21120.8062 Matrix Qualification Report to Verify the Performance Characteristics of the BioFire FilmArray® Respiratory Panel Assay using Nasopharyngeal and Oropharyngeal Swab Specimens in Universal Transport Med

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Initial version

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Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
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1.0	Approved and Current	Initial version	16-May-2019	20-May-2019	Indefinite

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Matrix Qualification Report to Verify the Performance Characteristics of the BioFire FilmArray® Respiratory Panel Assay using Nasopharyngeal and Oropharyngeal Swab Specimens in Universal Transport Media (UTM)

A. Introduction / Objective

This verification report was intended to provide documented evidence of the diagnostic accuracy and intra-inter assay precision of a multiplexed nucleic acid test intended for use with the BioFire FilmArray Instrument for the qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids using nasopharyngeal (NP) and oropharyngeal (OP) swabs.

B. Scope

The intended use of the assay was for the qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids using nasopharyngeal swabs (NPS) and oropharyngeal swabs (OP).

The test has the ability to identify the following organisms:

Adenovirus (ADV)
Coronavirus (CoV - 229E, HKU1, NL63, OC43)
Enterovirus (EV)
Human Rhinovirus (HRV)
Human Metapneumovirus (hMPV)
Influenza A (Flu A) subtypes H1, H1-2009 and H3
Influenza B (Flu B)
Parainfluenza Virus 1 (PIV1)
Parainfluenza Virus 2 (PIV2)
Parainfluenza Virus 3 (PIV3)
Parainfluenza Virus 4 (PIV4)
Respiratory Syncytial Virus (RSV)
Bordetella pertussis
Chlamydophila pneumoniae
Mycoplasma pneumoniae

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Table 1. Summary of verification criteria for Verification of BioFire FilmArray Respiratory Panel

Performance Characteristic	Acceptance Criteria	Results	Pass/Fail
(b) (4)			Passed
Precision Assay Reproducibility	(b) (4)		Passed
Accuracy			Passed

(b) (4)

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C. Materials

The following materials (or suitable equivalents) were used:

- FilmArray System including:
  - BioFire FilmArray Instrument
  - FilmArray Respiratory Panel Pouch Kit (BioFire Diagnostics, Inc.; cat# RFIT-ASY-0124)
  - FilmArray Pouch Loading Station
- ZeptoMetrix NATrol Respiratory Verification Panel, cat# NATRVP-IDI.
- Universal Viral Transport Media (3 mL), with flock, (b) (4), cat# (b) (4)
- For validating nasopharyngeal and oropharyngeal swabs, (b) (4) were used in this qualification. (b) (4)

(b) (4)

D. Methods

General methods

The FilmArray Respiratory Panel pouch is a closed, disposable system that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple respiratory pathogens within a single specimen. Sample preparation followed BioFire *"Protocols for Laboratory Verification of Performance of the FilmArray® Respiratory Panel (RP) EZ" technical note, specifically the "Simple Protocol" option and adapted for FilmArray Respiratory Panel.*

The FilmArray Respiratory Panel was run according to SOP *21120.2380 Procedure for Biofire FilmArray Respiratory Panel and Pneumonia Panel*

Performance Verification: Organism Pooling

Table 2. Overview of Verification Protocols

Verification Protocol	Organisms per Pool	Number of Sample Pools	Replicates Per Sample Pool	Pouches Required	Expected Positive Results	Expected Negative Results	Approximate Days of Testing
Protocol for 1 instrument	2 or 3	7	4	28	4 per organism	24 per organism	6(b) (4)

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The protocol described below utilize samples prepared by pooling together 2 or 3 different organisms (ZeptoMetrix NATRVP-IDI control organism).

The pooling scheme (Table 2) was designed so that both positive and negative target results can be obtained in a time and resource-efficient manner.

Table 3. Organism Pooling Scheme

Organism	Approximate volume	Approximate Final Volume of Pool
Pool 1		
(b) (4)	0.6 mL	1.2 mL
	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		
(b) (4)	0.6 mL	1.8 mL
	0.6 mL	
	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		
Bordetella pertussis	0.6 mL	1.8 mL
Chlamydophila pneumoniae	0.6 mL	
Coronavirus HKU1	0.6 mL	

[1]In the validation protocol Table 3, (b) (4) Their correct locations in Pools 1 and 4 are shown in the above version of Table 3 in agreement with manufacturer documentation (*"Protocols for Laboratory Verification of Performance of the FilmArray® Respiratory Panel (RP) EZ" technical note, specifically the "Simple Protocol" option and adapted for FilmArray Respiratory Panel)*.

