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Clinical Feasibility of a Multiplexed Molecular Diagnostic Test for the Identification of Wound Infection Pathogens from Clinical Wound Swab Specimens on BIOFIRE® FILMARRAY® 2.0 and Torch Instruments

Brittany C Collins, Jason Nielson, Andrew Erickson, Sidney Webster, Kimberly Celona, Marianne Kim, David Rabiger
BioFire Defense, LLC, Salt Lake City, Utah, USA

Abstract

Introduction: Commensal or exogenous pathogens routinely infect combat, traumatic, and surgical site wounds posing a great health risk. A diagnostic that identifies ESKAPEE group and other pathogens, and AMR markers directly from wound swabs could help guide treatment decisions for injured warfighters. This work was supported by the Naval Medical Research Command (NMRC) - Naval Advanced Medical Development (NAMD) Program under MTEC award [MT22007.006].

Capability Description: The BioFire panel in development incorporates automated sample prep and multiplex PCR to identify microbial pathogens common to wound infections from a single sample. With minimal hands-on time, a comprehensive result is returned in about one hour. The panel is being developed to discriminate between background levels of commensal species and infectious levels of pathogen within a wound swab specimen.

Methods/Technical Approach: A BioFire panel is being developed to detect microbial pathogens and AMR markers directly from wound swab specimens. Assay sensitivity was titrated to discriminate between contaminating and infectious levels of pathogen using proprietary multiple melt protocols. Archived clinical wound swab specimens, residual from standard of care (SOC) testing, were tested on the panel to assess concordance with reported SOC results.

Results: This panel was evaluated with 433 archived clinical wound swab specimens. The overall positive percent agreement between the panel and standard of care test result was 92.8% [89.4-95.1], demonstrating high preliminary clinical sensitivity of the panel. Furthermore, data from this study suggest that refinements to assay sensitivity cutoffs could enhance the clinical specificity of the panel.

Applicability to Medical Roles of Care: The ability to diagnose wound infections would allow for rapid intervention before an advanced infection is established, reduce difficult-to-treat chronic infections, and reduce the development of antimicrobial resistant pathogens.

Impact to the Warfighter/Significance: The BioFire FilmArray System has been adopted worldwide for infectious disease testing because of its broad capability, ease-of-use, speed, and low logistics burden (no cold chain storage required). A panel for the diagnosis of wound infections in the injured warfighter would be a valuable addition to this portfolio.

Developmental Status of the Technology: TRL4

Disclaimer: The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Department of Defense or the U.S. Government.

Introduction

The term “wound” covers a variety of injuries which may be acute or chronic in nature. In the case of traumatic wounds, the presence of foreign bodies increases the likelihood of contamination with bacterial and fungal species and a resulting infection. Traumatic injuries also often involve damage to a large surface area or multiple areas of the body, significant blood loss, may require limb(s) amputation and/or multiple rounds of surgery, and overall present a major shock to the body. Healing extensive injuries thus presents a challenge even for an otherwise immunocompetent individual and increases the risk of infections caused by opportunistic commensal organisms. The presence of microorganisms in a wound is normal in itself, and microbiota of a wound is known to change over time. Initial microorganisms at the site of a wound may represent contaminants from the environment. After initial wound care, organisms present would be expected to reflect the diversity of commensal microbes found on the skin, with some evidence of pathogenic strains. If an infection develops, proliferating pathogenic microbes begin to dominate. An infection can progress from initial contamination and/or colonization through local infection to a spreading infection and systemic infection.

The prototype BioFire Wound Infection Panel is composed of assays targeting specific bacterial and yeast organisms, as well as separate assays designed to detect specific antimicrobial resistance (AMR) markers associated with the bacterial and yeast organisms in clinical wound swabs eluted in Amies transport medium for use with the BIOFIRE FILMARRAY Systems.

The Wound Infection Panel was developed to use multiple melt protocols, whereby melt analysis is performed after incremental cycling, not just at the end of PCR amplification. Only amplification products detected at an assigned melt cycle are reported as ‘Detected’. Using this approach, assay sensitivity was titrated to facilitate discrimination between a microbial level consistent with invasive infection (>1.0E+04 CFU/mL) and a contaminating microbial level (<1.0E+03 CFU/mL).

