Syndromic Testing using the Biofire FilmArray®

Strengths and Caveats

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Two Really Good References

- Abbott AN and Fang FC. Clinical Impact of Multiplex Sydromic Panels in the Diagnosis of Bloodstream, Gastrointestinal, Respiratory and Central Nervous System Infections. Clinical Microbiology Newsletter. 2017. 39 (19): 133-142.

- Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic Panel-Based Testing in Clinical Microbiology. Clinical Microbiology Reviews. 2018. 31(1): 1-28. PMID 29142077.

Syndromic Testing

- Clinical syndromes are rarely specific for a single pathogen
  - Sepsis
  - Acute respiratory illness
  - Acute gastroenteritis
  - Meningitis
  - Encephalitis
- Rapid & accurate microbial attribution for clinical syndromes identified as a major unmet diagnostic need[1]
  - Can promote directed rather than empirical therapy and/or prevent unnecessary antimicrobial therapy

1. PMID 24200831, 25456043

Multiplex Molecular Syndromic Testing
Potential Benefits

- Reduced turnaround time
- Improved sensitivity
  - Head-to-head with other methods
  - Allows detection of organisms not otherwise test-able
- Simplified testing algorithm
  - Reduction in specimens required for testing
  - Reduction in number of tests that must be ordered, performed
- Laboratory benefits
  - Simplified workflow
  - Potential cost reduction

- Improved clinical decision-making? Improved outcomes?

Multiplex Molecular Syndromic Testing
Current Options

- Respiratory
  - Upper Respiratory*
  - Pneumonia#
- Gastrointestinal*
- Blood culture*
- Meningitis/Encaphalitis#

- * Multiple commercial options
- # Biofire FilmArray® only

Blood Culture Panels

- Rapid & accurate identification, initiation of appropriate therapy is critical to survival in cases of sepsis and hypotension
  - ~3% increase in mortality risk for each hour[2]
- Panels are performed on POSITIVE blood cultures (not all bottles)
- Different approaches lead to differences in TAT, sensitivity, panel comprehensiveness, effectiveness in mixed infections, etc.

TABLE 1 FDA-approved/cleared panel-based molecular assays for detection of select microorganisms and select resistance genes in positive blood culture bottles

Parameter	FilmArray BCID	Verigene Gram-positive blood culture	Verigene Gram-negative blood culture
Total no. of targets	27	15	14
Ability to detect pathogen			
Gram-positive bacteria			
Staphylococcus species	✓	✓	
Staphylococcus aureus	✓	✓	
Staphylococcus epidermidis		✓	
Staphylococcus lugdunensis		✓	
Streptococcus species	✓	✓	
Streptococcus agalactiae	✓	✓	
Streptococcus pyogenes	✓	✓	
Streptococcus pneumoniae	✓	✓	
Streptococcus anginosus group		✓	
Enterococcus species	✓		
Enterococcus faecalis		✓	
Enterococcus faecium		✓	
Listeria species		✓	
Listeria monocytogenes	✓		
Gram-negative bacteria			
Klebsiella oxytoca	✓		✓
Klebsiella pneumoniae	✓		✓
Serratia marcescens	✓		
Proteus species	✓		✓
Acinetobacter species			✓
Acinetobacter baumannii	✓		
Haemophilus influenzae	✓		
Neisseria meningitis	✓		
Pseudomonas aeruginosa	✓		✓
Enterobacteriaceae	✓		
Escherichia coli	✓		✓
Enterobacter species			✓
Enterobacter cloacae complex	✓		
Citrobacter species			✓
Yeasts			
Candida albicans	✓		
Candida glabrata	✓		
Candida krusei	✓		
Candida parapsilosis	✓		
Candida tropicalis	✓		
Ability to detect presence of resistance gene			
mecA	✓	✓	
vanA	✓	✓	
vanB	✓	✓	
bla_{KPC}	✓		✓
bla_{NDM}			✓
bla_{OXA}			✓
bla_{VIM}			✓
bla_{IMP}			✓
bla_{CTX-M}			✓
Time to result (h)	~1	~2.5	~2

