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Implementation Plan to Integrate the Nanosphere Verigene Assay in a High-volume Reference Laboratory and the Anticipated Workflow Benefits to Adopting Rapid Blood Culture Testing

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Implementation Plan to Integrate the Nanosphere Verigene Assay in a High-volume Reference Laboratory and the Anticipated Workflow Benefits to Adopting Rapid Blood Culture Testing

By

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A culminating capstone project report submitted to the faculty of Dominican University of California in partial fulfillment of the requirements for the degree of Masters in Clinical Laboratory Sciences in the Biological Sciences.

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This capstone project paper, written under the direction of the candidate's thesis advisor and approved by the department chair, has been presented to and accepted by the department of Biological Sciences in partial fulfillment of the requirements for the degree of Masters in Clinical Laboratory Science. The content and research methodologies presented in this work represent the work of the candidate alone.

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ABSTRACT

Laboratory processing pathways have a significant impact on the overall management of patients with sepsis. Retrieval and isolation of the suspected pathogen from a patient blood culture specimen is required for a definitive diagnosis of bacterial septicemia. Reference laboratories are high-volume facilities most often located some distance away from the collecting facility. Given the lengthy work up already required for blood culture pathogen analysis, reference laboratories must identify ways to optimize every step of the blood culture pathway in the effort to decrease turnaround time and mitigate lag time to final pathogen identification incurred by prolonged collection-to-incubation times. Rapid molecular diagnostic methods independent of culture results is an available potential solution. The focus of this paper is to consider published literature on the evaluation of rapid blood culture testing to identify its potential benefits and ultimately layout a properly developed implementation plan that integrates the Verigene microarray-based rapid blood culture testing system (Nanosphere, Northbrook, IL, USA) into the blood culture workflow at a high-volume reference laboratory.

KEYWORDS: Blood cultures, gram-positive, sepsis, Verigene, workflow, process mapping, reference laboratory, turn-around time

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Introduction: The need for rapid blood culture testing

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. This definition of sepsis was recently revised in 2015 to 1) reflect the overall understood syndromic pathobiology of the clinical condition and 2) to differentiate its need for urgent recognition in comparison to other types of infections (1). It is reported that approximately 582,000 episodes of septic infections in North America are documented per year and at least 250,000 Americans die from sepsis each year, making sepsis the leading cause of death by infection in the United States (2,3).

An increase in mortality rate is directly related to delays in reportable results for infections considered medical emergencies thus, in the case of sepsis, any delays prove significant when appropriate therapy is dependent on pathogen identification and susceptibility turnaround time (4,5). Retrieval and isolation of the suspected pathogen from a patient blood culture specimen is required for a definitive diagnosis of bacterial septicemia. This process can take between 1 and 3 days after a presumptive positive flag has been identified (5).

Laboratory processing pathways have a significant impact on the overall management of patients with sepsis and delays to appropriate patient care can be observed anywhere from collection to reporting (8). Pre-analytical factors like whether a blood culture is processed in the same facility where bedside collection took place or processed at a core laboratory away from bedside collection location are important to consider when the concern for turnaround time is as urgent as is with blood culture testing. Core laboratories, also known as reference laboratories are high-volume facilities that process anywhere between 100,000 specimens a year to over 1,000,000 specimens a year and are most often located some distance away from the collecting facility (6). Given the lengthy work up already required for blood culture pathogen analysis, reference laboratories must identify ways to optimize every step of the blood

culture pathway in the effort to decrease turnaround time and mitigate delay time to final pathogen identification incurred by prolonged collection-to-incubation times.

