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Was a Biofire Verification and Validation Worth the Cost?

A Laboratory Evaluation of Adding

the Biofire Respiratory and Meningitis Panels

to West Valley Medical Center,

a 150-bed Hospital.

By Benjamin J Walsh

A Thesis

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Committee Approval

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The members of the committee appointed to examine the thesis of Benjamin J Walsh find it satisfactory and recommend that it be accepted.

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Was a Biofire Verification and Validation Worth the Cost? A Laboratory Evaluation of Adding the Biofire Respiratory and Meningitis Panels to West Valley Medical Center, a 150-bed Hospital.

Thesis Abstract – Idaho State University (2019)

In the age of modern technology, where molecular testing has become more prevalent in clinical laboratories, a large push for faster methodology with accurate organism identification has become the new expectation by patient providers. This thesis aims to discuss the necessity for validations and verifications in clinical facilities. It is also seeking to determine if the Biofire FilmArray, a molecular analyzer can be a beneficial and effective tool for 150-bed hospitals. In the case of West Valley Medical Center, implementation of the FilmArray cost the facility $28,776.50. With their current workload volumes, using only the CMS Financial schedule, it will take approximately 10.4 months to begin showing a profit. Standard culturing techniques of body fluids at the facility take on average 80 hours 25 minutes, which was vastly improved by adding the FilmArray technology. Respiratory specimens were completed within 1 hour and 9 minutes, while meningitis specimens took 1 hour 37 minutes. Importantly, each facility seeking to use the Biofire FilmArray must weigh the advantage of the decreased turnaround times, with the disadvantage of an increased laboratory charges, and further compare those with each patient's overall out-of-pocket hospital cost. If the cost is justifiable and looks to be more beneficial than detrimental to the patient, then the addition of the Biofire FilmArray is a favorable option.

Key Words: validation, verification, cost analysis, Film Array, molecular, laboratory medicine

Introduction

Meningitis and encephalitis are acute inflammatory conditions, in which the meninges of the brain are inhabited by a viral or bacterial infection. Often times a diagnosis of meningitis or encephalitis is made by clinical history along with cerebrospinal fluid (CSF) analysis. For both CSF and respiratory specimens, it takes approximately 48 hours for bacterial culture results, and 48-72 hours for viral culture results.[1] During this time, patients are typically exposed to broad-spectrum antibiotics and their adverse effects, which can sometimes be dangerous. The timeframe to identification by culture has created a push for faster methodology, in hopes that patients can receive accurate treatments, faster. The Biofire FilmArray meningitis/encephalitis panel (BMEP) is one such instrument, that was approved by the Food and Drug Administration near the end of 2015. The technology was the first of its kind, and is currently one of the only multiplex polymerase chain reaction (PCR) systems to simultaneously detect multiple pathogens from a variety of specimen types. For a medical laboratory scientist working alone, the setup time requires roughly two minutes, and approximately another hour to receive test results.[1-5] Molecular testing provides a significant improvement in time to results from the previously mentioned culture method timeframes of 48-72 hours. This improvement in time should improve the treatment decisions made by physicians.

Discussions have increased over the last couple of years between laboratory professionals, providers, and patients, on improving test turnaround time. Many hospitals are continually being pushed for improvement. Due to that pressure, they must continually monitor the cost of implementing newer methodology and how these improvements impact patient outcomes.

Adding a new methodology into a hospital laboratory may add tremendous cost, but this cost can also add value, both for laboratories and patients. As new methodology is introduced, cost of the instrumentation is often a large factor, but many other factors play a role in the true cost for adding new instrumentation. These costs may come in the form of the validations, verifications, quality control, reagent specific costs, and many others. In every case, the cost of implementation must be weighed with the benefits that it provides physicians and their patients. This thesis aims to describe the process of validation and verification for new instruments, and then seeks to discover if adding the Biofire Respiratory and Meningitis Panels to a small, 150-bed hospital, will have a positive or negative impact on both the laboratory and patient care. The hypothesis is that adding the Biofire FilmArray will have a positive impact on West Valley Medical Center (WVMC), and other small hospital facilities.

Validation/Verification

I. *Introduction*

In laboratory medicine, instrument validations and verifications must be performed to ensure patient results are being accurately measured and reported. A validation is a confirmation through evidence, that the requirements for the test system, and its intended uses, are accurate. Likewise, a verification is a confirmation through evidence that the specific validation requirements have been completed and are valid.[6,7] In other words, a validation is implemented to ensure that the correct analytes are being tested, and a verification works to ensure that the test is being performed correctly. In 1988, the United States Congress passed the Clinical Laboratory Improvement Amendments (CLIA), which are overseen by three federal

agencies: The Center for Medicare & Medicaid Services (CMS), The Food and Drug Administration (FDA), and The Center for Disease Control (CDC). Each agency ensures that quality standards are being met across all areas of laboratory testing. The FDA's main responsibility is the categorization of laboratory tests into 1 of 3 CLIA regulated categories: waived, moderate, or high complexity testing.[6,8]

The CLIA statute 42 U.S.C. § 263a(d)(3) states: waived tests "are laboratory examinations and procedures that have been approved by the Food and Drug Administration for home use or that, as determined by the Secretary, are simple laboratory examinations and procedures that have an insignificant risk of an erroneous result, including those that (A) employ methodologies that are so simple and accurate as to render the likelihood of erroneous results by the user negligible, or (B) the Secretary has determined pose no unreasonable risk of harm to the patient if performed incorrectly."[8-10] Waived tests are the easiest for laboratories to integrate into their daily workflow, because they require very little verification. The only applicable requirements are to follow all manufacturer instructions, which are found within package inserts. Moderate or high complexity testing is categorized based on their potential risk to cause inaccurate results, which may have an adverse effect on patient health. These two categories of testing are often grouped together and referred to as nonwaived testing. All laboratories that perform both waived and nonwaived testing must be inspected by outside agencies, have a CLIA certificate, and meet all quality specifications present within Title 42 Part 493 Subparts H, J, K and M.[6,10,11]

For reference, Subpart H of CLIA regulations, state that all laboratories that perform nonwaived testing must be involved in proficiency testing with a CMS-approved agency.[10] The

three largest organizations with “deemed status” from CMS, are The Joint Commission on Accreditation of Healthcare Organizations (JCAHO), The College of American Pathologists (CAP), and The Commission for Office Laboratory Accreditation (COLA). Deemed status means they demonstrate that their accreditation requirements meet or exceed those specified under CLIA. The West Valley Medical Center (WMVC) laboratory has chosen to be accredited by CAP. CAP has been given the authority by CMS to perform all necessary inspections and relay their findings to CMS as they see fit. [6,12] Subpart J, of CLIA regulations, govern laws that describe the administration aspects of nonwaived testing, which includes laboratory specification requirements, retention of records, and various other administration requirements. Subpart K describes quality requirements for nonwaived testing, and section M describes personnel requirements.[8,10,11]

It is important to point out that CLIA regulations do not have requirements for instrument manufacturers on validating medical devices, but they do require manufacturers to register new instrumentation with the FDA under 493.17.[10] The FDA evaluates clinical validity of a test during its premarket clearance and instrument approval process, and places all of the rules and regulations that must be adhered to by manufacturers within Title 21 CFR 820.[13] Because of these regulations, instrument manufacturers are required to perform validation studies on all instruments that seek to have an FDA approved status for medical laboratory testing. All testing that is approved by the FDA has been validated for a specific purpose. Each test typically has specific reagents, controls, and equipment, which have all been tested and have met FDA rules and regulations. The FDA provides companies, like Biofire Diagnostics, with guidance for performing these validations, but does not provide them with specific

requirements on how these validation studies should be performed. Because of this, the regulations are often loose, and it's up to the manufacturers to determine what an adequate representation of data looks like.[6]

All Biofire instrumentation and materials used for testing are FDA-approved[14,15], as long as there are no modifications to the test system, or the FilmArrays specified testing techniques. A modification, for example, might be the use of inappropriate collection swabs or storing specimens in inappropriate collection media. As long as laboratories are maintaining previously validated methods, CLIA only requires the demonstration that performance specifications for their new methodology are comparable to those provided within the FDA submitted manufacturer's validation studies.

II. Instrument Validations

Instrument Methodology

PCR, also known as a polymerase chain reaction, is a process that utilizes an enzymatic protein, known as a polymerase, which has a specificity to RNA or DNA, and short known segments of nucleic acid, called primers. Primers used in general PCR are complementary to some target nucleic acid sequence in an organism. Regions of unknown nucleic acids are amplified when appropriate primers and polymerase are used. This amplified sequence is known as an amplicon. The Biofire Diagnostics technology uses a PCR technique known as "nested PCR", which is a modification to PCR. Nested PCR uses two separate stages of PCR, utilizing two separate sets of primers. The nucleic acid sequence of the second set of primers is nested within the first set of primers, which is intended to improve specificity. In general PCR, many

factors need to be accounted for to avoid mispriming. Mispriming occurs when a primer anneals to an incorrect nucleic acid region. When a nested PCR system is created, only amplified sequences that contain the first primer sequence will be amplified. All strands of nucleic acid that are created by misprimes are essentially removed because the concentration is vastly outweighed by correct amplicon sequences.[16]

Specimen requirements for the FilmArray vary between panels. For the meningitis panels, CSF specimens are to be used without the addition of any transport media. Respiratory panel specimens are collected using a nasopharyngeal swab which is placed into viral transport media. All other specimen collection techniques require further validations from the facilities that are performing the testing.