2. PMID 16625125, 20048677, 28528569

Excerpt from Ramanan et al

Blood Culture (BCID) Panels
Factors to Consider

- Blood culture bottles are sterile, BUT NOT DNA FREE
  - Documented problems with FilmArray, false positives signals resulting from nucleic-acid contaminating lots
- Newer is NOT always better
  - Difficulty accurately distinguishing *S. pneumoniae* from other *Streptococcus* species
- BCID ≠ Stewardship
  - Implementation of rapid blood culture ID panels in the absence of active stewardship measures does not lead to more appropriate therapy, better outcomes or reduced costs[3]
- CAN help rapidly distinguish likely contaminants (ex. Coagulase-negative *Staphylococcus*) and reduce inappropriate antibiotic use[4]

3. PMID 26329038, 27196015, 27487951, 27678085
4. PMID 26639226, 27543412, 25445120, 26197846

Meningitis-Encephalitis Panel

- Annually in the US[5]
  - 4,100 cases of bacterial meningitis
  - 20,000 hospitalizations resulting from encephalitis
- Delays in antibiotic therapy associated with poor clinical outcome[6]
  - Up to 30% increase in unfavorable outcomes for each hour without appropriate therapy

TABLE 6 Organisms targeted by the FilmArray Meningitis/Encephalitis panel

Parameter	FilmArray Meningitis/Encephalitis panel
Pathogen detected	
Viruses	Cytomegalovirus, enterovirus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, human parechovirus, varicella-zoster virus
Bacteria	*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*
Fungi	*Cryptococcus neoformans-C. gattii*
Analysis platform	FilmArray system or FilmArray Torch
Acceptable specimen type	CSF
Time to results (h)	~1

5. PMID 21612470, 24384647
6. PMID 19000639, 27507415

Excerpt from Ramanan et al

Meningitis-Encephalitis (ME) Panel
Factors to Consider

- ME Panel shows superior sensitivity to culture for bacterial targets[7]
  - Discordances often explained by CSF collected after initiation of antibiotics
- False positives are a risk[8]
  - Low prevalence setting + highly sensitive test = False Positives
  - Common targets for ME and RVP panel – must avoid contamination
- Does NOT detect common causes of nosocomial and shunt-related CNS infections
  - *Staphylococcus*, *Cutibacterium acnes*, GNRs
- Evidence of clinical impact on outcomes, antibiotic use, length of stay and cost is limited[9]

7. PMID 27335149, 28114152
8. PMID 27335149
9. PMID 25542472, 27342782

Gastrointestinal Panel

TABLE 4 FDA-approved/cleared multiplex gastrointestinal panels[a]

Parameter	Verigene EP	Luminex GPP	BioFire GIP
Analysis platform	Verigene system	Magpix or Luminex 100/200 system	FilmArray system or FilmArray Torch
Acceptable specimen type	Stool in Cary-Blair medium	Fresh stool or stool in Cary-Blair medium	Stool in Cary-Blair medium
No. of targets	9	14	22
Ability to detect pathogen			
Bacteria			
Campylobacter species	✓	✓	✓
Salmonella species	✓	✓	✓
Shigella species/enteroinvasive *E. coli*[b]	✓	✓	✓
Vibrio species	✓		✓
Vibrio cholerae		✓	✓
Yersinia enterocolitica	✓		✓
Escherichia coli O157		✓	✓
Enterotoxigenic *E. coli*		✓	✓
Enteropathogenic *E. coli*			✓
Enteroaggregative *E. coli*			✓
Plesiomonas shigelloides			✓
Shiga toxin-producing *E. coli* (stx_1-stx_2)	✓[c]	✓	✓
Clostridium difficile (toxin A/B)		✓	✓
Viruses			
Norovirus GI/GII	✓	✓	✓
Rotavirus A	✓	✓	✓
Astrovirus			✓
Adenovirus 40/41		✓	✓
Sapovirus			✓
Parasites			
Cryptosporidium species		✓	✓
Entamoeba histolytica		✓	✓
Giardia lamblia		✓	✓
Cyclospora cayetanensis			✓
No. of samples (throughput)	1–32 (scalable)	24	1–12 (scalable)
Time to result (h)	<2	~5	~1