(b) (4)

NOTE: Samples were prepared by R&D personal, blinded and transferred to Clinical Laboratory operators to run the samples

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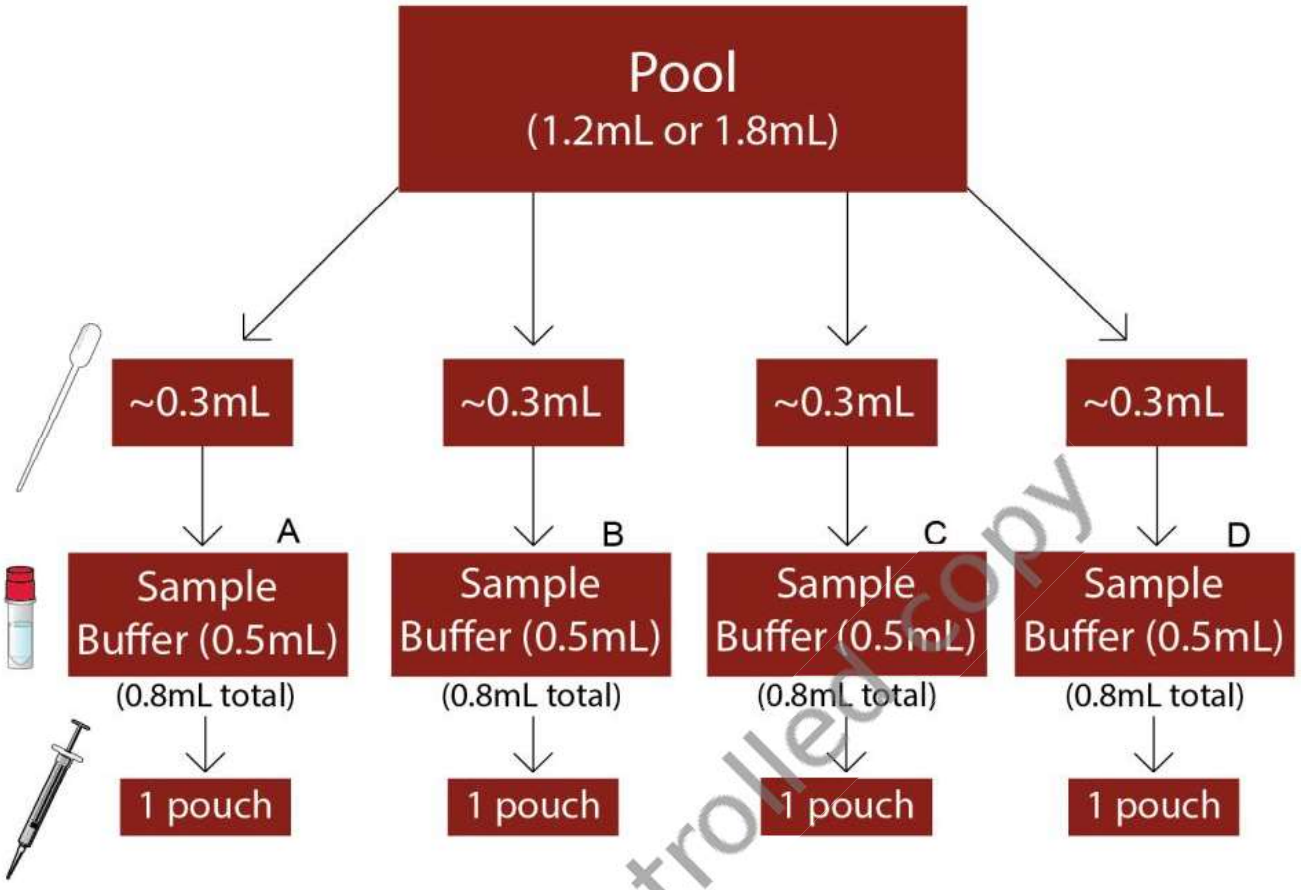

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Figure 1. Each sample pool had enough material for four tests (A-D) to assess variability from day to day and user to user. Workflow was repeated for each sample pool until all testing was completed.

A verification protocol with a schedule is outlined below.