The Biofire FilmArray system utilizes a small reaction pouch that contains all necessary reagents for a multiplexed, nested PCR. A FilmArray test is initiated when a laboratory scientist inserts the hydration solution into the right side of the pouch. Once inserted, the hydration solution liquifies lyophilized reagent to begin PCR analysis. Each pouch enables the system to perform three different stages of testing: preparation, amplification, and identification, all of which are completely automated. (Figure 1) During the preparation phase of testing, cell lysis occurs by mechanical abrasion from magnetic silica beads, which isolate, wash, and transfer samples of DNA/RNA between the cell lysis chamber

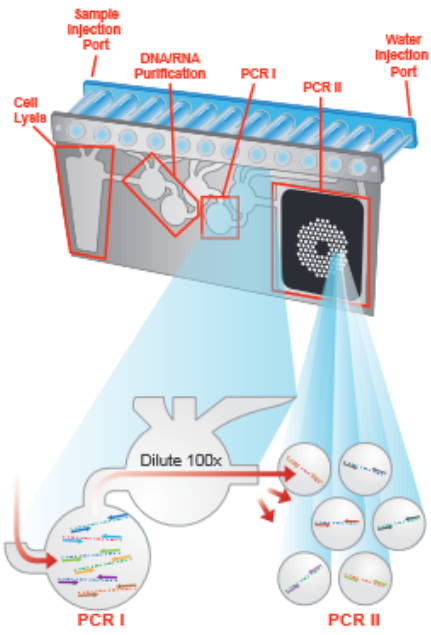


Figure 1. FilmArray Testing Pouch.[17]

and the DNA/RNA purification chamber/blister. Once DNA/RNA has been eluted from the sample, multiplexed DNA amplification begins. If RNA is present, reverse transcriptase converts the nucleic acid into complementary DNA (cDNA) before the first round of PCR begins, otherwise, standard PCR occurs in the blisters immediately following the purification blisters. After the sample DNA has been diluted, the sample mixture is then transferred to the array for nested PCR. Following the nested PCR reaction, amplicon

is melt curve analysis takes place charting the loss of signal over different temperatures and time. Amplicon melt curve analysis is the process of heating the amplicon until the fluorescence from the previously bound florescent binding dye has been completely eliminated. Melt curve analysis for the FilmArray takes place starting at 63^{o}C, and the organism's identification begins, using a linear ramp, between 68^{o}C to 95^{o}C, at a rate of 0.5^{o}C/second.[14,15] Each organism within the FilmArray will display a unique melt curve profile which is used to appropriately identify each organism on the panels.

Two internal controls are performed during testing, to ensure testing maintains accurate performance. The first control is composed of mRNA from *Shizosaccharomyces pombe* yeast cells, which is freeze dried within the cell lysis reaction chamber, and carried all the way through the second stage of PCR side-by-side with patient specimens. This control helps to improve test reliability, and ensures that the test pouch performed adequately through all stages of testing. The second control is a set of primers and a known target DNA ligand that has been attached at three points within the array. This control is specifically used to ensure that the PCR in stage 2 is performed adequately. If either the *S. pombe* or the short DNA primers aren't identified correctly during the identification stage, the entire test run is invalidated.[14,15]

Meningitis (ME) Panel Validation

For any medical device validation study, the most crucial step is to identify the exact purpose for the test, and what it will be used for. The Biofire FilmArray ME panel is intended to be a qualitative, multiplexed, nucleic acid-based, in-vitro, diagnostic test, which has the capability to simultaneously detect bacterial, viral, and yeast organisms, directly from patient CSF.[14] A total of nineteen different analytes can be identified from the panel. A comprehensive list of organisms on the ME panel can be seen in Appendix A. Biofire Diagnostics submitted their validation material for the ME Panel to the FDA in early 2015, and it was approved later that year.[14] As required by the FDA, Biofire Diagnostics analyzed performance characteristics for their test system, which met and adhered to all federal regulations found in Title 21. These performance characteristics include reproducibility, controls, detection limit, analytical specificity, interfering substances, and specimen stability.

Reproducibility

Reproducibility, or method precision, is difficult to perform for qualitative testing because a spectrum of values are not available. For qualitative testing results are typically in the format of either positive or negative. Reproducibility is often completed to demonstrate analytical consistency with a manufacturer's given methodology. Studies on reproducibility are generally performed to show that the test will give the same results regardless of operator, day, lot, or any other variable.[6] In the Biofire Diagnostic study, testing occurred using two separate instruments; the older FilmArray, and the newer FilmArray 2.0. The first generation FilmArray was tested using multiple instruments at three different testing sites, while the Film Array 2.0

was tested at three different locations within the Biofire diagnostics facility. The validation included the testing of all organisms present within the ME panel at three different concentrations: negative, the limit of detection (LOD) x 1, and the LOD x 3. Additional variants were checked throughout the entire validation study. These included changing pouch lots, operators, and instrumentation.[14] A total of 366 runs were successfully completed on the FilmArray. Of the 366, six runs had to be repeated due to invalid results. One of those was caused by invalid internal controls, while all the others were due to instrumentation or software errors. During the FilmArray study, seven unexpected false positives occurred. Six were from *Streptococcus pneumoniae,* and one from Human Herpes Virus 6. The FilmArray 2.0 had a total of 365 successfully completed runs, but five runs had to be repeated. Three were caused by operator error, and two were caused by instrument error.[14]

Controls, Calibrators, or Methods

Title 21 CFR 820.70 requires every manufacturer to develop, conduct, control, and monitor every stage of their production process.[13] This regulation is in place to ensure their device meets the stated specifications across all stages of testing. For the FilmArray, each pouch is created with two internal controls, the RNA control and the PCR2 control. Both must be positive in order for the test system to be valid. Biofire Diagnostics also state within the FilmArray package insert, that external controls are recommended. External controls for the FilmArray are required to be completed with every new lot, every new shipment, and every thirty days. External controls are used to ensure that each instrument is identifying positive and negative specimens as expected, while also ensuring that the entire test system has not been

contaminated. Laboratory external control requirements should be determined based on accrediting agency standards, and what each agency requires of their laboratory.[14,15]

Detection Limit

Also known as clinical sensitivity, the detection limit defines the lowest allowable concentration of the organism before false negatives begin to occur, which is positively identified at or above the manufacturers stated sensitivity values.[6] In order to evaluate the effectiveness of the FilmArray with multiple organisms, a detection limit had to be established. A study was performed to determine at which point organisms failed to be reactive on the FilmArray. For the majority of organisms on the meningitis panel, when the concentration fell below $2x10^3 \pm 1x10^3$ CFU/mL for bacterial, $1x10^4 \pm 9 x10^3$ copies/mL for viruses, or 300 ± 200 CFU/mL for yeast, the organism showed false negative results. Deviations within each group of organisms varied based on which organisms were being tested.[14] The limit of detection for each organism can be seen in Appendix A.

Analytical Specificity

During a validation for a molecular microbiology test, it is also important to determine if test analytes are going to cross-react with other unexpected analytes, and/or with each other. Identifying cross-reactivity helps to identify those organisms that have the potential to cause false positive results. During the FilmArray validation, 107 off-panel organisms were tested to determine if they could produce a positive result with one of the nineteen on-panel organisms. Cross-reactivity was shown for *Haemophilus influenzae* when tested with *Haemophilus*

haemolyticus. Since, *H. haemolyticus* is not frequently found in CSF specimens, it would be extremely rare for it to cause a false positive under *H. influenzae*. So, during the study, *H. haemolyticus* was shown as a possible organism for error, but isn't listed with *H. Influenzae* on the panel. Furthermore, rhinovirus showed positive reactivity with enterovirus. For this reason, all rhinovirus organisms are labeled rhino/enterovirus for resulting purposes. Lastly, *Cryptococcus amylolentus* was positive for *Cryptococcus neoformans/gattii*. Since *C. amylolentus*, is not isolated from humans, it too is excluded from the panel.[14]

Interfering Substances

Manufacturers for medical devices are also required to report all substances that are capable of interfering with their test assay, and are required to determine which substances could potentially alter their test methodology. In this case, that would include any interfering substance that might come in contact with a patient's CSF, either during sample collection or sample processing. It was found when samples contained high protein levels (albumin >15mg/dL), false negatives appeared for both E. coli and Enteroviruses. Furthermore, any time bleach, a common disinfectant for molecular testing, was greater than 0.1% (v/v), false negatives would occur for multiple FilmArray analytes.[14]

Specimen Stability

Testing on all CSF specimens stored at room temperature should be completed within 24 hours. If placed into a refrigerated environment upon collection, the specimens are good for up to seven days. Specimens that were stored in CSF transport media were not evaluated

during the validation. If a laboratory wanted to use this option, completion of further validations would be required by the testing laboratory.[14]

Respiratory Panel 2 Validation

The qualitative Respiratory Panel 2 (RP2) is intended to simultaneously detect multiple bacterial and viral nucleic acids from nasopharyngeal swabs stored in viral transport media. The test is to be used as a supplemental diagnostic tool for patients who are suspected to have respiratory infections. A total of twenty-two different analytes can be identified on the panel, and a comprehensive list of organisms on the RP2 panel can be seen in Appendix B. The Respiratory Panel 2 was submitted to the FDA in early 2017 and approved in mid-2017.[15] As required, Biofire analyzed performance characteristics for their test system to adhere to the federal regulations found in Title 21.[15]

Reproducibility

In order to test analytical precision on the RP2 panel, a study was performed between three different testing sites. To introduce more variability, organisms were tested over a period of five days, using seven different operators, fifteen different FilmArray 2.0 instruments, nineteen different FilmArray Torch modules, and three different reagent pouch lots. A total of four different samples were created, each containing known quantities of different RP2 analytes, and with every organism being introduced at least once across the four samples. Once created, the samples were then distributed to the testing sites for evaluation. The sites tested six replicates of each sample for five different days, with the exception of one site, which

performed twelve replicates per day on two different instruments. Valid results were completed for 480 of 489 attempts. Of the invalid attempts, eight were due to control failures, and one was due to an instrument failure. All invalid results were successfully completed upon replication of the test samples.[15]

Controls, Calibrators, or Methods

Requirements for the RP2 panels were exactly the same as those described in the meningitis/encephalitis section. Briefly, each pouch is created with two internal controls, both must be positive in order for the test system to be valid. Additionally, external controls must be performed with each new shipment, lot change, and every thirty days.

Detection Limit

The limit of detection for each analyte on the respiratory panel was determined by performing serial dilutions for each analyte, using a known concentration. Once a loss in detection was observed, twenty replicates were performed at that dilution to confirm the true loss of detection.[15] Detection limits for all organisms tested within the RP2 panel can be seen in Appendix B.