[a]EP, enteric pathogens; GPP, gastrointestinal pathogen panel; GIP, gastrointestinal panel.
[b]The Verigene EP and Luminex GPP do not specifically target enteroinvasive *E. coli*.
[c]The Verigene EP has separate targets for stx_1 and stx_2.

Excerpt from Ramanan et al

Gastrointestinal Panel

- Annually in the US[10]
  - 175 million cases
  - 25 million outpatient visits
- Conventional testing options are fragmented/piecemeal and not comprehensive
  - Bacterial: Culture (incapable of growing most diarrheagenic *E. coli*, variable sensitivity for other pathogens)
  - Parasitic: O&P (variable sensitivity, special stains required) and Rapid Antigen Testing (pathogen-specific)
  - Viral: Limited (molecular) options
- Clinical laboratory testing is the backbone of Infection Control and Public Health awareness and investigations

10. PMID 26656915

Gastrointestinal (GI) Panel
Factors to Consider

- More expensive than conventional… but not really
  - Consider labor, "all of the above" ordering practices
- Greater detection of Shiga-toxin-producing *E. coli* helps prevent potentially-harmful antibiotic use[10]
- Recent clinical impact study shows[12]
  - Increased rates of detection
  - Reduced turnaround time
  - Overlap in clinical acuity between those detectable by culture and those detected by GI panel
  - More rapid, more targeted antibiotic prescription
- MUST REMEMBER clinical guideline recommendations:
  - Moderate/severe or prolonged symptoms; immunocompromised

11. PMID 25926491, 26917812
12. PMID 29697761

Respiratory Panel

TABLE 3 FDA-approved/cleared multiplex respiratory panels[a]

Parameter	FilmArray	Verigene	x-TAG RVP	x-TAG RVP Fast	NxTAG-RPP	eSensor RVP	ePlex
Analysis platform	FilmArray system or FilmArray Torch	Verigene system	Luminex 100/200	Luminex 100/200	Luminex Magpix	eSensor	ePlex system
No. of targets	20	16	12	8	20	14	17
Ability to detect pathogen							
Viruses							
Adenovirus	✓	✓	✓	✓	✓	✓ (differentiates subgroup B/E from C)	✓
Coronavirus							✓
Coronavirus HKU1	✓				✓		
Coronavirus NL63	✓				✓		
Coronavirus 229E	✓				✓		
Coronavirus OC43	✓				✓		
Human bocavirus					✓		
Human metapneumovirus	✓	✓	✓	✓	✓	✓	✓
Influenza A virus	✓	✓	✓	✓	✓	✓	✓
Subtype H1	✓	✓	✓	✓	✓	✓	✓
Subtype H3	✓	✓	✓	✓	✓	✓	✓
Subtype 2009 H1N1	✓					✓	✓
Influenza B virus	✓	✓	✓	✓	✓	✓	✓
Parainfluenza virus 1	✓	✓	✓		✓	✓	✓
Parainfluenza virus 2	✓	✓	✓		✓	✓	✓
Parainfluenza virus 3	✓	✓	✓		✓	✓	✓
Parainfluenza virus 4	✓	✓			✓		✓
Respiratory syncytial virus	✓			✓			
Respiratory syncytial virus A		✓	✓		✓	✓	✓
Respiratory syncytial virus B		✓	✓		✓	✓	✓
Rhinovirus/enterovirus	✓	✓	✓	✓	✓	✓	✓
Bacteria							
Chlamydophila pneumoniae	✓				✓		✓
Mycoplasma pneumoniae	✓				✓		✓
Bordetella pertussis	✓	✓					
Bordetella parapertussis-Bordetella bronchiseptica		✓					
Bordetella holmesii		✓					
Time to result (h)	~1	~2–3	~8	~6	~4	~6	~1.5

[a]The acceptable specimen type for all panels is a nasopharyngeal swab. RVP, respiratory virus panel; RPP, respiratory pathogen panel.

