Development of a Laboratory Verification Protocol for Concurrent Detection of Bacterial, Fungal, and Antimicrobial Resistance Genes in a Multiplex Syndromic Joint Infection Panel

M. Cronin[1]; T. Fadgen[1]; B. Hoelcle[2]; R. Young[1]; L. Ogden[1]; J. Green[1]; S. Thatcher[1]

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

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Background

Performance verification is a critical component of implementing a diagnostic test in a clinical lab and can be time consuming and costly. A verification protocol and organism panel were developed in collaboration with ZeptoMetrix®, LLC to verify all analyte detections for the BioFire® Joint Infection (JI) Panel*. The proposed BioFire Joint Infection Panel detects 31 potential pathogens and 8 potential antimicrobial resistance (AMR) genes associated with joint infections from synovial fluid specimens.

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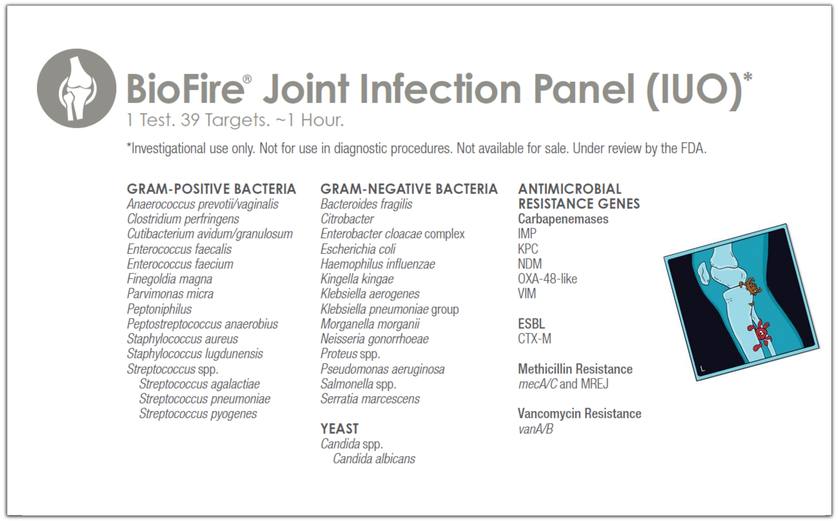
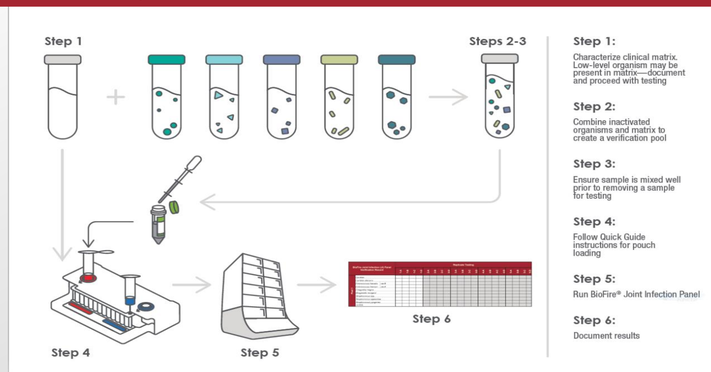


Methods

A protocol was developed using pilot NATtrol™ controls from ZeptoMetrix®, synovial fluid, and the BioFire® FilmArray® 2.0 and the BioFire® FilmArray® Torch Systems. Control materials were tested in the presence of synovial fluid from pooled human donors. Synovial fluid was characterized for Joint Infection Panel targets by screening the specimen on the BioFire Joint Infection Panel prior to starting the verification procedure. The 32 targets required for all analyte detections were divided into 5 pools of 6 to 7 analytes and then tested over multiple days on several systems.

Laboratory Verification Workflow for the BioFire Joint Infection Panel



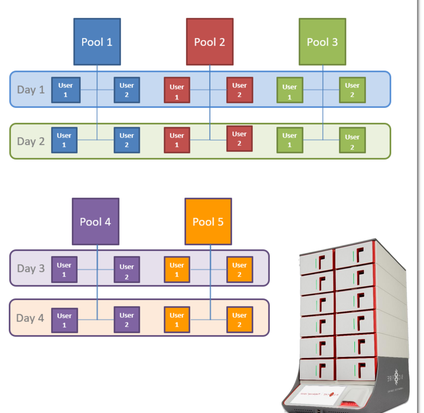
Verification Results for the BioFire Joint Infection Panel