The BioFire Wound Infection Panel		
Organisms		
Gram-positive Bacteria		
<i>Clostridium perfringens</i>	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.
<i>Enterococcus faecalis</i>	<i>Staphylococcus epidermidis</i>	<i>Streptococcus pyogenes</i>
<i>Enterococcus faecium</i>	<i>Staphylococcus lugdunensis</i>	
	<i>Staphylococcus aureus</i>	
Gram-negative Bacteria		
<i>Acinetobacter calcoaceticus-baumannii</i> complex	<i>Klebsiella aerogenes</i>	<i>Pseudomonas aeruginosa</i>
<i>Enterobacter cloacae</i> complex	<i>Klebsiella pneumoniae</i> group	<i>Stenotrophomonas maltophilia</i>
<i>Escherichia coli</i>	<i>Proteus</i> spp.	
Yeast		
<i>Candida</i> spp.		
<i>Candida auris</i>		
Antimicrobial Resistance Targets		
<i>bla</i> _{CTX-M}	<i>bla</i> _{VIM}	<i>aac</i> (3)
<i>bla</i> _{IMP}	IS <i>Aba1</i> (ADC, OXA23, OXA51)	<i>aac</i> (6')
<i>bla</i> _{EP} C	<i>mecA/C</i> , MREJ	<i>ant</i> (2'')
<i>bla</i> _{NDM}	<i>qnr</i> (<i>qnrA</i> , <i>qnrB</i> , <i>qnrC</i>)	<i>ant</i> (4')-II
<i>bla</i> _{OXA-48-like}	<i>vanA/B</i>	<i>aph</i> (3')

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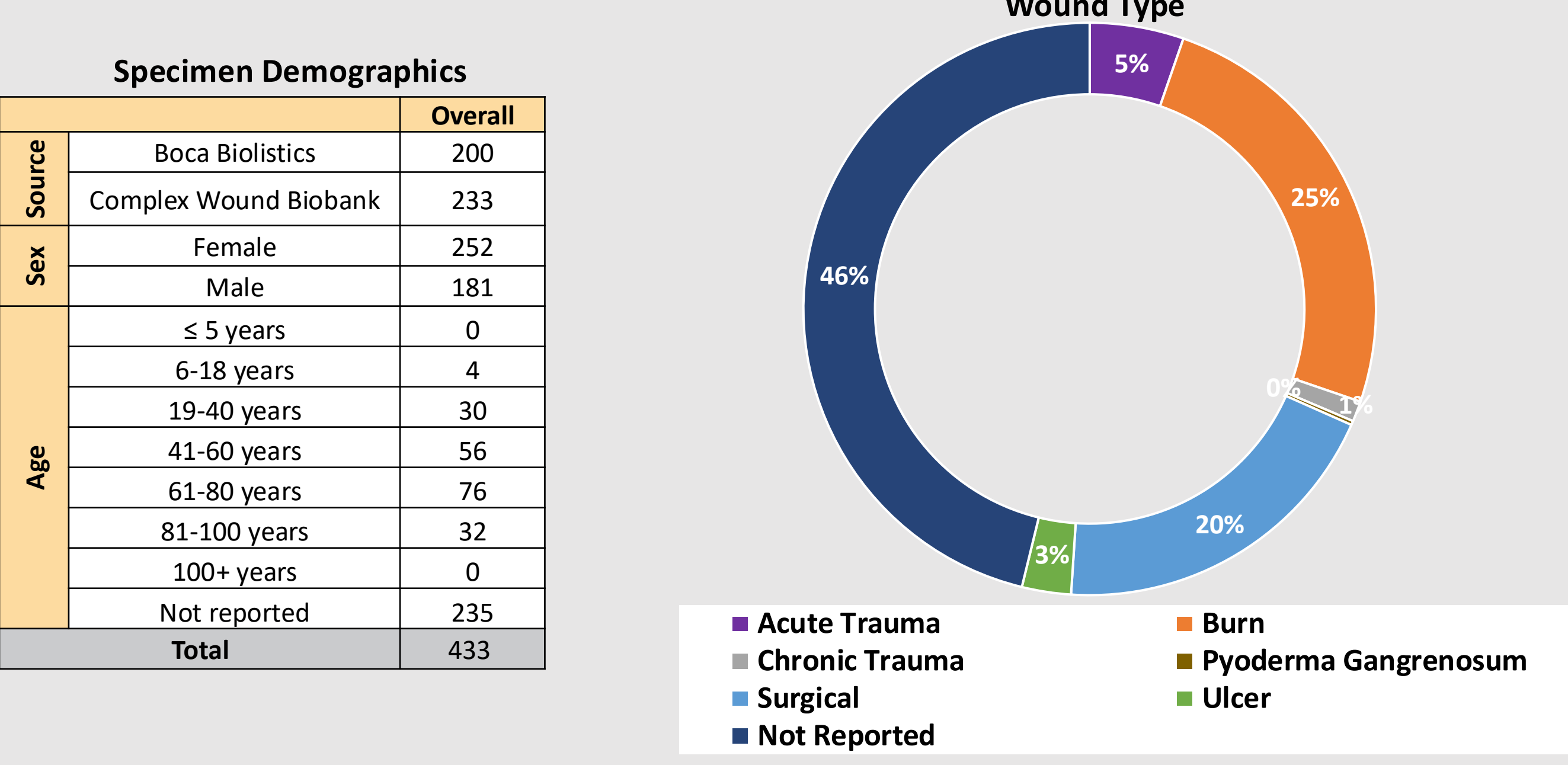