Excerpt from Ramanan et al

Respiratory Panel

- Acute respiratory illness (ARI) is among the most common reasons U.S. patients seek ambulatory care, and the most common reason for antibiotic prescription on an ambulatory setting[13]
- Diagnostic alternatives include
  - Culture: Sensitive but slow
  - Rapid diagnostics (antigen, NAAT): Fast but with variable sensitivity, pathogen-specific
- Potential benefits
  - Efficiency and simplicity
  - Reduction in antibiotic use
  - Epidemiological insight

13. PMID 30622156

Respiratory (RP) Panel
Factors to Consider

- Demonstrating clinical impact and/or cost effectiveness has been challenging
  - Use in combination with biomarkers (e.g. procalcitonin) might be necessary to ensure sufficient NPV to reduce antibiotic therapy
  - Clinical interpretation/therapeutic implications debated
    - Bacterial - OK
    - Influenza - OK
    - RSV - OK
    - “OTHER” ???
  - Over-utilization is a risk
- Remain attractive for testing high-risk populations
  - Pediatrics
  - ICU
  - Immunocompromised
  - Chronic lung conditions

Pneumonia Panel
(Just received FDA Approval)

Bacteria
(Semi-Quantitative)

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

Atypical Bacteria

Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

Viruses

Adenovirus
Coronavirus
Human Rhinovirus/Enterovirus
Human Metapneumovirus
Influenza A
Influenza B
Parainfluenza Virus
Respiratory Syncytial Virus

Antimicrobial Resistance Gene

METHICILLIN RESISTANCE
- *mec* A/C and MREJ

CARBAPENEMASES
- KPC
- NDM
- Oxa-48-like
- VIM
- IMP

ESBL
- CTX-M

Sample Requirements:
Sputum (including ETA) and BAL (including mini-BAL)

Respiratory (RP) Panel
Factors to Consider

- Potential Benefits (per the company)
  - Increase Diagnostic Yield
  - Decrease turn around time
  - Decrease time to optimal therapy
  - Reduce risk of mortality
  - Reduce Length of Stay, ICU Days, Ventilator Days
  - Aid in 30 day Pneumonia Readmissions
  - Reduce adverse drug effect of empiric antibiotics (i.e. nephrotoxicity, CDI)
- Challenges – Positive Predictive Value and Clinical Interpretation (i.e. “Infection” vs. “Colonization”)
- TBD….