While the need for rapid microbiology results was discussed in literature dating back to the 1980's, rapid molecular testing platforms within microbiology laboratories have only begun to be a norm during the last decade (8). Culture-based methods to identify pathogens remain the gold standard and prove necessary as comprehensive susceptibility information is required for appropriate therapy however, results obtained from rapid molecular testing platforms, independent of culture, have recently been acknowledged as necessary (9). The focus of this paper is to consider published literature on the evaluation of rapid blood culture testing to identify its potential benefits and ultimately layout a properly developed implementation plan that integrates the Verigene microarray-based rapid blood culture testing system (Nanosphere, Northbrook, IL, USA) into the blood culture workflow at a high-volume reference laboratory. Due to unforeseen proprietary issues the high-volume reference laboratory name and location will remain undisclosed.

Benefits to implementing rapid blood culture testing

Most documented benefits to adopting rapid blood culture testing are centered around the clinical aspect of patient care. Separate studies performed by MacVane et al., and Perez et al., demonstrate a significant impact to effective therapy selection as well as a more rapid approach by providers to utilize narrow-spectrum antibiotics (11,12). This prompt de-escalation of antimicrobial therapy is relevant not only to hospitalized patient outcomes but also directly related to hospital costs. Documented analysis of patient hospital length of stay and total hospital cost between two controlled studies at one hospital, identified nearly $20,000 average reduction in cost when comparing pre- and post-rapid pathogen identification platform integration (12). It is important to note that the aforementioned studies demonstrate maximum benefits are obtained when appropriate action is taken by antimicrobial stewardship programs in place (11,12).

A more global benefit to implementing rapid blood culture testing is the additional role that clinical microbiology laboratories can play on decreasing the selective pressure for pathogen resistance. Rapid blood culture testing platforms provide pathogen identification and information about clinically relevant resistance markers with high accuracy when compared to conventional methods (10). Reducing the time required for identification and susceptibility reporting from 48 – 72 h to less than 24 h, after positive flag, may have a potential impact on lessening the spread of multidrug resistant bacterial strains through the de-escalation of antimicrobial therapy. The United States government, in line with Center for Disease Control guidelines, identifies the need for the use of rapid and innovative diagnostic tests for the identification and characterization of resistant bacteria (14). Delays associated with the normal turnaround time of conventional microbiology methods leads to extended periods of treatment with broad-spectrum empiric therapy and although considered the appropriate choice for treating early stages of septicemia, can add to the selective pressure for pathogen resistance (13). Sautter et al. looked at the role that Labs have on lowering infection rates of multi-drug resistant infections and point out that laboratory administrators should consider the value in information that laboratory results provide on a larger scale when making decisions to support equipment that provide rapid diagnostic results (13).

Time is of the essence when it comes to definitively diagnosing sepsis. Blood culture bottles should be delivered to the processing laboratory soon after collection to avoid instrument incubation loading delays and ultimately time to pathogen detection delays (13). Pre-analytical delays incurred by processing blood cultures at a reference laboratory may inadvertently pose a risk to patient treatment by prolonging the time to detection of organisms. Although manufacturers specify collected blood culture bottles may be held at 25°C for up to 24 h prior to loading into a continuous-monitoring blood culture instrument without compromising results, a controlled study suggests temperature and holding time before incubation can lead to an increase in time to positive detection as well as a decrease in organism detection as a whole (13). Rapid

blood culture testing platforms offer clinically useful information on average 1.5-1.7 days sooner than the results obtained by conventional methods (11,15). If pre-analytical factors like specimen transport cannot be modified then the opportunity for improving turnaround times through testing platforms that provide rapid diagnostic results should be strongly considered.

Comparable assays: Verigene vs FilmArray

The main focus of this paper is to develop an implementation plan to integrate the Verigene assay into the positive blood culture workflow of a high-volume reference laboratory. Although a number of nucleic acid diagnostic assays are available for rapid pathogen detection directly from blood culture, the Verigene platform has been previously chosen by administrators and validated for use by research and development at the reference laboratory. Reasoning for choosing the Verigene platform over others on the market was not disclosed. In an effort to make an informed conclusion about the Verigene assay a brief comparison to FilmArray (BioFire Diagnostics, Salt Lake City, UT), a similar rapid blood culture identification assay, will be made. Both Verigene and FilmArray are qualitative multiplexed testing platforms that use slightly different nucleic acid technologies to detect multiple pathogens and select genetic determinants directly from positive blood culture bottles (16-18).