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Study Design

Previously characterized residual (leftover from clinician-ordered standard of care (SOC) testing) wound swab specimens in Amies Media were tested on the prototype BioFire Wound Infection Panel. Panel results were compared to the SOC result to evaluate preliminary concordance. The overall objective of this study was to demonstrate the feasibility of the panel using clinical specimens.

A total of 433 de-identified wound swab specimens were tested on the panel. Specimens were acquired from the commercial biorepository, Boca Biolistics and the clinical site, Complex Wound Biobank (CWB). Testing was performed using three lots of prototype panel pouches split evenly between BIOFIRE 2.0 systems and BIOFIRE Torch systems.



Concordance of the Prototype BioFire Wound Infection Panel and SOC Culture Results

WI Panel	SOC Result		WI Panel Performance	Two-sided 95%CI
	Positive	Negative		
	TP	FP	PPA =100% x (TP / (TP + FN))	Wilson Score Confidence Interval
	FN	TN	NPA = 100% x (TN / (TN + FP))	Wilson Score Confidence Interval
Total	TP + FN	TN + FP		

Organism	PPA	95% CI	NPA	95% CI
<i>Acinetobacter calcoaceticus-baumannii</i> complex	0/0	N/A	400/433 (94.5%)	89.5% - 94.5%
<i>Candida auris</i>	0/0	N/A	428/433 (99.5%)	97.3% - 99.5%
<i>Candida</i> spp.	59/59 (100%)	93.9% - 100.0%	330/374 (91.1%)	84.6% - 91.1%
<i>Clostridium perfringens</i>	0/0	N/A	426/433 (99.2%)	96.7% - 99.2%
<i>Enterobacter cloacae</i> complex	1/2 (50.0%)	9.5% - 90.5%	393/431 (93.5%)	88.1% - 93.5%
<i>Enterococcus faecalis</i>	28/28 (100%)	87.9% - 100.0%	276/405 (72.5%)	63.5% - 72.5%
<i>Enterococcus faecium</i>	7/8 (87.5%)	52.9% - 97.8%	401/425 (96.2%)	91.7% - 96.2%
<i>Escherichia coli</i>	19/19 (100%)	83.2% - 100.0%	350/414 (87.7%)	80.7% - 87.7%
<i>Klebsiella aerogenes</i>	0/0	N/A	424/433 (98.9%)	96.1% - 98.9%
<i>Klebsiella pneumoniae</i> group	21/21 (100.0%)	84.5% - 100.0%	363/412 (90.9%)	84.6% - 90.9%
<i>Proteus</i> spp.	25/26 (96.2%)	81.1% - 99.3%	349/407 (88.8%)	82.0% - 88.8%
<i>Pseudomonas aeruginosa</i>	21/22 (95.5%)	78.2% - 99.2%	308/411 (78.9%)	70.5% - 78.9%
<i>Staphylococcus aureus</i>	115/116 (99.1%)	95.3% - 99.8%	200/317 (68.2%)	57.7% - 68.2%
<i>Staphylococcus epidermidis</i>	5/6 (83.3%)	43.6% - 97.0%	227/427 (57.8%)	48.4% - 57.8%
<i>Staphylococcus lugdunensis</i>	0/0	N/A	345/433 (83.2%)	75.6% - 83.2%
<i>Staphylococcus</i> spp.	103/122 (84.4%)	77.0% - 89.8%	135/311 (49.0%)	38.0% - 49.0%
<i>Stenotrophomonas maltophilia</i>	0/0	N/A	409/433 (96.2%)	91.9% - 96.2%
<i>Streptococcus pyogenes</i>	1/1 (100%)	20.7% - 100.0%	423/432 (98.9%)	96.1% - 98.9%
<i>Streptococcus</i> spp.	36/37 (97.3%)	86.2% - 99.5%	327/396 (86.0%)	78.5% - 86.0%

- Summary
- A majority of observed on panel organisms had a PPA >90%
 - Some organisms with small sample sizes had lower PPA values with wide confidence intervals
 - NPA was more variable amongst the targets ranging from 49.0%-99.5%
 - Staphylococcus* spp. assay (and other *Staphylococcus* species) had comparably lower PPA and NPA values