Summary

Table 5. Comparison of multiplex panels and conventional testing

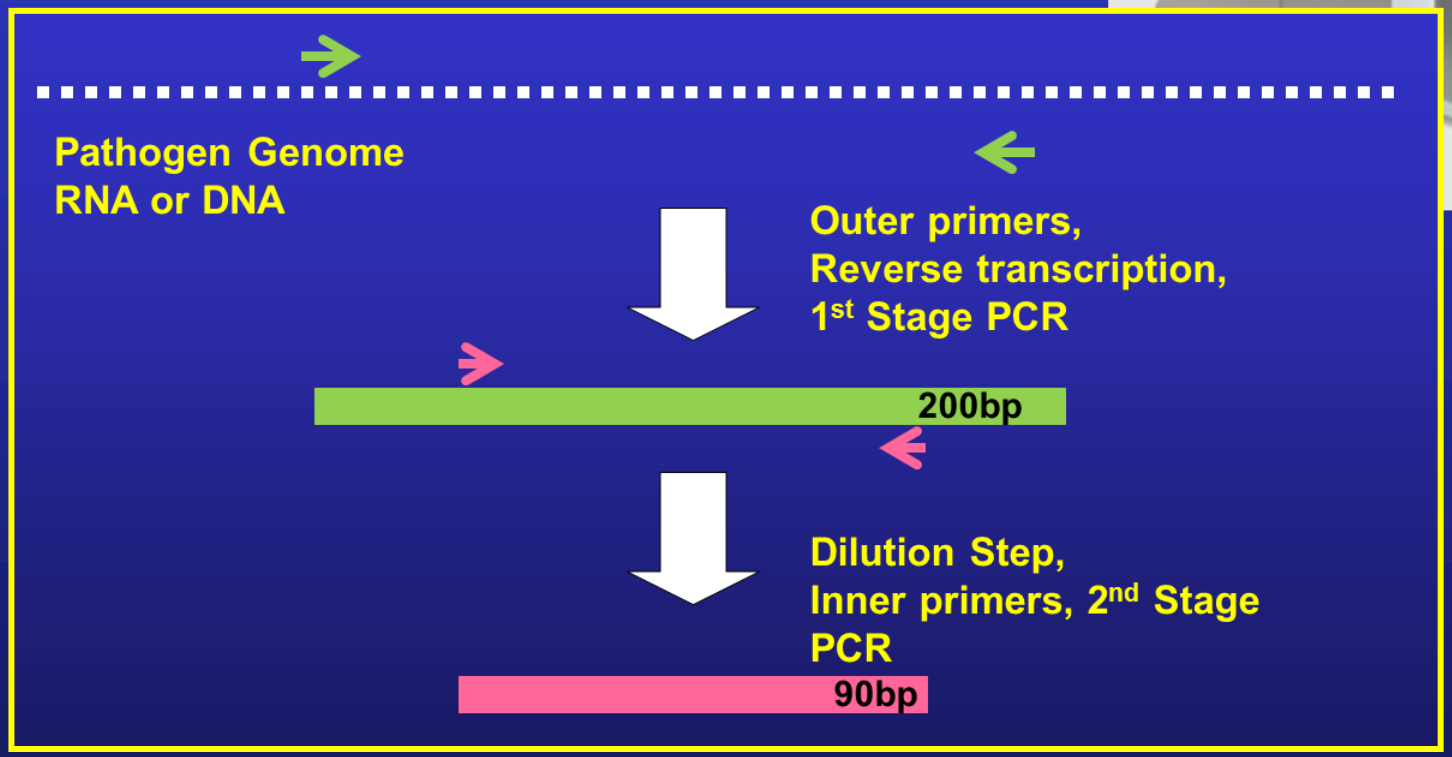
Panel	Advantage	Disadvantage
General considerations	Rapid clinically actionable results	Instrumentation investment
	Improved throughput and workflow	Limitation to targets on panel
	Consolidation of methods	High reagent cost per test
	Enhanced sensitivity	Potential contamination with amplification-based tests
	Reduced dependence on clinical presentation	
	Improved infection prevention and control	
Blood	Enhanced ability to detect resistance mechanisms	Increased laboratory costs
	Targeted therapy	Need to retain conventional testing
	Discontinuation of unnecessary antibiotics	
	Improved clinical outcomes	
	Reduced overall health care costs	
GI	Enhanced detection, including viruses and parasites	Need to retain targeted conventional testing for public health and susceptibility testing
	Reduced transport and storage requirements	Inability to confirm some results
		Lack of therapeutic implications for some detected pathogens
Respiratory	Fewer specimens required	Potential for reduced specificity
	Enhanced detection of viruses and difficult-to-culture bacteria	Lack of therapeutic implications for some detected pathogens
	Ability to track prevalence of circulating viruses	
ME	Improved antimicrobial stewardship	High percentage of false-positive results
	Potential for better clinical outcomes	Need to retain conventional testing