Day 1

1. Samples from ZeptoMetrix NATRVP-IDI control material were pooled and prepared according to Table 3.
   a. A pipette was used to remove the entire contents from two vials per pathogen of the ZeptoMetrix organism vial (approximately 1.2 mL x 2 vials) and transfer to a new vial or tube.
   b. A second (and/or third) organism was combined with one or two more organisms, according to Table 3, into a single vial or tube (approximately 2.4 mL total volume for two organisms or 3.6 mL for three organisms).
   c. The pooled sample was mixed by vortexing to ensure was fully mixed.
   d. (b) (4)
      (b) (4)
   e. (b) (4)
      (b) (4)

Note: It was important to prepare only the number of sample pools that were tested within 3 days of preparation. The suggestion to prepare 3 sample pools on day 1 was based on an (b) (4) testing (b) (4) The number of samples prepared were based on the laboratory's work schedule.

Note: Pool samples were stored at refrigeration temperature (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

2. Four aliquots from a single pool (i.e. pool #1) were prepared to be tested (i.e. A and B, see Figure 1) on Day 1 and 2. All sample pools were randomized and blinded to the final operators. For each sample:
   a. Instructions from the FilmArray Respiratory Panel Instruction Booklet were followed for pouch preparation and pouch hydration.
   b. Preparation of sample mix was as follow: Use the Transfer Pipette provided with the FilmArray RP kit and draw from sample pool (approximately 0.25–0.3 mL). Add sample to the red-capped Sample Buffer vial and gently pipette up and down to mix.
   c. Instructions from the FilmArray Respiratory Panel Instruction Booklet were followed for sample loading and FilmArray RP testing.

3. Step 2 was repeated for the remaining sample pools (i.e. pools #2, #3 and #4) to be tested that day.

Day 2

To evaluate day-to-day variation, the remaining aliquots were tested from the same sample pools prepared on Day 1.

Day 3

On day 3, 4 new sample pools were prepared (i.e. pools #4, #5, #6, and #7) as described in Step 1 and test according to Step 2.

Day 4

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

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E. Performance Characteristics Evaluation - Verification Testing

Each sample pool had enough material for four tests (A-D) to assess variability from day to day and user to user. Workflow was repeated for each sample pool until all testing was completed. At least two different scientists participated in the verification.

F. Result interpretation

Positive results were reported as Detected.
Negative results were reported as Not Detected.

G. Run Acceptance Criteria

The acceptance criteria for all the studies was on qualitative results, Detected or Not Detected.

- (b) (4) of all specimens expected to be positive were detected.
- (b) (4) of all specimens expected to be negative were non-detected.

H. Results and conclusions

Overview. A total of 19 different pathogens were organized in 7 different pools according to Table 3 and then tested for the presence (detected) or absence (non-detected) of the interrogated analyte in the sample. The pathogen results are shown in Table 4. In addition, a set of negative ZeptoMatrix panel samples (Table 5) and negative OP/UTM (Table 6) were tested. Results from all samples are summarized in Tables 4 through 6. 100% of observed results are in concordance with expected results. The results presented are derived from run packets BioFire Panel Run/Batch 1/2, BioFire Panel Run/Batch 3/4, BioFire Panel Run/Batch 5/6 and BioFire Panel Run/Batch 7/8 in binder # B-2019-042.

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Table 4: Summary of Respiratory Verification Pool Panel Samples from ZeptoMetrix

FilmArray® Instrument Verification Record

Instrument Serial #: (b) (4)
FilmArray Respiratory Panel Kit Part #: RFIT-ASY-0124 Lot #: 156019
Organism/Sample Source and Lot#: Cat# NATRVP-IDI Lot #: 319931

Organism	Detected?	# Detected	# Not Detected	# of days tested	# users	Pass/Fail
Adenovirus	☑ Yes ☐ No	(b) (4)				Pass
Bordetella pertussis	☑ Yes ☐ No					Pass
Chlamydophila pneumoniae	☑ Yes ☐ No					Pass
Coronavirus 229E	☑ Yes ☐ No					Pass
Coronavirus NL63	☑ Yes ☐ No					Pass
Coronavirus OC43	☑ Yes ☐ No					Pass
Coronavirus HKU1	☑ Yes ☐ No					Pass
Human Metapneumovirus	☑ Yes ☐ No					Pass
Human Rhinovirus/Enterovirus	☑ Yes ☐ No					Pass
Influenza A subtype H1	☑ Yes ☐ No					Pass
Influenza A subtype H1-2009	☑ Yes ☐ No					Pass
Influenza A subtype H3	☑ Yes ☐ No					Pass
Influenza B	☑ Yes ☐ No					Pass
Mycoplasma pneumoniae	☑ Yes ☐ No					Pass
Parainfluenza virus 1	☑ Yes ☐ No					Pass
Parainfluenza virus 2	☑ Yes ☐ No					Pass
Parainfluenza virus 3	☑ Yes ☐ No					Pass
Parainfluenza virus 4	☑ Yes ☐ No					Pass
Respiratory Syncytial Virus	☑ Yes ☐ No					Pass