The BioFire Wound Infection Panel Antimicrobial Resistance (AMR) Gene Detections and Concordance to Standard of Care (SOC) Antimicrobial Susceptibility Test Results

AMR targets	AST Resistance Phenotype
CTX-M, NDM	Amoxicillin
CTX-M, IS <i>Aba1</i>	Augmentin
CTX-M, NDM	Cefotaxime
IMP, VIM	Ciprofloxacin
CTX-M, NDM	Co-Amoxiclav
<i>mecA/C</i> and MREJ (MRSA)	Flucloxacillin
IMP	Gentamicin
<i>mecA/C</i> and MREJ (MRSA)	Methicillin
CTX-M, NDM	Penicillins
CTX-M, NDM	Piperacillin

AMR Target	Total Panel AMR Detections	Panel AMR Detections Concordant with Reported SOC AST Phenotype
<i>aac</i> (3)	29	0
<i>aac</i> (6')-33	0	0
<i>aac</i> (6')-aph(2'')	174	0
<i>aac</i> (6')-I	22	0
<i>aac</i> (6')-II	3	0
<i>ant</i> (2'')	23	0
<i>ant</i> (4')-II	0	0
<i>aph</i> (3')-Ia	55	0
<i>aph</i> (3')-IIb	81	0
<i>aph</i> (3')-IIIa	184	0
<i>aph</i> (3')-VI	2	0
<i>aph</i> (3')-XV	0	0
CTX-M	41	1
IMP	0	0
IS <i>Aba1</i>	4	0
KPC	7	0
<i>mecA/C</i>	161	0
<i>mecA/C</i> and MREJ (MRSA)	59	12
NDM	4	0
OXA	2	0
<i>qnr</i>	32	0
<i>vanA/B</i>	30	0
VIM	1	0

All data presented were obtained with research use only (RUO) versions of the panel. The BioFire Wound Infection Panel has not been evaluated by the FDA or other regulatory agencies for In Vitro Diagnostic use.

BioFire Wound Infection Panel Detections in Specimens Reported as Negative for All Panel Targets by SOC

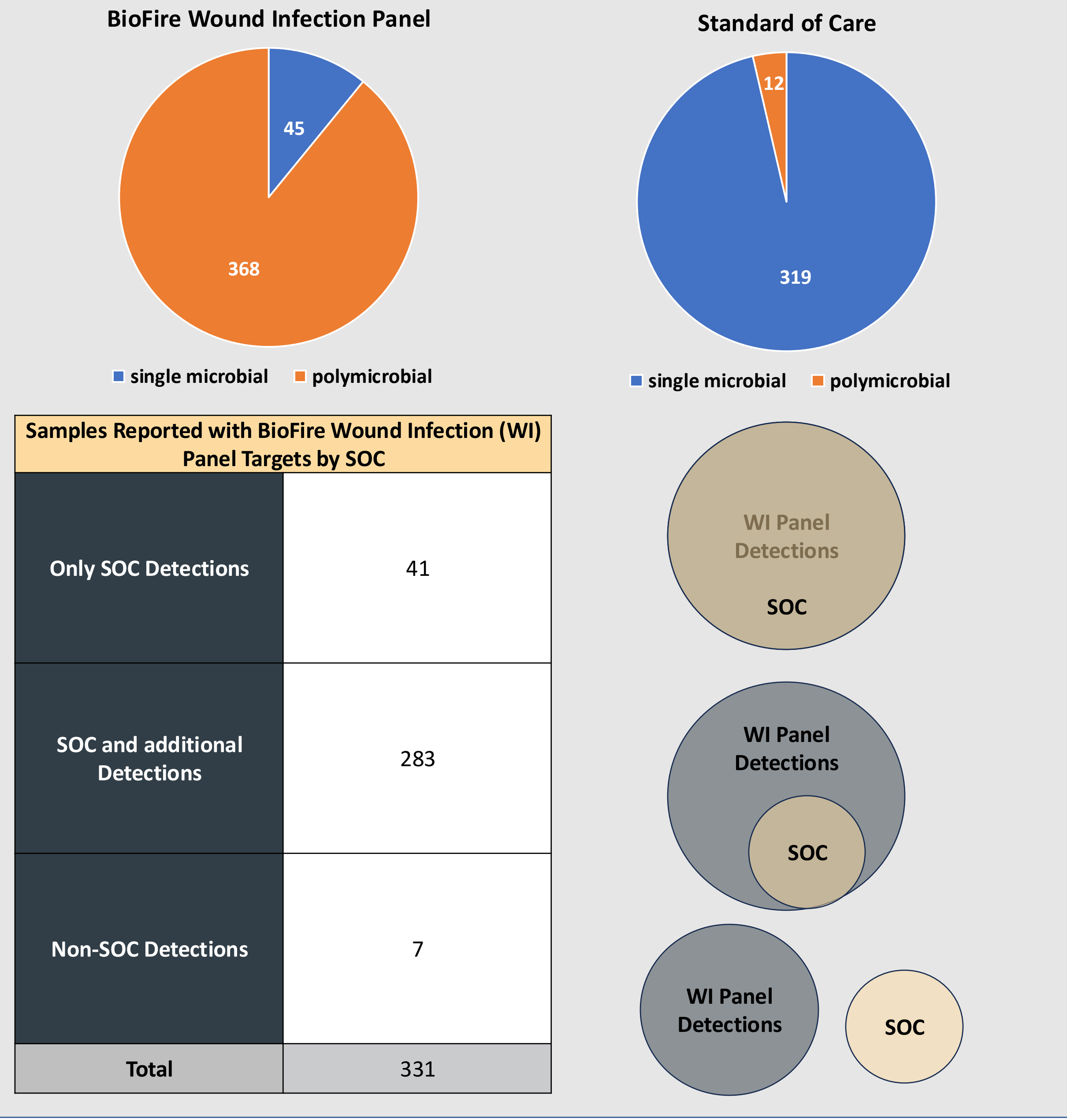
Of the 433 specimens tested 102 were reported as negative by SOC for all Wound Infection Panel targets. Of the specimens reported as negative by SOC the Wound Infection Panel reported one or more detections in 83 specimens (83/102 81.4%). The majority of detections were for *Staphylococcus* species.