Analytical Reactivity

A reactivity study was performed on the RP2 panel to determine if the test methodology was specific for one variant, or could capture a larger population of species variants. A total of 177 isolates of known clinical significance were tested in this study, across all analyte types

within the panel, and reacted as expected. As shown within the study, species of *Bordetella bronchiseptica* that contain *IS1001* will inevitably test positive for *Bordetella parapertussis* (IS*1001)*, resulting in a false positive.[15]

Analytical Specificity

As stated previously, analytical specificity is a very important part of a validation for a microbiology molecular assay. To test cross-reactivity on the panel, two separate experiments were set up. First, on-panel organism testing was performed in order to determine if organisms on the panel would cross-react with one another. It was discovered that swine origin Hsw1N1, at high concentrations, has the potential to cross react with Influenzae A H1-2009. Furthermore, all species of Bordetella that carry the pertussis toxin pseudogene, notably *B. parapertussis* and *B. bronchiseptica*, when they are present at high concentrations, can potentially be detected on the panel for *B. pertussis*. Of important note, if the situation does occur where a Bordetella sp. creates a false positive, both species should show positive on the panel, as long as both are available. Lastly, false positives were observed due to human rhino/enterovirus primer pairs that are similar to *B. pertussis* and *parapertussis*. In clinical care, Bordetella sp. are rarely seen, and definitely less frequent than the common cold, so this finding should be rare. But, a more concerning factor occurs when a patient is positive for a Bordetella species. In this case, additional care should be taken to rule out a concurrent rhino virus infection.[15]

The second experiment tested off-panel organisms, which were selected based on the following criteria: their relatability to analytes on the panel, the likelihood that they could be in

a nasopharyngeal specimen, and to those organisms genetically similar in DNA regions to assay primers found within the RP2 pouch.[15] Fifty similar off-panel organisms were tested to identify panel interactions, and of the fifty, only *Bordetella bronchiseptica* showed significant interactions on the panel. Because some variants of *B. bronchiseptica* contain *IS1001* and pertussis toxin pseudogenes, the organism was then able to amplify targeted sequences and be detected as *B. pertussis* and *B. parapertussis*. It was also shown, but not visualized during the validation experiments, that some strains of *B. bronchiseptica* contain genes that are closely similar to the human rhino/enterovirus, and thus have the capability to show additional false positive results. It was also noted that because *B. bronchiseptica* is a rare human pathogen more commonly found in canines, the probability of it coming in contact with a nasopharyngeal specimen is low.[15]

Interfering Substances

Remarkably, in a study that tested close to forty different endogenous and exogenous sources of potentially competing analytes, only when bleach was greater than 0.1% (v/v) was it found to interfere and cause false negatives. Due to the effect of bleach on the degradation of nucleic acids. [15]

Specimen Stability

A study on specimen stability was performed during the first iteration of the respiratory panel submitted to the FDA. This study tested organisms up to four hours at room temperature, three days at refrigerated temperatures, and thirty days at temperatures below 0 degrees

celcius[18] During the study it was shown that nucleic acids begin to break down, at three days refrigerated and thirty days frozen, resulting in false negative values. For this fact, it was strongly recommended by the manufacturer to test specimens within four hours of collection. It was also recommended, that once a panel pouch has been displaced from the vacuum seal, users should load the pouch with reagent and specimen within thirty minutes, and test the specimen on the FilmArray within one hour. The probability of false negatives occurring outside this time allotment also increases the longer the pouch stays outside of the vacuumed environment.[18]

III. Instrument Verifications

The majority of material found within this section will focus on those topics found within Subpart K, more specifically, Title 42 CFR 493.1253, which is the laboratory standard for the establishment and verification of instrument performance specifications. This statute requires accredited laboratories to demonstrate in a clinical setting, that they can obtain performance specifications comparable to those established by the manufacturer. Specifications are obtained during validation studies for certain performance characteristics. These characteristics include the accuracy, precision, reportable ranges, and the assurance that the manufacturer assigned reference intervals are appropriate for the laboratory's patient population.[6,10] All instrumentation in use before April 24th, 2003, and currently in use by a laboratory facility, is exempt from verification requirements set forth within that specific regulation. The majority of instruments in use today fall outside those parameters. For this reason, regulations require an instrument verification if they are labeled as a non-waived instrument. Furthermore, all CLIA accredited laboratories participating in proficiency testing must maintain all verification data

for at least two years after the discontinuation of the instrument, as declared in 493.1105.[10] All verifications must be performed in the exact location where patient testing will be performed. If any instrumentation is moved, a new verification must be performed to verify changes in test conditions, which include changes in temperature, humidity, storage conditions, and all other conditions outlined in 492.1252. The instrument verification will ensure that the testing performed on patients is not adversely altered by the change to the testing environment.[6,10]

At West Valley Medical Center (WVMC), located in Caldwell, Idaho, a verification of the Biofire FilmArray meningitis panel and respiratory panels was completed to ensure the test system followed all manufacturer recommendations and CLIA regulatory requirements. The verification process, in its entirety, took approximately twenty-five days, which included the instrument test verifications and the development of the Internal Quality Control Plan (IQCP). Zeptometrix PCR kits, as recommended by Biofire Diagnostics, were used for verification of the Biofire FilmArray systems. The respiratory panel Zeptometrix kit (NATRVP2-BIO) included twenty-two test specimens, plus a negative control. The meningitis panel kit included a total of fourteen test specimens, with no negative control. During the verification process, the respiratory specimens each contained approximately 0.6 mL of test solution for each analyte, and each of the meningitis analytes contained approximately 0.4 mL.

As described in Biofire Diagnostics validation submissions to the FDA, many test variations were required to ensure that the test was being performed correctly and for its intended use. [14,15] At WVMC, many of the variations of test variables were similar to the FilmArray validation studies. Verification testing occurred over four days, which helped test for any day-to-day variations. A total of nine different laboratory scientists were included in the

verification, to ensure procedures were being followed adequately, and to test for person-to-person variability. In addition, two FilmArray instruments were used during the verification, to help ensure the manufacturer performance specifications were valid, completed, and accurate. All guidelines for the performance of the verification procedures, as provided by Biofire Diagnostics, were completed to ensure the intended verification results were obtained.[19,20] CLIA has recommended that all verification results are approved each laboratory facility, and it is the laboratory director's responsibility to determine when appropriate results have been obtained. In this study, the WVMC laboratory director determined one outlier for the verification fell within the verification perimeters.[21]

With the addition of the respiratory and meningitis panels at West Valley Medical Center, the facility now utilizes the FilmArray with three different panels. It was necessary then, with the addition of the new panels, to retrain all laboratory scientists that have frequent interaction with the instrument. All individuals were required to demonstrate knowledge of the different panel pouch types and testing requirements for each panel. Each person was also trained on the appropriate procedures for donning and doffing gloves, and when the use of gloves was appropriate to help mitigate cross contamination. This gloving procedure includes putting on fresh gloves as you enter the molecular room, discarding gloves as you leave the room, and immediately after you remove completed pouches from the FilmArray instrument.

The requirements as provided under the CLIA regulations for each verification at WVMC are shown in the following sections.

Meningitis Panel Verification

During the meningitis panel verification, Zeptometrix meningitis kits, which included known specimens of each analyte found on the meningitis panel, were used to test the performance specifications set by the manufacturer.[22] During the meningitis verification studies, each control organism was separated into one of three pools. Prior to pooling, each control vial was vortexed to ensure that no dilutional errors would occur when specimens were aliquoted into their pooled environments. All control organisms were pooled as is, without the addition of any CSF matrix, which is a variable that is capable of changing from lab to lab.

Accuracy

To determine accuracy, known pooled zeptometrix organisms were used, which allowed WVMC to test for viable detection of each organism, and simultaneous detection of multiple organisms, while also reducing the amount of test runs required for the verification. All pooling information and verification data for the meningitis panel can be seen in Appendix C. Organisms on the panel were tested a combined total of fifty-six times, over a two-day period. Of those fifty-six tests, no false negatives occurred, which gave a total accuracy over that time frame of 56/56 (100%).

Precision

To determine precision, replicates for each zeptometrix specimen were completed on two instruments, over multiple days, to ensure that the same set of organisms were obtained each time. During the verification, a total of five different laboratory scientists were used to determine any person-to-person deviation. It was intended to exclude repeat sample testing by

the same scientist, but due to limited tech time available, one repeat was performed during the meningitis verification, as seen in Appendix C. Each day, both instruments ran one replicate for each pool during the verification. This was to ensure that the same organisms were being identified between pools and that there was no deviation between instruments, scientists, or days. Of the sample pools completed, a total of twelve pooled replicates were completed successfully, giving a verification precision of 100%.

Reportable Range

Because the meningitis panel is a qualitative molecular test, the reportable range for each patient specimen is reported as either negative or positive for each organism on the panel.

Reference Intervals

The normal values for patient CSF tested at WVMC is negative. All positive values are critical, as CSF is considered a sterile fluid.

Respiratory Panel 2 Verification

During the respiratory panel verification, Zeptometrix RP2 kits, which include known specimens of each analyte found on the respiratory panel, were used to test the performance specifications set by the manufacturer. [23] During the respiratory verification studies, each control organism was separated into one of four pools. Prior to pooling, as mentioned previously, each control vial was vortexed to ensure that no dilutional errors would occur when

specimens were aliquoted into their pooled environments. Viral transport media can sometimes cause minimal unforeseen interferences with patient specimens. For this verification, to avoid the chance of interference, all control organisms were then pooled without the use of viral transport media. All viral transport media used by WVMC is recommended by the manufacturer, and follows guidelines presented within the manufacturer's package insert for the RP2 FilmArray panel. From the manufacturer's validation studies, the viral media used has not been shown to cause any negative interferences with the RP2 methodology.