Table 5: Summary of Respiratory Verification Negative Panel Sample from ZeptoMetrix

FilmArray® Instrument Verification Record

Instrument Serial #: (b) (4)
FilmArray Respiratory Panel Kit Part #: RFIT-ASY-0124 Lot #: 156019
Organism/Sample Source and Lot#: Cat# NATRVP-IDI, Negative Sample Lot #: 319931

Organism	Detected?	# Detected	# Not Detected	# of days tested	# users	Pass/Fail
Adenovirus	☐ Yes ☑ No	(b) (4)				Pass
Bordetella pertussis	☐ Yes ☑ No					Pass
Chlamydophila pneumoniae	☐ Yes ☑ No					Pass
Coronavirus 229E	☐ Yes ☑ No					Pass
Coronavirus NL63	☐ Yes ☑ No					Pass
Coronavirus OC43	☐ Yes ☑ No					Pass
Coronavirus HKU1	☐ Yes ☑ No					Pass
Human Metapneumovirus	☐ Yes ☑ No					Pass
Human Rhinovirus/Enterovirus	☐ Yes ☑ No					Pass
Influenza A subtype H1	☐ Yes ☑ No					Pass
Influenza A subtype H1-2009	☐ Yes ☑ No					Pass
Influenza A subtype H3	☐ Yes ☑ No					Pass
Influenza B	☐ Yes ☑ No					Pass
Mycoplasma pneumoniae	☐ Yes ☑ No					Pass
Parainfluenza virus 1	☐ Yes ☑ No					Pass
Parainfluenza virus 2	☐ Yes ☑ No					Pass
Parainfluenza virus 3	☐ Yes ☑ No					Pass
Parainfluenza virus 4	☐ Yes ☑ No					Pass
Respiratory Syncytial Virus	☐ Yes ☑ No					Pass

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Table 6: Summary of Respiratory Verification Negative UTM Sample

FilmArray® Instrument Verification Record

Instrument Serial #: (b) (4)
FilmArray Respiratory Panel Kit Part #: RFIT-ASY-0124 Lot #: 156019
Organism/Sample Source and Lot#: OP/UTM, Negative Sample Lot #: 20190415

Organism	Detected?	# Detected	# Not Detected	# of days tested	# users	Pass/Fail
Adenovirus	☐ Yes ☑ No	(b) (4)				Pass
Bordetella pertussis	☐ Yes ☑ No					Pass
Chlamydophila pneumoniae	☐ Yes ☑ No					Pass
Coronavirus 229E	☐ Yes ☑ No					Pass
Coronavirus NL63	☐ Yes ☑ No					Pass
Coronavirus OC43	☐ Yes ☑ No					Pass
Coronavirus HKU1	☐ Yes ☑ No					Pass
Human Metapneumovirus	☐ Yes ☑ No					Pass
Human Rhinovirus/Enterovirus	☐ Yes ☑ No					Pass
Influenza A subtype H1	☐ Yes ☑ No					Pass
Influenza A subtype H1-2009	☐ Yes ☑ No					Pass
Influenza A subtype H3	☐ Yes ☑ No					Pass
Influenza B	☐ Yes ☑ No					Pass
Mycoplasma pneumoniae	☐ Yes ☑ No					Pass
Parainfluenza virus 1	☐ Yes ☑ No					Pass
Parainfluenza virus 2	☐ Yes ☑ No					Pass
Parainfluenza virus 3	☐ Yes ☑ No					Pass
Parainfluenza virus 4	☐ Yes ☑ No					Pass
Respiratory Syncytial Virus	☐ Yes ☑ No					Pass

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Intra-Precision

Intra-precision was defined as a (b) (4)
(b) (4)
(b) (4) See Table 7

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Table 7: Intra-Precision

(b) (4)