Excerpt from Abbott AN and Fang FC

BioFire FilmArray®

Nested Multiplex PCR (nmPCR)

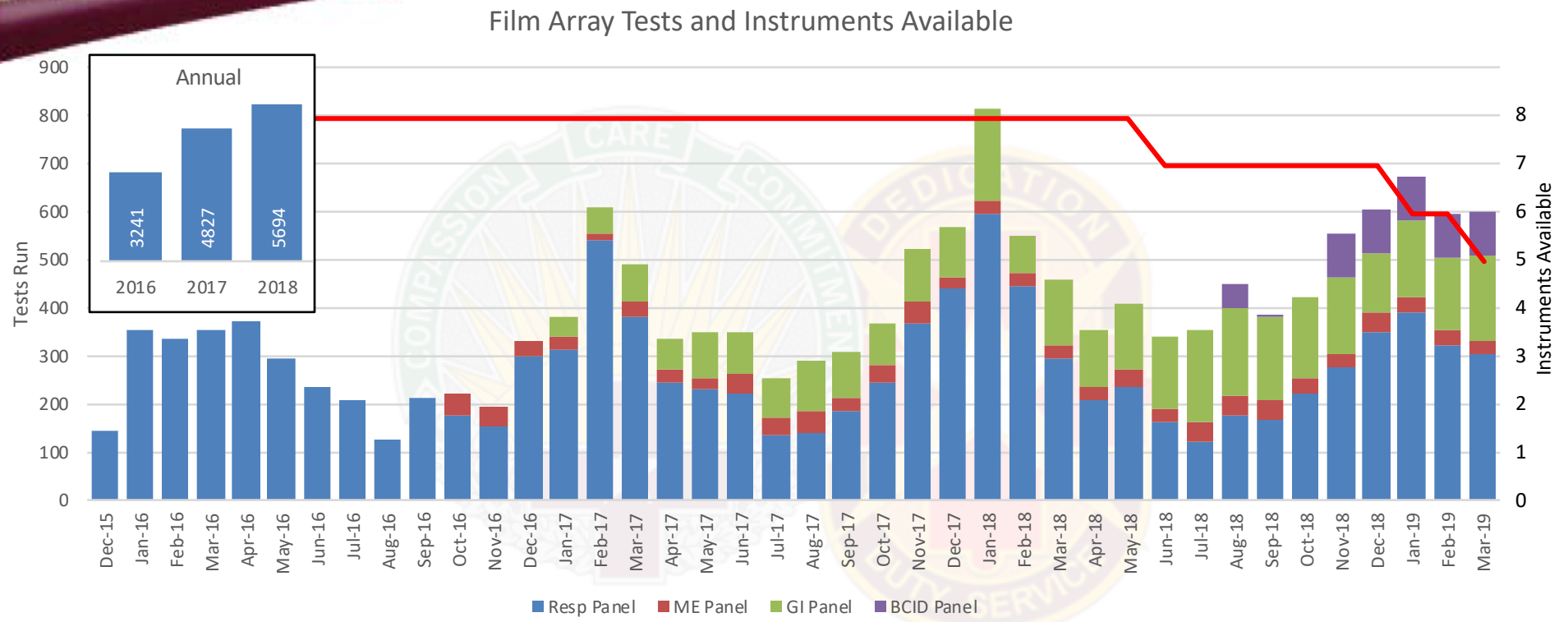
- High-sensitivity assay for large panel of biological agents