Accuracy

As mentioned, accuracy was determined by pooling known zeptometrix specimens. Pooling these organisms enabled WVMC to test the assay's ability to simultaneously detect multiple organisms. The use of pooled specimens helps determine analytical specificity. This tests if a value is positive in a group of other organisms or substances, while also ensuring that no specimen cross-reactivity occurs. Testing pooled specimens also tests for analytical sensitivity, which helps to ensure that the organisms are accurately detected when the concentration is low.

Pooling samples also decreases the number of runs and time required to effectively complete the verification process. All pooling information and verification data for the respiratory panel can be seen in Appendix D. During the verification, eighty-eight organisms were intended to be identified, however, with the addition of one false positive, eighty-nine organisms were identified. On one run, the Human Metapneumovirus was found to be a

contaminate of the respiratory panel, sample pool 2. Upon repeat analysis of the same sample pool, all organisms were completed and correctly identified. Upon subsequent analysis, to try and find the source of the contaminate, no significant positives could be found. Because all the organisms within each pool were correctly identified on the first completed run, even with the addition of the contaminate, the overall accuracy was 88/89 (98.9%).

Precision

Replicates for each zeptometrix pooled specimen were completed on two instruments, over multiple days, to ensure that the same set of organisms were obtained each time. During the verification, seven different laboratory scientists were employed to determine any person-to-person deviation. As intended, each replicate sample was completed by a different laboratory scientist to test for any person-to-person variation. No significant test deviations were seen when transitioning between scientists. Each day, during the verification, both instruments ran one replicate for each pool. This was to ensure that the same organisms were being identified between pools, and that no deviation between instruments, scientists, or days occurred. Of the sample pools completed, a total of sixteen pooled replicates were completed successfully, giving a precision of 100%.

Reportable Range

Because the respiratory panel is a qualitative molecular test, the reportable range for each patient specimen is reported as either negative or positive for each organism on the panel.

Reference Intervals

A normal value in respiratory samples at WVMC is negative. Since organisms on the panel are known respiratory pathogens, a positive value is an indication of an upper respiratory infection.

IV. Conclusion

Validations and verifications are extremely important for the success of medical laboratories and the assurance of complete and accurate patient testing. The completion of validation and verification procedures are not only done because they are required by accreditation agencies, but also to ensure safety for all patients. Finishing a thorough review of instrumentation before patient interaction helps to avoid analytical errors, and most importantly, helps to avoid inaccurate information sent to patient providers. Inaccurate testing can lead to inaccurate diagnoses, which may lead to incorrect treatments and further patient suffering.

Cost Analysis

I. Introduction

An important question for anyone seeking medical treatment is, “how much is this going to cost?” A key goal for many hospitals is mitigating a patient’s hospital cost, while also ensuring that the patient is receiving the best care possible. Laboratory medicine undoubtedly has a significant impact on cost, and these costs are often directly related to the tests performed as ordered by the providers. Both accuracy of test results and test turnaround time, are two of the most important factors contributing to how much debt a patient might accrue

during their hospital stay. Laboratory testing has an impact on nearly every decision that is made by physicians. This testing helps determine how long a patient stays, whether a patient receives antibiotics, and even the duration of an antibiotic used. Furthermore, laboratories have a significant impact on the overall outcome of patient care, and therefore are a crucial part to every hospital. Over recent years, new laboratory methodology and instrumentation has improved efficiency of laboratory testing, by improving accuracy of results, and test turnaround times, which inevitably helps to reduce patient costs.

Implementing new laboratory testing is very costly, and requires an in-depth understanding of what the new testing will bring to the hospital. As mentioned previously, bringing in new instrumentation requires validations or verifications of the new test system. This instrument verification requires clinical laboratory scientist time, instrument costs, and additional reagent costs. In addition, introducing a new instrument requires laboratories to perform quality control, as specified by the manufacturer, sometimes once a month, once a week, or with every new lot of reagent. Each of these costs accumulate over time, and each must be measured to determine whether the benefit of having the new instrumentation is worth the cost of obtaining and maintaining it.

In the United States, all hospitals and laboratories that accept Medicaid and Medicare patients must use accurate current procedural terminology (CPT) codes to ensure proper reimbursement. All financial reimbursement for Medicare patients is established by the Center of Medicare and Medicaid Services (CMS), and published yearly in the CMS financial schedule.[24] Listed within the financial schedule, are the maximum payable amounts for testing based on the CPT codes created for the majority of clinical departments. In laboratory medicine, the

Medicare financial schedule is often used as a baseline for cost, because it often determines the minimum reimbursement amount that a laboratory may receive. As shown in numerous studies, and also mentioned by the Medicare payment advisory commission (MedPAC), Medicare fees are consistently lower than private insurance payments.[25] In a study provided by Dykeman and associates to MedPAC, it was found that most private insurance companies' health plans use the Medicare payment models with the addition of their own specific conversion factors.[26] So, if private insurers use the Medicare model as their baseline, their reimbursement amounts should be higher.

Determining actual amounts laboratories receive from patient to patient deviates significantly, based on insurer, a patient's ability to pay, testing received, and a variety of other factors. Thus, finding a baseline to calculate probable returns, is almost always more effective when trying to determine if the implementation of new test methodology is worth the cost. The study below seeks to determine if the cost of adding the Biofire ME and Respiratory panels is worth the cost of implementation in relatively small hospitals.

II. Cost of Verification

The cost to verify new test methodology in clinical laboratories often makes the difference between obtaining the new methodology and sticking with a laboratory's older methodology. In order to verify the meningitis and respiratory panels at West Valley Medical Center, the institution was required to purchase verification kits, instruments, and scientists' time. WVMC leased two Biofire FilmArray instruments for $1,122.50 a month. Creating a monthly lease benefits the hospital by reducing large upfront instrument costs. Furthermore,

leasing enables the facility to upgrade when new test methodologies become available. The RP2 verification kit, which included all necessary reagents to perform the respiratory verification, cost WVMC $4,718.00, and likewise, the meningitis verification kit totaled $5,980.00.

In order to create the Internal Quality Control Plan (IQCP), which enables WVMC to perform QC monthly instead of daily, twenty days of quality control (QC) for both the meningitis and respiratory panel were required. This added an additional $3,628.40 and $2,295.00, respectively in reagent pouch costs. Remaining verification quality control specimens were used to mitigate the cost of purchasing additional unnecessary quality control reagent. Monthly control specimens are obtained from Maine Molecular. M262 kits are used for the meningitis QC, and M315 kits are used for respiratory QC.[27,28] The cost for this QC was $532.00 and $371.00, respectively. Each QC kit includes six positive specimens and six negative specimens, where one positive and one negative are used each month, and for every new lot of reagent pouch. Reagent pouch kits, which are required for QC and patient samples, include thirty testable reagent pouches. The meningitis kit sums to $5,442.60, and the RP2 pouch kit sums to $3,442.50. The time of verification and the development of the IQCP took approximately fifty hours of scientist time. The average Bureau of Labor Statistics (BLS) salary provided for a medical laboratory scientist within the United States in 2017 was $24.89/hour.[28] Using this information, verification cost WVMC an additional $1,244.50 in scientist time. The total price for verifying and performing the IQCP on the Biofire FilmArray at WVMC was approximately $18,988.40. Additionally, QC specimens and reagent pouches had to be

Table 1. Cost of Verification at WVMC	
Item	**Price**
Lease Biofire FilmArray	$1,122.50/month
RP2 Verification Kit	$4,718.00
Meningitis Verification Kit	$5,980.00
Reagent Pouch for IQCP	$5923.40
Controls for IQCP	Included in Verification Kits
Scientist time @ 50 hours	$1244.50*
Total:	$18,988.40
*Bureau of Labor Statistics salary data for 2017 at $24.89/hour	

purchased, which added another $9,788.10, so that patient specimens could begin. This brought the total cost of implementation to $28,776.50.

III. Specimen Turnaround Time

In clinical laboratories, test turnaround time has one of the biggest impacts on the outcomes of patients, and the patient care that is received. The gold standard for traditional laboratory testing of meningitis and respiratory specimens involved direct examination by gram stain and culture. A major drawback to culture is that turnaround times can be between 24 and 72 hours or longer, which requires physicians to frequently make short-term management decisions for the administration of antibiotics, antivirals, or antifungals. These decisions are usually based strictly on the patient's underlying symptoms, which is frequently ineffective.[29] With the advent of new technology, like the Biofire FilmArray, organism identification, if performed in-house, can be provided to the physician in roughly 1.5 hours. At many hospitals, rapid molecular testing isn't available, and if a physician requires the information from a molecular panel, these laboratories are required to send out the specimen, which adds addition time to identification.

From January to December 2017, no positive CSF cultures were isolated on culture plates at WVMC, and the average turnaround time, from received to resulted, was approximately 72 hours. Because there were no positive CSF isolates, the search was expanded to include all body fluids for 2017, which included pleural, peritoneal, and synovial fluid. Since setup time and identification are similar between other body fluids and CSF, positive body fluid cultures were included to give a good representation of positive culturing. A total of twelve positive body fluids were isolated and identified from culture plates, as seen in Appendix E. The average turnaround time for all body fluids was 80 hours and 25 minutes.

As shown in Appendix G, between April 2017 and December 2017, the average turnaround time for send-out molecular meningitis testing was 11 days, 18 hours, and 6 minutes, when all data points were included. Omitting two heavy outliers, caused by tech error, the average turnaround time changed to 17 hours and 8 minutes. Implementation of the new Biofire molecular system in 2018 at WVMC, over the introductory three-month period (January thru March), had an average sample turnaround time of 1 hour 37 minutes for the meningitis panel, while the respiratory panel had a turnaround time of 1 hour 9 minutes. Over the first three months of use, the meningitis panel was run by six different laboratory scientists and had a sample size of thirteen patients. The testing showed only a single positive result, varicella zoster, with twelve negative patients, as can be seen in Appendix F. The respiratory panel was also performed by six different laboratory scientists, and saw a sample size of fourteen patients: three negative, two rhinovirus/enterovirus, one rhinovirus/enterovirus with RSV, two human metapneumovirus, one human metapneumovirus with RSV, three RSV, one adenovirus, and one Flu B. These results are listed in Appendix H.