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Inter-Precision

Inter-precision is defined as (b) (4)
(b) (4)
(b) (4)
(b) (4) See Table 8

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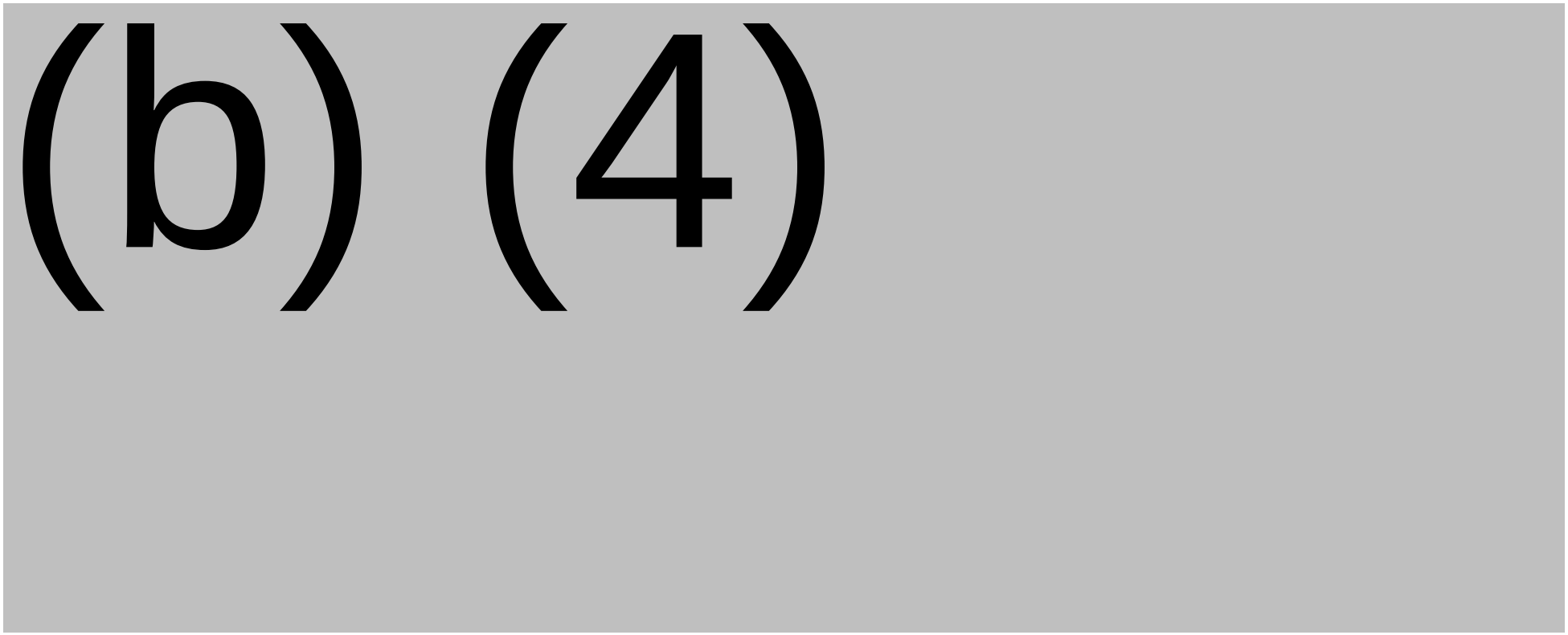
Table 8: Inter-Precision