The Verigene molecular assay detects nucleic acid targets of the pathogens listed in Table 1 via a microarray-based capture and mediator oligonucleotide system in the self-contained single use blood culture gram-positive test cartridges (BC-GP) and the single use blood culture gram-negative test cartridges (BC-GN). Verigene technology (Processor SP and Verigene Reader) requires minimal hands on time for nucleic acid extraction; after blood culture gram stain evaluation, 350uL and 700uL of well mixed positive blood culture specimen is respectively added into the required BC-GP or BC-GN test cartridge aliquot well and programed into the Processor SP (VPSP). In this closed system, bacterial DNA is extracted, denatured, fragmented and allowed to hybridize

with target specific oligonucleotides covalently bound to a glass microarray slide inside the Verigene Test Cartridge (VTC).

TABLE 1 : IDENTIFICATION AND RESISTANCE TARGETS

TABLE 1. Identification and resistance targets for Verigene BC-GP/BC-GN and FilmArray BCID assays obtained from Verigene Package Insert and FilmArray Instruction Booklet respectively.

Verigene				FilmArray			
BC-GP		BC-GN		BCID			
Bacterial Targets	Resistance Targets	Bacterial Targets	Resistance Targets	Bacterial Targets			Resistance Targets
Staphylococcus spp.	*mecA*	*Acinetobacter spp.*	CTX-M (*bla* $_{CTX-M}$)	Gram negative	Gram Positives	Fungi	mecA
Staphylococcus aureus	*vanA*	*Citrobacter spp.*	KPC (*bla* $_{KPC}$)				vanA/vanB
Staphylococcus epidermidis	*vanB*	*Enterobacter spp.*	NDM (*bla* $_{NDM}$)	*Enterobacteriaceae*	*Staphylococcus spp.*	*Candida albicans*	KPC
Staphylococcus lugdunensis		*Proteus spp.*	VIM (*bla* $_{VIM}$)	*Escherichia coli*	*Staphylococcus aureus*	*Candida glabrata*	
Streptococcus spp.		*Escherichia coli*	IMP (*bla* $_{IMP}$)	*Enterobacter cloacae complex*	*Streptococcus spp.*	*Candida krusei*	
Streptococcus pneumoniae		*Klebsiella pneumoniae*	OXA (*bla* $_{OXA}$)	*Klebsiella oxytoca*	*Streptococcus agalactiae*	*Candida parapsilosis*	
Streptococcus pyogenes		*Klebsiella oxytoca*		*Klebsiella pneumoniae*	*Streptococcus pyogenes*	*Candida tropicalis*	
Streptococcus agalactiae		*Pseudomonas aeruginosa*		*Serratia marcescens*	*Streptococcus pneumoniae*		
Streptococcus anginosus group [1]				*Proteus spp.*	*Enterococcus spp.*		
Enterococcus faecalis				*Acinetobacter baumannii*	*Listeria monocytogenes*		
Enterococcus faecium				*Haemophilus influenzae*			
Listeria spp.				*Neisseria meningitidis*			
				Pseudomonas aeruginosa			

[1] Includes *S.anginosus, S. constellatus,* and *S. intermedius*

Additional steps with mediator oligonucleotides, gold nanoparticle probes, and a silver enhancement step is required to efficiently detect presence of bacterial targets (Fig. 1). According to manufacturer specifications once hybridization is complete within the VPSP, the VTC can be loaded into the Verigene Reader within 12 hours for accurate detection. A 2- 2.5 h turnaround time from specimen loading to initial results is documented. Technology specifics were obtained from Verigene BC-GP and BC-GN FDA 510(k) Summary submissions (17).