In a follow-up study, between April and October of 2018, to determine if any shifts had occurred to sample volumes, a total of thirty-seven respiratory specimens were tested: twenty negatives, ten Rhinovirus/Enterovirus, two adenovirus, two parainfluenza, one B. pertussis, one parainfluenza with rhinovirus, and one coronavirus. In addition, the meningitis panel was completed a total of twenty-two times, with twenty negatives, one human herpes virus 6, and one parechovirus.

IV. Laboratory Reimbursement

A necessary and integral part of every hospital laboratory is the ability to recover expenses paid for time, reagents, equipment, and all other outgoing expenses of a functional laboratory. Laboratories are constantly working to improve costs to help improve the hospital's profitability. As mentioned previously, laboratory reimbursement is extremely variable from patient to patient, and therefore, the financial fee schedule as provided by CMS, is an effective method to calculate lowest possible yield. The cost for performing a CSF culture, positive or negative, to an uninsured patient at West Valley Medical Center is $119.09. This value changes significantly between insurance companies, and likely isn't the reimbursement that the WVMC laboratory will see. Following the CMS financial fee schedule, the minimum reimbursement amount that the laboratory might see can be broken down, which is also the maximum payable amount provided for Medicare or Medicaid patients. Included in a culture and identification for CSF specimens are CPT codes, 87205, 87070, 87073, and 87186, which may all be completed. These codes, in their respective order, enable us to perform a gram stain, aerobic culture and identification, an anerobic culture and identification, and allows for the use of microscan plates

for susceptibility studies. The maximum reimbursement amount through CMS for performing this CSF culture would come to $38.24, which is significantly lower than the $119.09 billed. With the new in-house molecular system, WVMC looked to improve the cost to patients from send out molecular testing. The hospital cost for uninsured patients is $1,200.00 for the meningitis panel, and $1,300.00 for each respiratory panel. The Biofire meningitis and respiratory panels (CPT 87483, CPT 87633) have a national Medicare limit, as can be seen in the fee schedule, of $514.55 in 2018.[24] A reimbursement evaluation of the old versus new technology helps to determine if the cost of the new methodology will be detrimental or beneficial to laboratories. If used correctly, this study has shown that the new molecular technology is largely beneficial to laboratory revenue, but is detrimental to patient out-of-pocket costs.

V. Laboratory Cost of Methodology

Meningitis and respiratory specimens ordered at WVMC prior to the Biofire FilmArray had to be sent out to another laboratory for identification. Send-out testing charged to WVMC for meningitis panels was $1,319.00 per patient sample, and WVMC charged patients $1,349.00, which included an outside miscellaneous charge of $30.00 to cover addition expenses, such as shipping the specimen to other laboratories. Prior to the addition of Biofire FilmArray for upper respiratory specimens, it was uncommon to send out respiratory specimens at WVMC. Generally, a physician needed to have a specific organism of interest, which could then be sent out for follow up molecular testing. The typical plating procedure for CSF at WVMC includes TSA II 5% SB agar, Chocolate II agar, and BHI broth. The laboratory cost

for performing a single negative CSF culture is approximately $9.50: $7.98/BHI Broth, $0.19/Choc II Agar, $0.18/TSA II 5% SB, and approximately $0.50 for the microbiologist's time required for setup and identification. Overall cost for microbiologist's time deviates largely based on sample plate volume, and overall workflow required daily. Therefore, the number used here isn't a perfect number, but rather a reference point. Positive culture costs can vary largely depending on organism replating, use of the microscan panels, gram stains, secondary biochemicals, and various other testing. No testing is going to be exactly the same from patient to patient, because of the nature of microbial growth. This study isolates a few common biochemicals tested in the microbiology laboratory to help determine the cost of performing a positive culture at WVMC.

The cost for a few ancillary microbial tests and plates can be seen in Appendix J. To complete the patient as suggested during the reimbursement analysis section, which included CPT codes 87205, 87070, 87073, and 87186, WVMC would need to supply reagents for a gram stain, both aerobic and anaerobic culture plates, biochemical identification reagents, and also a microscan plate for susceptibility analysis. This patient would represent the highest possible cost for this laboratory. If an organism appeared as a gram-positive coccus on the gram stain, a workup would include differentiation between a staphylococcus and a streptococcus. Therefore, catalase reagent would need to be used, if the test appeared positive, coagulase would then be used. If the catalase test appeared negative, streptococcus antigen typing would be performed in this specific laboratory. After presumptive identification, testing is targeted towards identification and antimicrobial susceptibilities via a microscan panel. All testing for a patient that yielded a catalase positive organism, up through the microscan, cost the laboratory

$19.29 in reagent and plating costs, (see Appendix L for a breakdown of the calculations). For a negative catalase gram positive coccus patient, laboratory costs come to roughly $23.14. Adding all addition costs into testing this patient, which includes scientist time, quality control, replating, etc., will likely bring the total cost per patient to the laboratory to roughly $25.00.

At WVMC, the laboratory cost for running the Biofire FilmArray is significantly more, as price per cartridge is $181.42 for each meningitis panel, and $114.75 for each respiratory panel. Additionally, quality control must be completed once per month, and with every new lot of pouch reagent. As stated previously, for respiratory panels, the cost of quality control through Maine molecular is $371.00, and the cost for the meningitis quality control is $572.00, which includes enough QC to last for either six months or six lots of reagents, or any variation of the two, six times.[27,28] Furthermore, all CSF meningitis specimens are also plated, and any positive bacterial specimens are worked up as if no molecular testing has been performed, as a test confirmation. Therefore, if a positive meningitis bacterial organism were found, an additional $25.00 would need to be added to laboratory cost, per positive patient tested. Regardless, per WVMC plating procedure, all CSF specimens are plated as described previously. So, all negative plates add an additional $9.81, between plating and gram staining, to the cost of molecular testing.

Discussion

Molecular testing over the last decade has significantly increased the amount of work that can be done in clinical microbiology laboratories. This molecular testing has reduced the amount of time it takes from receiving specimens, to resulting causative agents for underlying

diseases. Since the Biofire Meningitis/Encephalitis Panel (BMEP) and the Biofire Respiratory Panels (BRP) are multiplexed assays, they enable their users to target many potential pathogens at the same time, eliminating the need for guess work. This form of extensive testing has shown to be most beneficial when physicians are unsure of the causative agents. As mentioned previously, a huge advantage of the FilmArray is that the Meningitis/Encephalitis panel specifically contains seven bacterial pathogens and six viral pathogens, while the Respiratory Panel tests for seventeen different viruses and four bacterial pathogens, each of which can quickly be identified in approximately one hour. This separates the FilmArray methodology from other molecular instrumentation, like the Alere-I, in that the FilmArray can do multiplexed identification, but in a slightly longer time frame. The Alere-I, like many other molecular instruments, have extremely quick identification processes, but only have the ability to identify one organism at a time.

The FilmArray instrumentation, along with many other advancements in technology, opens up the opportunity for medical laboratories to reduce the amount of hands-on, scientist time required for testing. The five-minute setup time for the FilmArray is a significant reduction in comparison to identification using older culturing methodologies. The reduction in scientist time provided by newer technologies gives laboratories the ability to allocate scientist time more effectively, enabling scientists to focus more of their attention on other tasks, or to reduce the overall number of scientists working each shift.

In this study, it was determined that the Biofire FilmArray improved turnaround times significantly, in comparison with culturing methods. Introducing the BMEP testing at WVMC provided physicians with CSF test results in 1 hour 37 minutes, which was a vast improvement

from send out and culture testing, at 17 hours 8 minutes, and 80 hours 25 minutes, respectively. The drastic reduction in CSF and respiratory results to physicians, provides a huge advantage for patient care. It rapidly enables a physician to prescribe accurate pharmaceuticals, and decreases the cost of using broad-spectrum drugs. Doing so, also gives the patient a better chance at overcoming their disease, by allowing a smaller window of time before accurate treatments are received. In a study by Soucek and colleagues, it was shown that, depending on hospital size and use, there is a significant cost savings when using the Biofire FilmArray. As seen in the study, in a 350-bed hospital, the savings often came because of decreased broad-spectrum antimicrobial consumption, which completely offset the increased cost of molecular testing per patient. In addition, if a patient was negative for all pathogens on the BMEP, physicians had the ability to stop antibiotic use within as little as 2 hours, versus 48-72 hours, which is typical for traditional methods.[2] This provides two distinct advantages for patients: it decreases the adverse side effects of inappropriate antibiotic use, which could have a small impact on the reduction of antibiotic strains, and also decreases the amount that a patient will be required to pay for that antibiotic use, which will help to decrease the patients total hospital out-of-pocket cost.

In addition to length of time, modern techniques using culture plating and gram stains don't give clinical laboratories the ability to find many viral pathogens discovered in upper respiratory or meningitis infections. Viral cultures are not routinely performed in clinical laboratories because of the time and difficulty of performing them effectively. Typically, viral cultures require a specialized microbiologist with the skills to specifically identify a large variety of viral cytopathic effects. This is often not cost effective for standard clinical laboratories.

Another advantage for choosing the FilmArray over traditional methods is the low volume required for testing. When CSF specimens are collected, they are typically classified as low volume fluids, and generally require multiple tests to be completed on the low volume that is available. This is one of the distinct advantages the Biofire FilmArray has over modern culturing techniques, because the system only requires 200 to 300 µl of fluid. Anytime standard culture is being performed, at least 1 mL of fluid is required, especially if a gram stain is also required. Furthermore, when looking for bacterial meningitis in a CSF specimen, the bacteria may only be detected 50-70% of the time. This poor sensitivity is often due to low concentrations of bacteria present within CSF, as well as the high limit of detection for gram staining (10^4 to 10^5 colony-forming units [CFU]/ml). Approximately 40-50% of patients with bacterial meningitis have bacterial counts less than this, with many specimens containing fewer than 20 to 500 CFU/ml.[30] Testing with the FilmArray essentially eliminates the false negatives that occur when only performing culture and gram staining.