BioFire FilmArray®

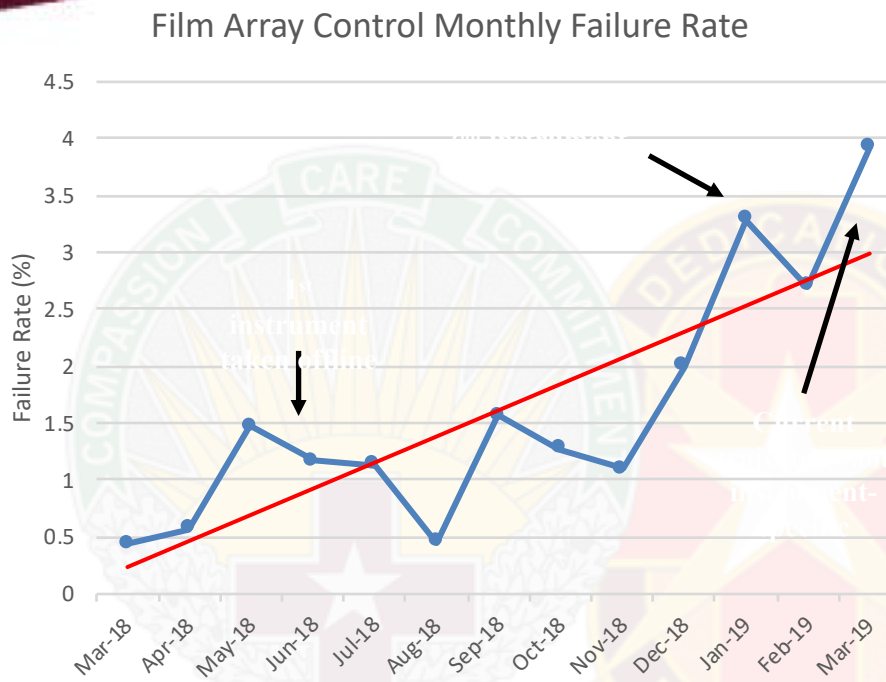
- FDA-approved
  - Respiratory Panel – 20 pathogens
  - GI Panel – 22 pathogens
  - Blood Panel – 24 pathogens and 3 antibiotic resistance markers
  - Meningitis/Encephalitis – 16 pathogens
  - Pneumonia – 26 pathogens and 7 antibiotic resistance markers

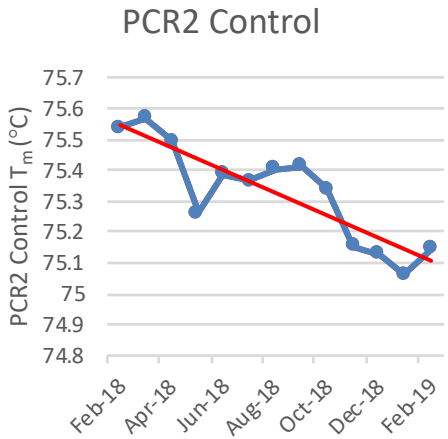
Analysis



- Annual reagent cost: ~$900,000 (Cost to send to LabCorp: $1,250,000)
- Peak Film Array testing occurs during annual respiratory infection season around November to March
- A secondary peak during summer months is possible, depending upon demand for GI testing
- One instrument is reserved exclusively for the ME Panel to mitigate cross-contamination risk

Analysis





- Total instrument failure is preceded by a rising rate of test failures
- Test failures occur when the internal controls do not meet defined specifications (i.e. temperature range) as measured by the instrument's sensors
- Eventually*, the sensors fail and the instrument becomes inoperable

* Per communication with Biofire, instrument failure generally occurs between 1,000-1,200 runs

Analysis

- **Underlying Cause:** Workload exceeded design of instrument
  - Platform was not designed to handle high-throughput testing as employed (Biofire has subsequently released 2nd generation platform [FA Torch])
  - Additional panels (MEP, GIP, BCID) were added after initial implementation, expanding the scope and volume of use
  - Unconstrained ordering of RVP and GIP with year-over-year rises in ordering

- **Result:** Life-cycle projection for instruments was seven (7) years, but we have begun to exceed the MEL in approximately 3.5 years

- **Risk:** Loss of of capability to perform rapid organism identification in cases of bacteremia (BCID), suspected meningitis/encephalitis (MEP)

Thank You