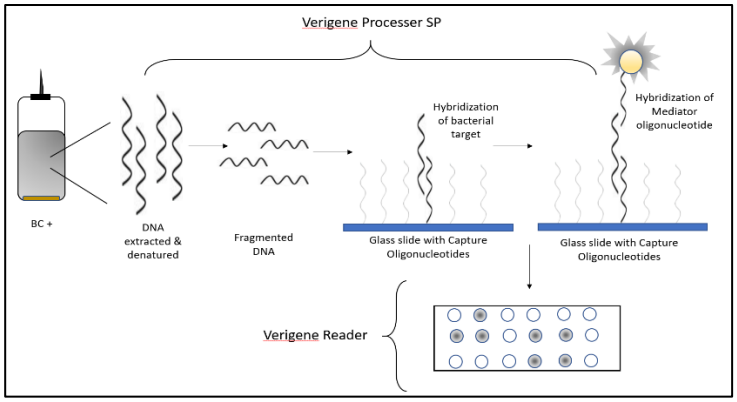

Figure 1. Basic illustration outlining the steps of Verigene technology. Capture and mediated oligonucleotide assay utilizing gold-nanoparticle probe and silver enhancement.

FIGURE 1 : BASIC ILLUSTRATION OF VERIGENE TECHNOLOGY

FilmArray Blood Culture Identification Panel (BCID) detects all of its bacterial nucleic acid sequences (Table 1) simultaneously in one closed system known as the Blood Culture Identification Panel (BCID), through two stages of PCR and an end analysis

of melt curves in replicate. The FilmArray platform has two main components 1) the Loading Station and 2) the FilmArray Instrument (FilmArray Torch). Minimal hands on time is also required to initiate nucleic acid extraction. After hydration fluid has been injected to properly reconstitute the freeze-dried enclosed reagents, 200ul of positive blood culture specimen mixed with sample buffer is added to the sample injection port of the FilmArray Pouch. The FilmArray pouch is described as an enclosed circuit that allows compartmentalized nucleic acid purification in which the first PCR reaction, the second PCR reaction and the end melting temperature analysis takes place. The first PCR reaction is a multiplexed PCR reaction performed to enrich the target nucleic acids in the sample while the second PCR reaction is diluted and mixed with a double stranded fluorescent binding dye to be performed in an array of individual wells, specifically for each target in triplicate, for every diagnostic target. The presence or absence of diagnostic targets are identified by DNA melt curve data collected and compared with internal control data. Technology specifics were obtained from FilmArray (BCID) Panel 510(k) Decision Summary (18).

Pathogen identification differences between the two assays are present and each platform has advantages over the other. The FilmArray BCID for example, detects *Haemophilus influenzae* and *Neisseria meningitidis* both of which are not detected by the Verigene BC-GN assay. Rapid diagnosis of both, *H. influenzae* and *N. meningitidis,* is deemed clinically significant since in both cases patients may worsen rapidly. The Verigene BC-GP panel identifies three Staphylococcus species to genus level: *Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus lugdunensis* whereas the FilmArray assay only identifies *Staphylococcus aureus* to species level. In 2012, a review of sepsis incidence and sepsis pathogen recovery, implicated gram-positive organisms as the most common organisms isolated from positive blood cultures and accounting for 52% to 77% of bacterial sepsis (19). It is an advantage to rapidly identify coagulase negative staphylococcus species (CNS) like *S.epidermidis* and *S.lugdunensis* because of their more recently understood roles as pathogens (19,20). Verigene BC-GP and BC-GN combined offer the ability to detect 9 of the most common

resistance markers including some of the most concerning carbapenamase resistance genes (Table1). Since the first *Enterobacteriaciae* carbapenemase resistant organism was identified in 1993 the concern to rapidly identify these multidrug resistant species has grown (21). The FilmArray BCID assay does not offer detection for extended spectrum beta-lacatam (ESBL) resistance genes, however, it does detect one other gram-negative pathogen to species level that the Verigene BC-GN does not, *Acinetobacter baumanii*. This *Acinetobacter sp.* is recognized as a major drug-resistant organism implicated in nosocomial infections (16).