The FilmArray technology is classified under CLIA as being moderate complexity testing, which enables the instrument to be used by both certified professionals and trained professionals without certification.[3,6,10,11] The use of moderate complexity testing has many benefits for patient care. Moderate complexity testing enables laboratories to hire individuals without certification, at a lower wage, which benefits a laboratories productivity costs. This allows them to allocate money to other laboratory facilities where it can better be used for patient care. The use of moderate complexity testing also enables nursing staff to perform laboratory testing at or near the patient bedside, eliminating the need for laboratory professionals to receive specimens.[31] Inevitably, this speeds up turnaround time, because

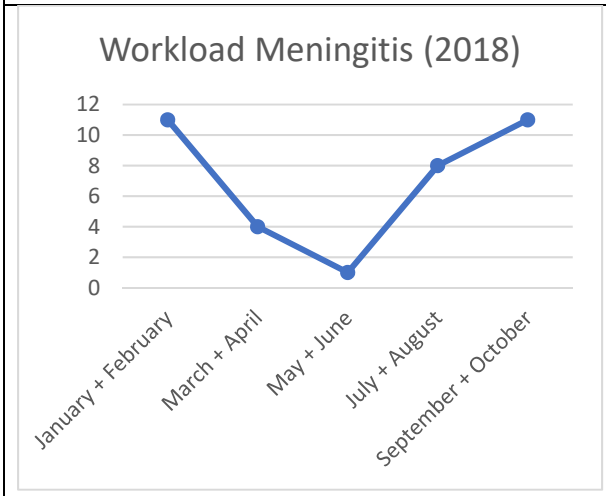
nursing staff can receive specimens directly from the physician's hand, and go directly to test setup.

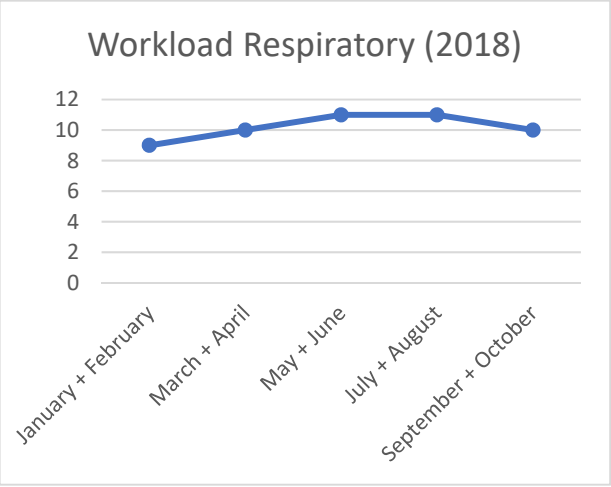
The downside to moderate complexity, is that testing has the opportunity to leave the laboratory, which no longer adds any benefit to laboratories. Moderate complexity testing leads to a decrease in medical laboratory scientist positions, because moderate complexity testing no longer requires individuals who are knowledgeable with test methodology. Instead, individuals must only be knowledgeable with instrument use. This loss of laboratory professionals leads to a rapid decline in people who have spent numerous hours in education learning about test methodology, and inevitable leads to decreased patient safety. Trained personnel will need to adhere to strict standards, and not deviate from procedures learned in their training. In a study by Leber and her colleagues, it was found that multiplex PCR is generally more sensitive than traditional culturing techniques, and antigen detection.[1-3] In molecular testing, if instruments or the work area become contaminated, accurate resulting would then become dependent on personnel who may have little understanding of the test methodology, which could lead to inappropriate pharmaceutical use.

One disadvantage of the FilmArray is seen in the testing of immunocompromised individuals. Many meningitis and encephalitis pathogens can be latent in immunocompromised patients, which can present issues during the testing. For the immunocompromised patient, the physician may not be able to determine if the organism is latent or a true pathogen. This may lead to unnecessary testing and additional costs to the patient. [32]

Unfortunately, these panels do not detect all potential pathogens, and physicians will still need to be aware that a negative panel does not exclude an infection.[1-3,33,34] Examples are

Figure 2. Testing volumes for the Meningitis and Respiratory Panels at WVMC from January thru October 2018.





the exclusion of the Epstein-Barr virus (EBV) on the BMEP, which can have a significant impact on children; the lack of almost all fungal meningitis pathogens, including Histoplasma; and the absence of the West Nile Virus (WNV) on the panel for geographically prevalent populations. [4] If a physician does not recognize that many organisms are missing from these panels, they may delay accurate patient treatment, or fail to accurately treat patients for the microbial agents causing their disease.

Biofire Diagnostics recommends that all negative panels be cultured .[14,15] One reason is to ensure that bacterial false positives are not occurring on the panels. Presented previously, was the example of a non-*Bordetella pertussis* species containing the *IS1001* gene that tested positive for *B. pertussis*. To identify the correct organism, a culture is necessary. The second reason is due to antibiotic resistance, which frequently occurs in clinical settings. The FilmArray only tests for a few resistance genes, which can be an advantage in quickly determining the likelihood for resistance on select organisms. But facilities would still need to determine, through other methods, which antibiotics are best for each patient. Because of these factors,

patient cost will be increased. This places a burden on facilities to accurately identify if the speed of identification for each patient justifies the cost.

As shown during the cost analysis, the overall cost of implementing the FilmArray methodology was $28,776.50. In the following analysis, the methodology is broken down into a monthly basis, with the upfront reagent costs calculated on a month to month accrual. Doing this drops the cost of the instrumentation to just the validation and verification cost alone, at $18,988.40. Each month $1,122.50 accrues for instrument leasing. After the initial $903.00 for quality control (QC) reagents, the purchase of additional QC will be necessary. Based on the current testing volumes performed at WVMC, (as shown in Figure 2), additional QC must be purchased every four months for the respiratory panel, and every five months for the meningitis panel. Using these estimates for QC, an approximate monthly cost is $199.15.

At WVMC, a total of thirty-five meningitis and fifty-one respiratory specimens were completed over ten months in 2018. Adding the tests performed over this time period, allows for a total cost of pouch consumption per month. Approximately 3.5 meningitis specimens and 5.1 respiratory specimens are completed each month. Using Appendix J for pouch cost, an additional monthly cost of $634.97 for meningitis pouches and $585.23 for respiratory pouches can be added. Tech time required for patient and QC samples, brings the total tests performed to nine per month. At five minutes per sample, a total of 45 minutes of tech time is required per month. Using the Bureau of Labor Statistics salary information, as mentioned previously, there would be a total cost of $18.67 per month for staffing.[29] Furthermore, all monthly meningitis specimens are required to be plated onto media, which adds an additional $34.34 per month for negative specimens. Each positive bacterial panel would elevate this number by

Table 2. Monthly Outgoing Charges at WVMC	
Item	**Charges**
Instrument Lease	$1,122.50
QC	$199.15
Reagent Pouches	$1,220.20
Scientist Time @ 45 minutes	$18.67
Plating Specimens	$34.34
Total	$2,594.86

Table 3. Monthly Reimbursement at WVMC	
Item	**Reimbursement**
Panels x 8.6	$4,425.13

an additional $15.00 to $20.00 per patient. Since no CSF panels have been positive in the last year this number is omitted from the final calculation. Using all of these numbers, and excluding external miscellaneous charges such as electricity, paper, etc., the overall cost to perform meningitis and respiratory specimens per month at WVMC is approximately $2,594.86.

Using the 8.6 specimens that are performed monthly, a determination of overall patient income received at WVMC for meningitis and respiratory specimens, using CMS fee schedule charge limit, brings in a monthly total of $4,425.13. The current breakeven point for WVMC, after recovering all of the implementation costs, is projected to be about 10.4 months. Remarkably, no significant findings were identified with specimen workload, from initial startup to nearly a year later. Granted, it's likely that the test is still new enough that providers are still learning to recognize when the methodology could be used effectively.

A few limitations to this study need to be addressed, and are good indicators for future studies in clinical laboratory medicine. Due to the limitations in WVMC laboratory information system, information on patients, prior to the Biofire FilmArray, who had positive CSF specimens requiring a culture workup, could not be found. This limited the ability to correlate length of stay (LOS) between patients prior to the FilmArray, and after implementation of the FilmArray.

LOS is one of the main patient quality indicators, and is important because it directly impacts the risk of hospital-acquired conditions. (LOS also directly impacts the financial, operational, and clinical outcomes by decreasing the costs of patient care, facility expenses, and laboratory supplies and staffing. Additionally, this study did not perform an antibiotic evaluation, which would be required to determine if physicians use the findings from the FilmArray to change their patient's antibiotic consumption.

Future research could build upon this topic by identifying those 150-bed facilities looking to adopt the FilmArray methodology and comparing LOS before and after implementation and identifying any significant differences. In addition, antibiotic studies could also be performed while using the Biofire, to determine if any correlation can be found between decreased antibiotic usage and rapid results provided by the Biofire, as described by Soucek and colleagues in their 350-bed hospital study.[2]

According to the data found in this study, implementation of the FilmArray at WVMC has been largely beneficial to the laboratory, especially with the low sample volumes. A return on investment of less than a year is good for any instrumentation in laboratory medicine. Even with limiting the panels to nine a month, a return of their investment in less than a year, has shown that smaller laboratories can transition to this instrumentation without overwhelming costs, which supports the initial hypothesis.

There are many advantages for implementation of the FilmArray methodology, and the data shown here supports that implementation. The decreased turnaround time in obtaining test results potentially leads to a decrease in patient length-of-stay and an overall increase in the quality of patient care. The recovery time for implementation costs is short which allows

laboratories to benefit from the increase in laboratory test revenue. This added revenue allows laboratories further opportunities to explore new technologies. And because of the presence of highly qualified testing personnel in the laboratory, the FilmArray technology is not difficult to implement, perform, or maintain. The usefulness of the FilmArray is beginning to be more prevalent as laboratories are turning to molecular methods, and its added value should not be underestimated. The added value may lead to unforeseen improvements to laboratories and patient care, which only time can tell.