Samples Reported as Negative for All Wound Infection Panel Targets by SOC	
Negative by SOC	84
Positive for One or More Organisms Not Reported by Wound Infection Panel	18
Total	102

Organism	Number of Detections in Samples Reported as Negative for All Panel Targets by SOC
<i>Staphylococcus</i> spp.	50/102 (49.0%)
<i>Staphylococcus epidermidis</i>	48/102 (47.1%)
<i>Staphylococcus aureus</i>	30/102 (29.4%)
<i>Enterococcus faecalis</i>	24/102 (23.5%)
<i>Pseudomonas aeruginosa</i>	21/102 (20.6%)
<i>Staphylococcus lugdunensis</i>	20/102 (19.6%)
<i>Escherichia coli</i>	13/102 (12.7%)
<i>Candida</i> spp.	12/102 (11.8%)
<i>Streptococcus</i> spp.	12/102 (11.8%)
<i>Proteus</i> spp.	11/102 (10.8%)
<i>Enterobacter cloacae</i> complex	10/102 (9.8%)
<i>Enterococcus faecium</i>	9/102 (8.8%)
<i>Acinetobacter calcoaceticus-baumannii</i> complex	7/102 (6.9%)
<i>Klebsiella pneumoniae</i> group	7/102 (6.9%)
<i>Stenotrophomonas maltophilia</i>	7/102 (6.9%)
<i>Streptococcus pyogenes</i>	2/102 (2.0%)
<i>Klebsiella aerogenes</i>	1/102 (1.0%)

Polymicrobial Detection Analysis

Of the 433 specimens tested 331 were reported as Positive by SOC for at least one BioFire Wound Infection (WI) Panel targets. Of these 331 specimens, the WI Panel failed to detect the reported SOC result in only 7 specimens. In the remaining 324 specimens, the panel detected only the detected SOC 41 times and detected the SOC along with additional detections 283 times.



Conclusions

- Preliminary data demonstrates high clinical sensitivity of the panel, while also suggesting specificity may need to be refined.
- The variable specificity could have many contributing factors including the panel having higher sensitivity than the culture method, culture results not being reported or being incorrectly reported, or the assays having non-optimal sensitivity cutoffs.
- The *Staphylococcus* spp. assay had the highest rate of unexpected positives and lowest NPA, even with the most conservative melt cycle assignment. There has not been a *Staphylococcus* species detection that was not also covered by the species-specific assays on the panel in the specimens tested. The clinical need for this assay may need to be reassessed.
- Software processing to adjust assay sensitivity cutoffs could be utilized to attempt to tune sensitivity of the panel to what is observed from culture.
- The panel could be modified to provide semi-quantitative results if it is preferred to not tune sensitivity to culture.
- Overall, it can be concluded that the panel has much greater sensitivity than traditional culture methods.