Assay limitations exist for both platforms and a number of them have been compiled in Table 2. The following limitations described, although not all inclusive, appear to show significant variability in performance and should be considered by laboratory scientists upon final result review.

TABLE 2: ASSAY LIMITATIONS

TABLE 2. Assay Limitations for Verigene BC-GP/BC-GN and FilmArray BCID assays

Verigene BC-GP/BC-GN[1]	FilmArray BCID[2]
Gram stain required for appropriate panel choice	Resin beads contained in blood culture media can cause pouch control failures
Decreased performance in polymicrobial infections	Unable to test for organisms in charcoal containing media
Mixed cultures with *S.epidermidis* and *S.auerus* , *mecA* target cannot be assigned to either isolate	Risk of false positive or false negatives results from improperly collected, transported, or handled specimens
Failure to identify slow growing *Streptococcus sp.*	Decreased perfromance in polymicrobial infections
S. oralis misidentified as *S. anginosus*	If three or more distint organisms detected, retesting is recommened
S.mitis/oralis misidentified as *S.pneumoniae*	Blood culture media may contain non-viable organisms/nucleic acid at levels detected by BCID panel
Unable to distinguish *E.coli* from *Shigella sp.*	Potential amplicon contamination due to inappropriate pouch disposal
Aerococcus spp . strains may cross-react with BC-GP *Staphylococcus spp* . Probes causing a false positive	Antimicrobial resistance cannot be linked to organisms in mixed cultures
Cross reactivity between *K.oxytoca* and *K.pneumoniae*	May not detect all *Streptococcus sp.*
CTX-M negative does not exclude presense of another ESBL resistance gene	May not detect all *S.pneumoniae* serotypes with same sensititvity
Potential for OXA false negative	Cross reactivity between *E.coli* and *Shigella sp.*
Detection of KPC, OXA, NDM, VIM, IMP; many not always infer resistance to carbapenems	Cross reacivity of *S.marsences* to *P.aueriginosa, P. putida, Pantoea sp.*
Invitro resistance to ceftazidime/ceftriaxone is not always demonstrated for organism with CTXM detected	*Routella ornitholytica* misidentified as *K.oxytoca*

[1] Obtained from Verigne BC-GP and BC-GN product inserts
[2] Obtained from FilmArray Instruction Booklet

Both FilmArray and Verigene BC-GP/BC-GN demonstrate reduced sensitivity in accurately detecting organisms directly from polymicrobial blood cultures (18,10). When compared to other rapid identification platforms, literature documents that the BC-GP assay more successfully identifies at least one organism from polymicrobial blood cultures (21). Misidentification of organisms is shared between FilmArray BCID and Verigene BC-GP/BC-GN; both platforms are unable to distinguish between *Escherichiae coli* and *Shigella sp.*, the BC-GP misidentifies *Streptococcus mitis/oralis* as *Streptococcus pneumoniae*, BC-GN shows cross reactivity between *Klebsiella oxytoca* and *Klebsiella pneumoniae*, BCID may not be able to detect all Streptococcus sp. and also shows misidentification of *Serratia marcesens* as *Pseudomonas aeuriginosa*, *Pseudomonas putida* or *Pantoea sp.* (10,18,21). Procedural limitations for FilmArray BCID include the inability to use charcoal containing media for testing and the potential for pouch control failures due to resin beads contained in blood culture media (18). The latter a source of error requiring careful attention by the microbiologist upon initial set up. A gram-stain is an essential part of positive blood culture work up procedures, nonetheless, it is considered a procedural limitation of the BC-GP and BC-GN assays when compared to FilmArray BCID. A correct gram stain interpretation is required in order to choose the appropriate panel for identification on the Verigene platform (16,17).

