Precision of the FilmArray® Pneumonia Panel

Considerations for Interpreting Relative Abundance of Bacterial Nucleic Acids in Lower Respiratory Specimens

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Introduction

Routine evaluation of lower respiratory specimens for diagnosis of pneumonia and other important lower respiratory tract infections involves various testing paradigms to identify the microorganisms that may be responsible for the infection. Though molecular methods may be used for viral detection, culture remains the gold standard for diagnosis of bacterial infection from lower respiratory specimens. Quantitative and/or semi-quantitative culture can help distinguish clinically relevant bacteria from colonizing bacteria or normal oral flora based on the abundance of single or multiple bacteria cultured from the specimen[1]. However, culture can be a time-consuming (~24-48 hours) and insensitive procedure, particularly for fastidious bacteria or when evaluating specimens collected after empiric antibiotic therapy has been administered.

The FilmArray® Pneumonia Panel is a molecular, multiplex device that can rapidly identify viruses, bacteria, and antimicrobial resistance genes in sputum-like and bronchoalveolar lavage (BAL)-like specimens obtained from individuals with signs of a lower respiratory tract infection (**Table 1**).

Table 1. Analytes Detected by the BioFire FilmArray Pneumonia Panel

Bacteria		
Acinetobacter calcoaceticus-baumannii complex	*Klebsiella oxytoca*	*Serratia marcescens*
Enterobacter aerogenes	*Klebsiella pneumoniae* group	*Staphylococcus aureus*
Enterobacter cloacae complex	*Moraxella catarrhalis*	*Streptococcus agalactiae*
Escherichia coli	*Proteus* spp.	*Streptococcus pneumoniae*
Haemophilus influenzae	*Pseudomonas aeruginosa*	*Streptococcus pyogenes*
Antimicrobial Resistance Genes		
CTX-M	IMP	KPC
NDM	OXA-48-like	VIM
mecA/C and MREJ		
Atypical Bacteria		
Chlamydia pneumoniae	*Mycoplasma pneumoniae*	*Legionella pneumophila*
Viruses		
Adenovirus	Human Rhinovirus/Enterovirus	Parainfluenza Virus
Coronavirus	Influenza A	Respiratory Syncytial Virus
Human Metapneumovirus	Influenza B	

For bacteria, the panel provides an estimate of nucleic acid abundance for 15 different groups or species. The estimate is based on real-time PCR relative to an internal standard material of known quantity and is reported in bins over a relevant range from 10^4 to ≥10^7 copies/mL. Each bin represents a 1-log range of values with a discrete lower and upper limit. (**Figure 1**).

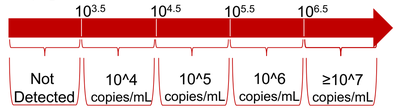

Figure 1. Schematic of FilmArray Pneumonia Panel Bins and Bin Limits

The objective of the bin result is to provide simple and accurate information about the relative abundance of bacteria in lower respiratory specimens in a manner that is similar in concept to current quantitative and semi-quantitative culture-based practices, yet faster and more sensitive (**Figure 2**).

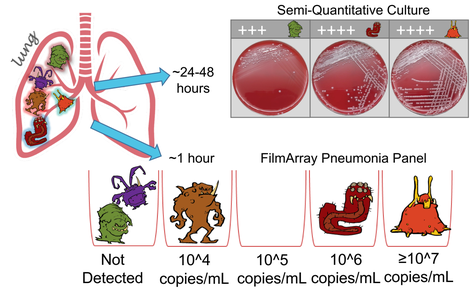

Figure 2. Approaches to Diagnosis of Bacterial Lower Respiratory Tract Infections: Semi-quantitative Culture and FilmArray Pneumonia Panel

When evaluating and validating the performance of the FilmArray Pneumonia Panel, it is important to recognize that the precision (repeatability and/or reproducibility) of the bin results is a function of the bin structure. Precision will vary depending on the quantity of bacterial nucleic acid measured and its relation to the upper and lower limits of the bins (see model in **Figure 4**). An evaluation of the predicted and observed precision of bin results is described in this poster.

Materials and Methods

To evaluate the precision of bin results, testing was performed with contrived BAL-like samples consisting of multiple bacteria titrated in 1-log dilutions (**Figure 3**; ~10^2 - 10^7 copies/mL of bacterial genomic DNA, measured by digital PCR). Samples were tested repeatedly over multiple days by different operators using different FilmArray systems (FilmArray, FilmArray 2.0 and FilmArray Torch) and instruments, as well as three different Investigational Use Only (IUO) reagent lots.

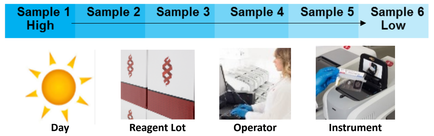

Figure 3. Variables Included in the Evaluation of Bin Precision for Bacteria Detected by the FilmArray Pneumonia Panel

Reference: [1] Garcia, Lynne S. *Clinical Microbiology Procedures Handbook, 3rd Edition*. Washington, D.C. American Society of Microbiology, 2010.