Accuracy

All accuracy samples were (b) (4)
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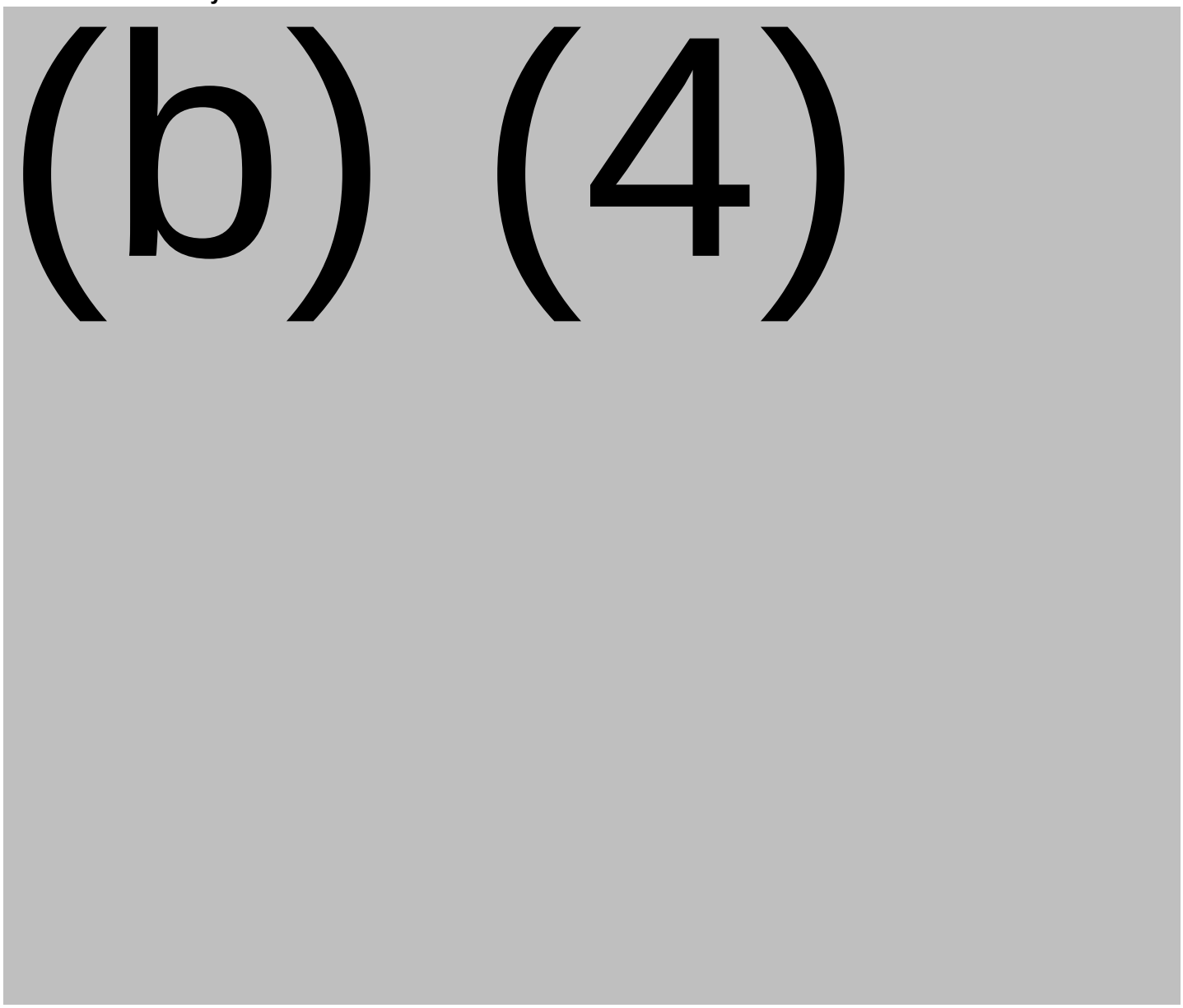
(b) (4)
(b) (4) See Table 9

Table 9: Accuracy



I. Deviations

Negatives Samples. The written validation plan did not indicate that negative controls would be run; however, the ZeptoMetrix panel negative and UTM negative samples were recommended by the manufacturer and Viracor respectively. Thus, both negative controls were run and the report includes the results. The only deviation was that the written validation plan did not indicate that the negative controls would be run.

J. Conclusions

The performance characteristics of the BioFire FilmArray® Respiratory Panel Assay using Nasopharyngeal and Oropharyngeal Swab Specimens met the acceptance criteria specified in the *Matrix Qualification Report to Verify the Performance Characteristics of the BioFire FilmArray® Respiratory Panel Assay using Nasopharyngeal and Oropharyngeal Swab Specimens.* (See Section I, Deviations). Therefore, nasopharyngeal and oropharyngeal specimen swabs in Universal/Viral Transport Media will be accepted for BioFire FilmArray® Respiratory Panel Assay.

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K. References

SOP *21120.2380 Procedure for Biofire FilmArray Respiratory Panel and Pneumonia Panel*

FilmArray Operator's Manual

FilmArray Respiratory Panel (RP) Instruction Booklet

Guidelines for Laboratory Verification of Performance of the FilmArray RP system

CLIA Interpretive Guidelines Section 493.1252

Li L, Chen QY, Li YY, Wang YF, Yang ZF, Zhong NS. Comparison among nasopharyngeal swab, nasal wash, and oropharyngeal swab for respiratory virus detection in adults with acute pharyngitis. *BMC Infect Dis.* 2013;13:281. Published 2013 Jun 20. doi:10.1186/1471-2334-13-281

Irving SA, Vandermause MF, Shay DK, Belongia EA. Comparison of nasal and nasopharyngeal swabs for influenza detection in adults. *Clin Med Res*. 2012;10(4):215-8.

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