Implementing molecular diagnostics in a clinical microbiology lab is largely dictated by cost thus it is necessary to mention differences in cost between the two platforms. A general list price inquiry comparison, reveals the unit price for Verigene to be more affordable than the newest FilmArray platform (FilmArray Torch). CAP Today, product comparison webpage, documents a list price of $40,000 for the Processor SP and Verigene Reader required to run the Verigene BC-GP and BC-GN assays (22). It is important to note that one Verigene Reader is adapted to connect up to 32 Processor SP units; moreover, each Processor SP is capable of running one BC-GP or BC-GN at a time, each of which cost $20,000 per unit (22). The list price for the newest FDA approved (2016) FilmArray instrument, the FilmArray Torch, is $85,000 for the base model which includes 2 modules and the system analyzer. Each module of the FilmArray Torch runs

one BCID pouch at a time and one analyzer can adapt up to 12 modules maximum. Additional FilmArray Torch modules are $10,000/2modules (Alex Sterling, Biofire Diagnostics, LLC, Sales Manager, personal communication). To further compare cost, it is important to consider cost per test, however, a definitive value is dependent on a number of variables that can differ among facilities. Blood culture positive volume at the evaluated high-volume reference laboratory could not be disclosed thus cost analysis was not included in this paper but the following values obtained from literature review can be used to approximate a difference in cost per test between assays: FilmArray BCID $129/per test, Verigene BC-GP/BC-GN $60-$99/per test (23-25). An additional reference point for cost was obtained from a direct survey with a laboratory manager at a 200-bed nonprofit community hospital whose microbiology department uses the Verigene BC-GP and BC-GN for rapid blood culture detection. When asked if BioFire FilmArray assay was considered upon integrating rapid blood culture testing, her response was "BioFire is too costly and reimbursement may be a problem for their panels. Verigene is a reasonable in price." Although reimbursement for testing should be considered, it may be more of an issue for a smaller hospital than it may be for a high-volume reference laboratory.

Current blood culture positive workflow and suggested modifications

Besides minor changes like call notification documentation, the blood culture workflow procedure at the evaluated high-volume reference laboratory has remained the same for years. Moreover, new instrumentation with the potential to change workflow processes has not been introduced in over 15 years. This section will aim to delineate the current blood culture positive workflow steps from the pre-analytical to the analytical as well as identify areas in need of improvement.

Before review of the current workflow was performed, a retrospective evaluation of laboratory information system (LIS) documented turnaround time was done for 108 nonduplicate specimens collected between December 2017 and February 2018. Average time from blood culture collection time to instrument load time, average

time to blood culture positive flag from documented load time, and the average time to organism identification from blood culture positive flag time, can be seen in Table 3. All blood cultures are collected in SA Standard Aerobic, SN Standard Anaerobic, PF Pediatric FAN bottles and are incubated in the BacT/ALERT 3D (bioMérieux, Durham, NC, USA) continuous monitoring instrument.

TABLE 3: AVERAGE TURN-AROUND TIME

nonduplicate specimens from December 2017 through February 2018

	3 Month Average	Collection Time to MS ID
Load Time[1]	8 hrs	
Positive Flag Time[2]	8 hrs	44 hrs
MS ID Time[3]	28 hrs	

[1] Collection time to instrument load time avereage

[2]Time to organims growth detected by instrument from load time

[3] Time to MS ID from load time

Steps in the pre-analytical phase cannot be addressed within the scope of this paper but it is important to acknowledge the average time of 8 hours that it takes for a blood culture to be loaded onto the incubation instrument from time of collection(Fig.2). The workup process for the current blood culture workflow follows the general principle of subculture, gram stain review, and gram stain result notification to appropriate provider. Emphasis is placed on prompt incubation of subculture media at optimal conditions (37°C/CO2) to allow adequate bacterial growth for analysis early enough on day two of blood culture positive workflow (Fig. 2).