Model for Bin Result Precision

When tested repeatedly, different measured values within the range of a bin will exhibit variable precision of the reported bin results. Bin precision may be as low as 50% for values at a bin limit and precision will increase (up to 90% or higher) as the distance of the measured value from a bin limit increases. If the bin size is at least two standard deviations (2σ) of the measured value, then the precision of bin results will follow the model illustrated in **Figure 4**:

- >90% at a bin center (Scenario 1)
- ~60 – 90% between a bin limit and bin center (Scenario 2)
- ~50% at bin limits (Scenario 3)

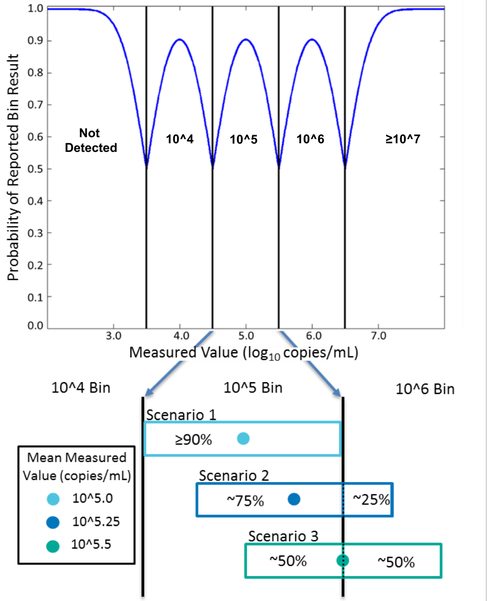

Figure 4. Model for Precision of FilmArray Pneumonia Panel Bin Results
Top: The probability of the same bin results for each replicate tested varies based on proximity of the measured value to a bin limit.
Bottom: Expected distribution of bin results at different mean measured values.

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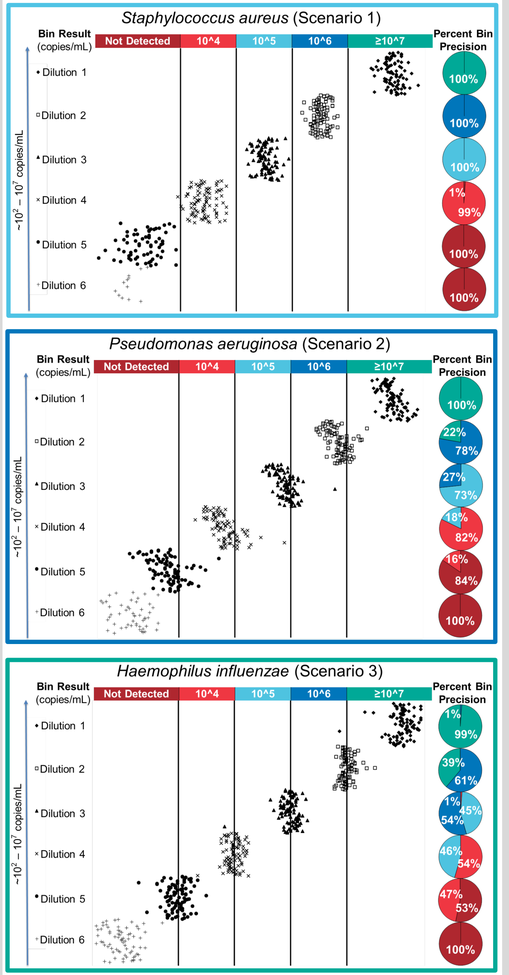
Precision of Bin Results



Summary and Conclusion

In the **Precision of Bin Results** section, the distribution of measured values (data points) and % bin precision (pie charts) for 90 replicates of three bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa,* and *Haemophilus influenzae*) at six different dilutions is shown. Results for twelve additional bacteria are shown in **Figure 5** and demonstrate, that the FilmArray Pneumonia Panel bin results have the expected precision of ~50% to >90% for all bins over the reportable range of the device.

Figure 5. Summary of the Precision of Bin Results for Bacteria Detected by the FilmArray Pneumonia Panel Reported percent (of 90 replicates) is graphed as a bar in each bin (x-axis) at each dilution tested (y-axis).

The FilmArray Pneumonia Panel is a molecular, multiplex device developed to identify bacterial and viral pathogens of lower respiratory tract infections and to rapidly and reliably report the approximate abundance of bacterial nucleic acids (±1-log copies/mL) in polymicrobial BAL and sputum-like specimens. Reporting results in bins with discrete limits dictates that at some concentrations, bin precision will be low (~50%) and at other concentrations, bin precision will be high (>90%). Variability in the precision of results should be considered when establishing criteria for device performance evaluations, verifications, and quality control procedures.

Note: The FilmArray Pneumonia Panel and the FilmArray Pneumonia Panel *plus*, which includes the detection of Middle East Respiratory Syndrome Coronavirus (MERS-CoV), have not been evaluated by the US FDA or other regulatory agencies.