/22	108/108	100%
MERS-CoV-1*	22/22	108/108	100%
MERS-CoV-2*	22/22	108/108	100%
Mycoplasma pneumoniae	22/22	108/108	100%
Parainfluenza virus	22/22	108/108	100%
Respiratory Syncytial Virus	22/22	108/108	100%

*Middle East Respiratory Syndrome Coronavirus (MERS-CoV) provided as two synthetic constructs that report "equivocal" detections when tested separately.

Qualitative Results for Antibiotic Resistance Markers

NATPPQ-BIO	Positives	Negatives	Concordance
CTX-M	64/64	66/66	100%
IMP	32/32	98/98	100%
KPC	22/22	108/108	100%
mecA/C and MREJ	32/32	98/98	100%
NDM	32/32	98/98	100%
OXA-48 like	32/32	98/98	100%
VIM	20/22	110/108	90.9%

A total of 130 BioFire Pneumonia Panel *plus* tests were performed using the protocol developed.
- **Expected positives: 798/802 (99.5%)**
- **Expected negatives: 2702/2716 (99.5%)**
- **Antibiotic resistance markers: correctly identified in 234/236 replicates when a correlated bacteria was present**
- **HIGH/LOW bin differentiation in 130/130 tests (100%)**

Semi-Quantitative Results for Bacteria Reported in Bins

NATPPQ-BIO	Positives	Negatives	Concordance	Target bin	LOW		HIGH		Target Bin Concordance
					10^4	10^5	10^6	≥10^7	
Acinetobacter calcoaceticus-baumanni complex[a]	30/32	100/98	93.8%	LOW	14	16	0	0	93.8%
Enterobacter aerogenes	22/22	108/108	100%	HIGH	0	0	4	18	100%
Enterobacter cloacae	32/32	98/98	100%	HIGH	0	1	27	4	96.9%
Escherichia coli (IMP)	32/32	98/98	100%	LOW	3	29	0	0	100%
Haemophilus influenzae	22/22	98/98	100%	HIGH	0	0	6	26	100%
K. pneumoniae (KPC-2)	22/22	44/44	100%	HIGH	0	0	19	3	100%
K. pneumoniae Z138 (CTX, OXA)	32/32		100%	HIGH	0	0	11	21	100%
K. pneumoniae Z460 (CTX, NDM)	22/22		100%	HIGH	0	0	24	8	100%
Klebsiella oxytoca	22/22	108/108	100%	LOW	0	22	0	0	100%
Moraxella catarrhalis	22/22	98/98	100%	LOW	21	11	0	0	100%
Proteus spp.	22/22	98/98	100%	LOW	2	30	0	0	100%
Pseudomonas aeruginosa (VIM)[b]	20/22	110/108	90.9%	LOW	13	7	0	0	90.9%
Serratia marcescens	32/32	98/98	100%	HIGH	0	0	5	27	100%
Staphylococcus aureus (MRSA)	22/22	98/98	100%	HIGH	0	0	8	24	100%
Streptococcus agalactiae	22/22	98/98	100%	LOW	12	19	1	0	96.9%
Streptococcus pneumoniae	22/22	98/98	100%	LOW	12	16	4	0	87.5%
Streptococcus pyogenes	22/22	108/108	100%	HIGH	0	4	18	0	81.8%

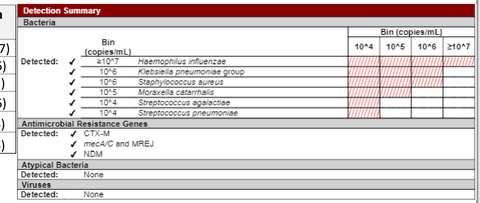
[a] 2 missed detections in sputum background on 2nd day of testing, organism degradation suspected
[b] 2 missed detections due to under filled tubes

ZeptoMetrix® Pneumonia Panel NATtrol™ Controls are packaged to allow maximum flexibility for laboratory validation and QC needs
NATPPQ-BIO -semi-quantitative bacteria/antibiotic resistance markers at log level 10^4–10^5 cp/mL (Low) or 10^6– 10^7 cp/mL (High) levels
NATPPA-BIO - qualitative viruses and atypical bacteria
NATMR-BIO - synthetic MERS-CoV for qualitative detection (for use with BioFire Pneumonia Panel *plus* only)

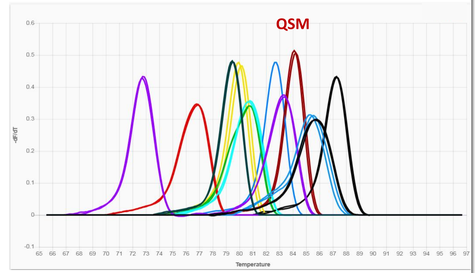
Detection of High and Low Levels of Semi-Quantitative Bacteria Within a Single Test

Pool 3- Pooling scheme for semi-quantitative bacteria, BioFire Pneumonia Panel *plus* report and Melt curves

ZeptoMetrix Organism	Target Level	Actual Bin Detection
Haemophilus influenzae	HIGH	HIGH (>10^7)
K. pneumoniae Z460 (CTX-M/NDM	HIGH	HIGH (10^6)
Moraxella catarrhalis	LOW	LOW (10^5)
Staphylococcus aureus (MRSA)	HIGH	HIGH (10^6)
Streptococcus agalactiae	LOW	LOW (10^4)
Streptococcus pneumoniae	LOW	LOW (10^4)



- An internal Quantified Standard Material (QSM) of known concentration is present in every test.
- The QSM and specimen are co-processed resulting in accurate semi-quantitative reporting in log level bins of 10^4, 10^5, 10^6 or ≥ 10^7 copies/mL.
- LOW bin - 10^4- 10^5 copies/mL
- HIGH bin - 10^6- ≥ 10^7 copies/mL
- The pooling scheme combines bacteria at different target levels to demonstrate that HIGH vs LOW prevalence can be distinguished in a single test run.
- Semi-quantitative reporting may help determine whether the reported bacteria is a colonizer or a pathogen.



Conclusions

- Efficient system verification is achieved by combining 30 organisms and 7 antibiotic resistance markers into 5 pools and can be completed with 20 test runs in 4 days.
- The pooling scheme provides multiple positive and negative detections for every target and sufficient material for running as many as 10 tests per pool.
- The workflow may be modified or expanded to meet a laboratory's specific criteria.
- The protocol accurately detects antibiotic resistance markers and consistently reports distinct HIGH and LOW organism levels in the same test for the 15 semi-quantitative bacteria.
- The protocol and controls serve as a useful tool for providing reliable detections of qualitative and semi-quantitative targets over multiple days, users and systems and offers a flexible solution for supporting verification or QC needs.
- The verification materials are packaged to allow maximum flexibility for meeting the laboratory's validation, quality control and IQCP needs.