	Organism and Resistance Genes		Summary # Positives	# Possible Positives	# Negatives	# Modules	% Positivity
Pool 1	*Candida*		8	8	33	8	100
	Candida albicans		8	8	33	8	100
	Enterococcus faecalis	*vanB*	8	8	33	8	100
	Enterococcus faecium	*vanA*	8	8	33	8	100
	Finegoldia magna		8	8	33	8	100
	Morganella morganii		8	8	33	8	100
	Streptococcus spp.		8	8	24	8	100
Pool 2	*Streptococcus agalactiae*		8	8	33	8	100
	Streptococcus pyogenes		8	8	33	8	100
	vanA/B		8	8	33	8	100
	Enterobacter cloacae complex		8	8	33	8	100
	Haemophilus influenzae		8	8	33	8	100
	Peptoniphilus		8	8	33	8	100
	Peptostreptococcus anaerobius		8	8	33	8	100
	Serratia marcescens		8	8	33	8	100
	Staphylococcus aureus	*mecA/C + MREJ*	8	8	33	8	100
	mecA/C + MREJ		8	8	33	8	100
Pool 3	*Bacteroides fragilis*		9	9	32	8	100
	Citrobacter		9	9	32	8	100
	Klebsiella aerogenes		9	9	32	8	100
	Neisseria gonorrhea		9	9	32	8	100
	Parvimonas micra		8	9	33	8	89
	Pseudomonas aeruginosa	VIM	9	9	32	8	100
	Streptococcus spp.		9	9	24	8	100
	Streptococcus pneumoniae		9	9	24	8	100
	VIM		9	9	24	8	100

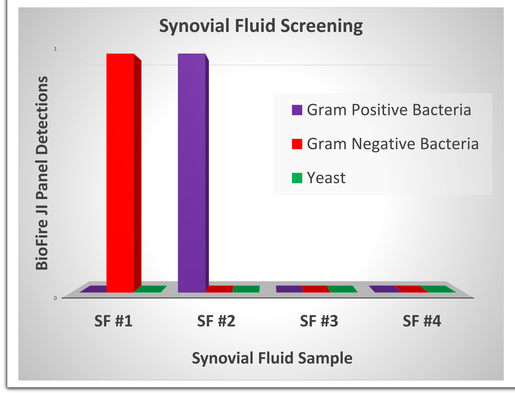
	Organism and Resistance Genes		Summary # Positives	# Possible Positives	# Negatives	# Modules	% Positivity
Pool 4	*Anaerococcus prevotii/ vaginalis*		8	8	33	8	100
	Cutibacterium avidum/ granulosum		8	8	33	8	100
	Escherichia coli	IMP	8	8	33	8	100
	Kingella kingae		8	8	33	8	100
	Proteus spp.		8	8	33	8	100
	IMP		8	8	33	8	100
Pool 5	*Clostridium perfringens*		8	8	33	8	100
	Klebsiella pneumoniae group	KPC-2 (KPC) Z138 (CTX-M & OXA-48 like) Z460 (CTX-M & NDM)	8	8	33	8	100
	Salmonella spp.		8	8	33	8	100
	Staphylococcus lugdunensis		8	8	33	8	100
	CTX-M		8	8	33	8	100
	KPC		8	8	33	8	100
	NDM		8	8	33	8	100
	OXA-48 like		8	8	33	8	100

A total of 41 BioFire Joint Infection tests were performed using the protocol developed and pilot control material (ZeptoMetrix® NATJIP-BIO).

- Expected positives: 263/264 (99.6%)
- Expected negatives: 1008/1008 (100%)
- Antibiotic resistance markers correctly identified when a correlated bacteria was present: 65/65 (100%)
- One missed detection (*P. micra*) was recovered upon re-testing and may be due sample handling of pilot verification materials, or manufacturing variability in the prototype test pouches.



Synovial Fluid Screening on BioFire Joint Infection Panel



Synovial fluid from pooled human donors was tested on the BioFire Joint Infection Panel prior to preparing verification pools.

- Two of the four samples were positive for panel analytes
  - SF#1 reported *Streptococcus* spp.
  - SF#2 reported *Enterobacter cloacae* complex
  - SF#3 and SF#4 were negative for panel targets
- Data presented here used SF #4
- Synovial fluid with JI panel detections may be used- but additional detections may be reported.

Conclusions

- Efficient performance verification may be achieved by combining 32 organisms/8 AMR into 5 pools and can be completed with 20 test runs in 4 days.
- Time to complete:
  - A single BioFire Joint Infection test run was completed in about 55 minutes.
  - Verification workflow of 20 tests completed in 18 hours 18 minutes of system run time.
- The pooling scheme provides multiple positive/negative detections for every BioFire Joint Infection target and sufficient material for running as many as 10 replicates per pool.
- The protocol and controls serve as a useful tool for providing reliable detections of targets over multiple days, operators and systems and offers a flexible solution for supporting verification needs.

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Contact Information: Monica Cronin
BioFire Diagnostics, LLC
Monica.Cronin@BioFireDx.com