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Appendix A

Meningitis Panel Organism List and Limit of Detection[14]		
Panel Organism	Strain/Organism Tested	LoD Concentration
Escherichia coli K1	*E. coli* K1, strain C5	1×10^3 CFU/mL
Haemophilus influenzae	*H. influenzae, strain AMC 36-A-1*	1×10^3 CFU/mL
Listeria monocytogenes	*L. monocytogenes,* strain 1071/53	1×10^3 CFU/mL
Neisseria meningitidis	*N. meningitidis*, strain M-1574	100 CFU/mL
Streptococcus agalactiae	*S. agalactiae*, type strain, G19, group B	1×10^3 CFU/mL
Streptococcus pneumoniae	*S. pneumoniae*, strain SV 1, serotype 1	100 CFU/mL
Cytomegalovirus	CMV, strain AD-169	4.3E+03 Copies/mL (100 $TCID_{50}$/mL)
Enterovirus	Coxsackievirus A6, species A	50 $TCID_{50}$/mL
	Coxsackievirus A9, species B	5 $TCID_{50}$/mL
	Coxsackievirus A17, species C, strain G-12	5 $TCID_{50}$/mL
	Enterovirus 10, species D	50 $TCID_{50}$/mL
Herpes simplex virus 1	HSV-1, strain MacIntyre	1.51E+03 Copies/mL (250 $TCID_{50}$/mL)
Herpes simplex virus 2	HSV-2, strain MS	1.29E+03 Copies/mL (50 $TCID_{50}$/mL)
Human herpesvirus 6A	HHV-6A, strain U1102	1×10^4 Copies/mL
Human herpesvirus 6B	HHV-6B, strain HST	1×10^4 Copies/mL
Human parechovirus	HPeV, type 3	500 $TCID_{50}$/mL
Varicella zoster virus	VZV, strain Ellen	1.66E+03 Copies/mL (0.10 $TCID_{50}$/mL)
Cryptococcus neoformans/gattii	*C. neoformans* var. *grubii*	100 CFU/mL
	C. gattii, strain A6MR38	100 CFU/mL

Appendix B

Respiratory Panel Organism List and Limit of Detection[15]		
Panel Organism	Strain Tested	LoD Concentration
Adenovirus	Adenovirus Species A, Serotype 18 ATCC VR-19	7.6E+03 Copies/mL (5.0E+00 $TCID_{50}$/mL)
	Adenovirus Species B, Serotype 7A Zeptometrix 0810021CF	3.9E+01 Copies/mL (5.0E-02 $TCID_{50}$/mL)
	Adenovirus Species C, Serotype 2 ATCC VR-846	3.7E+01 Copies/mL (2.0E+00 $TCID_{50}$/mL)
	Adenovirus Species D, Serotype 37 Zeptometrix 0810119CF	9.0E+00 Copies/mL (5.0E-02 $TCID_{50}$/mL)
	Adenovirus Species E, Serotype 4a S. Carolina/2004, UIRF	3.0E+03 Copies/mL (1.0E+01 $TCID_{50}$/mL)
	Adenovirus Species F, Serotype 41 Tak, ATCC VR-930	1.2E+02 Copies/mL (1.0E+00 $TCID_{50}$/mL)
Coronavirus 229E	Coronavirus 229E ATCC VR-740	6.5E+01 Copies/mL (4.0E-01 $TCID_{50}$/mL)
Coronavirus HKU1	Coronavirus HKU1 Clinical specimen a	2.0E+03 RNA Copies/mL
Coronavirus NL63	Coronavirus NL63 BEI NR-470	5.4E+01 Copies/mL (2.5E-01 $TCID_{50}$/mL)
Coronavirus OC43	Coronavirus OC43 ATCC VR-759	5.6E+02 Copies/mL (3.0E+01 $TCID_{50}$/mL)
Human Metapneumovirus	Human Metapneumovirus 16, Type A1 IA10-2003 Zeptometrix 0810161CF	1.2E+03 Copies/mL (1.0E+01 $TCID_{50}$/mL)
Human Rhinovirus/ Enterovirus	Enterovirus Species D, Serotype 68 ATCC VR-1823	2.6E+01 Copies/mL (3.0E+02 $TCID_{50}$/mL)
	Human Rhinovirus Type 1A Zeptometrix 0810012CFN	3.8E+01 Copies/mL (1.0E-01 $TCID_{50}$/mL)
Influenza A H1	Influenza A H1N1 A/New Caledonia/20/99 Zeptometrix 0810036CF	1.4E+02 Copies/mL (1.0E+03 $TCID_{50}$/mL)
Influenza A H1-2009	Influenza A H1N1pdm09 A/Swine/NY/03/2009 Zeptometrix 0810249CF	3.3E+02 Copies/mL (5.0E-01 $TCID_{50}$/mL)
Influenza A H3	Influenza H3N2 A/Port Chalmers/1/73 ATCC VR-810	2.1E+01 Copies/mL (1.0E-01 $TCID_{50}$/mL)
Influenza B	Influenza B B/FL/04/06 Zeptometrix 0810255CF	3.4E+01 Copies/mL (5.0E+00 $TCID_{50}$/mL)
Parainfluenza Virus 1	Parainfluenza Virus 1 Type 1 Zeptometrix 0810014CF	1.0E+03 Copies/mL (5.0E+00 $TCID_{50}$/mL)
Parainfluenza Virus 2	Parainfluenza Virus 2 Type 2 Zeptometrix 0810015CF	3.0E+01 Copies/mL (5.0E-01 $TCID_{50}$/mL)

Parainfluenza Virus 3	Parainfluenza Virus 3 Type 3 Zeptometrix 0810016CF	3.8E+01 Copies/mL (2.5E+00 $TCID_{50}$/mL)
Parainfluenza Virus 4	Parainfluenza Virus 4 Type 4a Zeptometrix 0810060CF	1.6E+03 Copies/mL (5.0E+01 $TCID_{50}$/mL)
Respiratory Syncytial Virus	Respiratory Syncytial Virus Type A Zeptometrix 0810040ACF	9.0E+00 Copies/mL (2.0E-02 $TCID_{50}$/mL)
Bordetella parapertussis (IS1001)	B. parapertussis A747 Zeptometrix 0801461	6.0E+01 IS1001 Copies/mL (4.1E+01 CFU/mL)
Bordetella pertussis (pxP)	B. pertussis A639 Zeptometrix 0801459	1.0E+03 CFU/mL
Chlamydia pneumoniae	C. pneumoniae TW183 ATCC VR-2282	6.6E+01 Copies/mL (1.0E-01 $TCID_{50}$/mL)
Mycoplasma pneumoniae	M. pneumoniae M129 Zeptometrix 0801579	4.6E+02 Copies/mL (1.0E+01 $TCID_{50}$/mL)

Appendix C

Meningitis/Encephalitis (ME) Panel Verification Results				
	Day 1	**Day 1 (Replicate)**	**Day 2**	**Day 2 (Replicate)**
Pool 1	**Operator: mge7224** *+ Escherichia coli K1* *+ Streptococcus pneumoniae* *+* Cytomegalovirus + Enterovirus *+* Human herpesvirus 6	**Operator: req9415** *+ Escherichia coli K1* *+ Streptococcus pneumoniae* *+* Cytomegalovirus + Enterovirus *+* Human herpesvirus 6	**Operator: mge7224** *+ Escherichia coli K1* *+ Streptococcus pneumoniae* *+* Cytomegalovirus + Enterovirus *+* Human herpesvirus 6	**Operator: Karen** *+ Escherichia coli K1* *+ Streptococcus pneumoniae* *+* Cytomegalovirus + Enterovirus + Human herpesvirus 6
Pool 2	**Operator: mge7224** *+ Neisseria meningitidis* *+ Streptococcus agalactiae* *+* Herpes simplex virus 1 *+ Crpytococcus neoformans/gattii*	**Operator: lwa7268** *+ Neisseria meningitidis* *+ Streptococcus agalactiae* *+* Herpes simplex virus 1 *+ Crpytococcus neoformans/gattii*	**Operator: mge7224** *+ Neisseria meningitidis* *+ Streptococcus agalactiae* *+* Herpes simplex virus 1 *+ Crpytococcus neoformans/gattii*	**Operator: hxu8719** *+ Neisseria meningitidis* *+ Streptococcus agalactiae* *+* Herpes simplex virus 1 *+ Crpytococcus neoformans/gattii*
Pool 3	**Operator: mge7224*** *+ Haemophilus influenzae* *+ Listeria monocytogenes* + Herpes simplex virus 2 + Human parechovirus + Varicella zoster virus	**Operator: mge7224*** *+ Haemophilus influenzae* *+ Listeria monocytogenes* + Herpes simplex virus 2 + Human parechovirus + Varicella zoster virus	**Operator: mge7224** *+ Haemophilus influenzae* *+ Listeria monocytogenes* + Herpes simplex virus 2 + Human parechovirus + Varicella zoster virus	**Operator: req9415** *+ Haemophilus influenzae* *+ Listeria monocytogenes* + Herpes simplex virus 2 + Human parechovirus + Varicella zoster virus
+ indicates positive organisms for each successive run * repeat replicate performed by the same laboratory scientist				

Appendix D

Respiratory Panel 2 (RP2) Verification Results				
	Day 1	**Day 1 (Replicate)**	**Day 2**	**Day 2 (Replicate)**
Pool 1	**Operator: mge7224** + Adenovirus + Coronavirus OC43 + Influenza A H1-2009 + Influenza B + Parainfluenza Virus 4	**Operator: hxu8719** + Adenovirus + Coronavirus OC43 + Influenza A H1-2009 + Influenza B + Parainfluenza Virus 4	**Operator: mge7224** + Adenovirus + Coronavirus OC43 + Influenza A H1-2009 + Influenza B + Parainfluenza Virus 4	**Operator: feu7429** + Adenovirus + Coronavirus OC43 + Influenza A H1-2009 + Influenza B + Parainfluenza Virus 4
Pool 2	**Operator: mge7224/MC** + Coronavirus 229E + Human Rhinovirus/Enterovirus + Influenza A H3 + Parainfluenza Virus 1 + Parainfluenza Virus 2 * Human Metapneumovirus	**Operator: feu7429** + Coronavirus 229E + Human Rhinovirus/Enterovirus + Influenza A H3 + Parainfluenza Virus 1 + Parainfluenza Virus 2	**Operator: mge7224** + Coronavirus 229E + Human Rhinovirus/Enterovirus + Influenza A H3 + Parainfluenza Virus 1 + Parainfluenza Virus 2	**Operator: Connie** + Coronavirus 229E + Human Rhinovirus/Enterovirus + Influenza A H3 + Parainfluenza Virus 1 + Parainfluenza Virus 2
Pool 3	**Operator: mge7224** + Adenovirus + Coronavirus NL63 + Influenza A H1 + Parainfluenza Virus 3 + Respiratory Syncytial Virus *+ Bordetella parapertussis (IS1001)*	**Operator: feu7429** + Adenovirus + Coronavirus NL63 + Influenza A H1 + Parainfluenza Virus 3 + Respiratory Syncytial Virus *+ Bordetella parapertussis (IS1001)*	**Operator: mge7224** + Adenovirus + Coronavirus NL63 + Influenza A H1 + Parainfluenza Virus 3 + Respiratory Syncytial Virus *+ Bordetella parapertussis (IS1001)*	**Operator: cax8378** + Adenovirus + Coronavirus NL63 + Influenza A H1 + Parainfluenza Virus 3 + Respiratory Syncytial Virus *+ Bordetella parapertussis (IS1001)*

Pool 4	**Operator: mge7224** + Adenovirus + Coronavirus HKU1 + Human Metapneumovirus + *Bordetella pertussis (ptxP)* + *Chlamydia pneumoniae* + *Mycoplasma pneumoniae*	**Operator: req9415** + Adenovirus + Coronavirus HKU1 + Human Metapneumovirus + *Bordetella pertussis (ptxP)* + *Chlamydia pneumoniae* + *Mycoplasma pneumoniae*	**Operator: mge7224** + Adenovirus + Coronavirus HKU1 + Human Metapneumovirus + *Bordetella pertussis (ptxP)* + *Chlamydia pneumoniae* + *Mycoplasma pneumoniae*	**Operator: Nicole** + Adenovirus + Coronavirus HKU1 + Human Metapneumovirus + *Bordetella pertussis (ptxP)* + *Chlamydia pneumoniae* + *Mycoplasma pneumoniae*
+ indicates positive organisms for each successive run * False positive result remedied by repeat testing. Repeat testing performed by Mark Covington, Biofire Representative (MC)				

Appendix E

Body Fluid Positives. Turnaround times for positive body fluid cultures at WVMC between January 2017 and February 2018.			
Specimen type	Organism ID	Received*	Resulted*
Peritoneal	Clostridium sp.	1.11.2018 (1306)	1.15.18 (1445)
Synovial	Coag positive Staph	2.21.2018 (1201)	2.24.2018 (0747)
Pleural	Coag negative Staph	12.23.2017 (1646)	12.27.2017 (1023)
Synovial	Coag negative Staph	12.12.2017 (1559)	12.14.2017 (1120)
IR Drainage	MRSA	9.22.2017 (1323)	9.24.2017 (0908)
Synovial	E. coli	8.21.2017 (1211)	8.24.2017 (1105)
Unknown	S. aureus	7.27.2017 (1001)	7.29.2017 (1326)
Synovial	Beta Strep	7.25.2017 (1654)	7.28.2017 (0952)
Unknown	S. aureus	6.12.2017 (0130)	6.15.2017 (1059)
Peritoneal	Coag negative Staph	4.3.2017 (1157)	4.5.2017 (1017)
Synovial	E. faecalis	3.23.2017 (1308)	3.26.2017 (1315)
Peritoneal	Corynebacterium sp.	1.4.2017 (1348)	1.11.2017 (0957)
		Average Completion Time:	80 hours 25 minutes
*Format: Date (Time)			

Appendix F

Introductory Meningitis Panel Turnaround Times (CSF) – January 2018 thru March 2018			
Organism ID	Tech	Received Time	Resulted Time
Negative	KMZ3361	2155	2344
Negative	FEU7429	1759	1928
Negative	RJO8150	1421	1551
Negative	FEU7429	2202	2322
Negative	FEU7429	1623	1747
Negative	FEU7429	1605	1733
Negative	KMZ3361	0139	0315
Negative	CAX8378	1546	1726
Negative	MLBJDG	1157	1412
Negative	CAX8378	1603	1741
Negative	MGE7224	0044	0222
V. Zoster	CAX8378	1721	1905
Negative	MLBJDG	1715	1850
		Average Completion Time:	1 hour 37.4 minutes

Appendix G

Send Out Turn Around Times (CSF)		
Organism ID	Received*	Resulted*
Negative	12.06.2017 (1401)	12.07.2017 (0923)
Negative**	09.16.2017 (1132)	10.02.2017 (1442)
Herpes Simplex Virus	04.04.2017 (1533)	04.05.2017 (0832)
Negative	08.20.2017 (1206)	08.20.2017 (2024)
Negative**	08.22.2017 (1453)	10.02.2017 (1448)
Negative	07.17.2017 (1355)	07.18.2017 (1316)
	Average Completion Time: Removing Outliers:	11 days 18 hours 6 minutes 17 hours 8 minutes
*Format: Date (Time) **Large Outliers based on time received to resulted.		

Appendix H

Introductory Respiratory Panel Turnaround Times			
Organism ID	Tech	Received Time	Resulted Time
Negative	FEU7429	2102	2157
RSV	CAX8378	1946	2045
Adenovirus	RJO8150	1147	1344
Human Metapneumovirus	OPT8136	1954	2130
Human Metapneumovirus	RJO8150	0903	1008
Flu B	IMY7268	2201	2319
Negative	FEU7429	1855	1959
Human Rhino/Enterovirus	OPT8136	2036	2154
RSV	MLBCMH	1415	1518
Human Rhino/Enterovirus + RSV	MLBCMH	1913	2011
RSV	RJO8150	1411	1510
Human Metapneumovirus + RSV	MLBCMH	1015	1106
Human Rhino/Enterovirus	RJO8150	0826	0924
Negative	RJO8150	1338	1443
		Average Completion Time:	1 Hour 9 Minutes

Appendix I

Medicare 2018 Test Reimbursement – Reimbursement values as provided in the CMS Laboratory financial schedule.[22]		
CPT Code	Test	Amount
87070	Culture, Upper Respiratory, CSF, Aerobic	10.64
87073	Culture CSF, Anaerobic	11.66
87081	Culture Screen only (Staph Aureus Screen)	8.18
87076, 87077	Culture Aerobic identity	9.97
87186	Microscan (Minimum Inhibitory Concentration)	10.67
87187	Microscan for susceptibility studies (minimum lethal concentration)	40.17
87205	Gram Stain	5.27
87483, 87633	Meningitis and Respiratory PCR	514.55

Appendix J

Miscellaneous Reagent Costs. Reagent costs to WVMC for purchasing reagents for use in their microbiology department.		
Item:	Cost: [per patient]	Usage Information
Catalase (hydrogen peroxide)	$0.68 / 16 oz bottle (473.176 mL) [0.01¢]	1 drop (or 50 microliters) / test
Coagulase	$66.44 / kit [0.55¢]	120 tests / kit
Strep typing	$219.93 / kit [$4.40]	50 tests / kit
Oxidase	$19.90 / box [0.04¢]	10 tests / ampule 50 ampules / box
Glass slides	$291.00 / shipment [0.19¢]	1500 slides / shipment
Crystal violet	$26.35 / 3785 mL [0.01¢]	1 mL / slide
Decolorizer	$177.00 / 3785 mL [0.05¢]	1 mL / slide
Safranin	$147.11 / 3785 mL [0.04¢]	1 mL / slide
Iodine	$21.21 / 1000 ml [0.02¢]	1 mL / slide
MicroScan panels (+ / -)	$55.04 / 20 panels [$2.75]	1 panel / patient
Brucella anaerobic plates	$2.27 / plate	Approx. 1 plate / patient
Bruc / BBE anaerobic plates	$5.05 / plate	Approx. 1 kit / patient
TSA II 5% SB agar	$0.18 / plate	-
Chocolate II agar	$0.19 / plate	-
BHI broth	$7.98 / Broth	-
Meningitis pouches	5442.60 / Box [181.42]	30 / Box
Respiratory pouches	3442.50 / Box [114.75]	30 / Box
[] – values rounded to the nearest penny		

Appendix K

WVMC FilmArray Test Volumes - Volume of testing performed at WVMC by month from January 2018 thru November 10, 2018.		
Month	Tests Completed	
	Meningitis	Respiratory
January + February	11	9
March	2	5
April	2	5
May	1	6
June	0	5
July	2	4
August	6	7
September	7	2
October	4	8
November 1 – November 10	2	0

Appendix L

Thesis Calculations. A reference for the calculations used throughout the thesis.
Staph = 0.01 + 0.55 + 0.19 + 0.01 + 0.05 + 0.04 + 0.02 = 0.87 (Biochemicals w/ microscan 3.62) + plates = $19.29
Strep = 0.01 + 4.40 + 0.19 + 0.01 + 0.05 + 0.04 + 0.02 = 4.72 (Biochemicals w/ microscan 7.47) + plates = $23.14
Total Implementation Cost = Lease (1122.50) + RP2 Verification Kit (4718) + Meningitis Verif. Kit (5980) + IQCP Meningitis Reagent Pouches (3628.40) + IQCP RP2 pouches (2295) + Scientist Salary (1244.5) = $18988.4 + (Starting reagent) 9788.10 = $28,776.5
Monthly Costs WVMC = Lease (1122.50) + Meningitis Pouches (634.97) + Respiratory Pouches (585.23) + Staffing (18.67) + Plating Costs (34.34) + QC Reagent Costs (199.15) = $2594.86
Monthly Panel Income = Test Performed (8.6) x CMS limit (514.55) = $4425.13
Time to Break even = Implementation Cost (18988.4) ÷ [Monthly Income (4425.13) - Monthly Costs (2594.86)] = 10.38 months