The evaluated reference laboratory operates on a 24-hour 3 shift system with groups of employees starting at various times: Shift 1 includes groups starting every hour between 6 AM and 10 AM; groups in Shift 2 start every hour between 2 PM and 4 PM (including a limited number starting at 3:30PM); and all employees on Shift 3 start at 10 PM. Scheduling priority is given to the blood culture assignment to avoid gaps between shifts that may potentially lead to unnecessary delays in processing blood culture positives. Moreover, two skilled bacteriologists are scheduled per shift. All shift

members are to continuously perform tasks outlined in the analytical day 1 process (Fig. 2) up to 6 hours of their assigned shift allowing the last two hours for post analytical documentation review of LIS documentation, slide archiving, and paper worksheet completion. The post-analytical steps are not included in Figure 2 nor Figure 4 as they do not affect the overall process of blood culture positive handling.

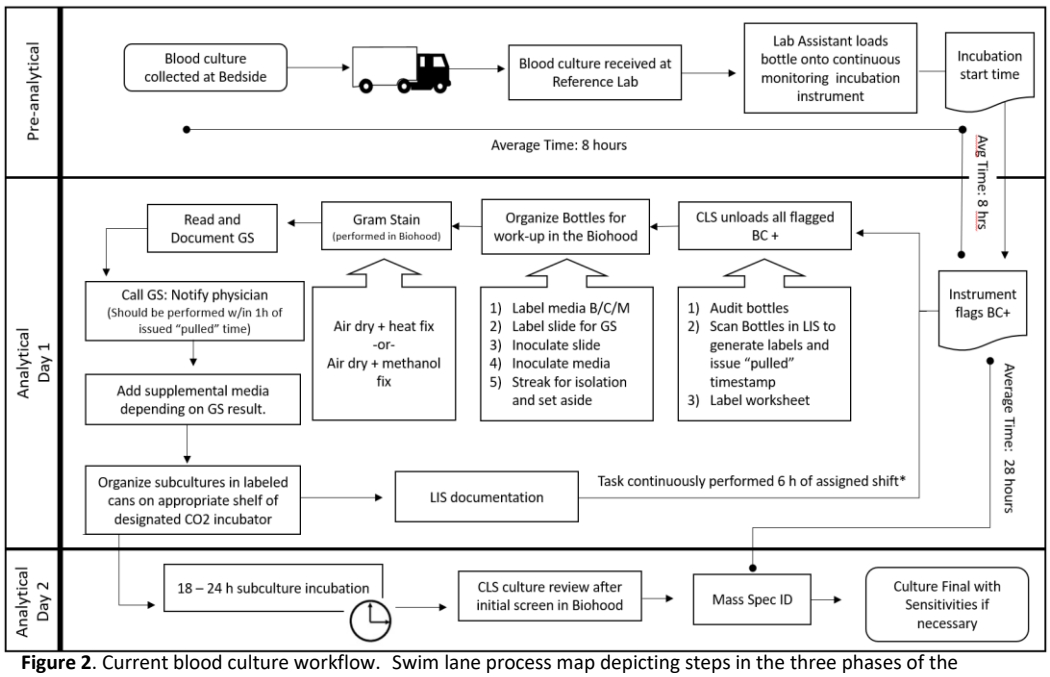

Figure 2. Current blood culture workflow. Swim lane process map depicting steps in the three phases of the current blood culture workflow. Clock symbol indicates incubation time required. "Pulled" refers to positive blood culture bottle removed from continuous monitoring incubation instrument.

FIGURE 2: CURRENT BLOOD CULTURE WORKFLOW

Although an advantage of using MALDI-TOF MS (Matrix assisted laser desorption ionization-time of flight mass spectrometry) for pathogen identification on day 2 of analysis exists, a review of the current blood culture workflow process highlights the prolonged amount of time it still takes for organism identification information to reach clinicians (Fig. 3). Summing the average amount of time it takes to update a report with a pathogen ID obtained on MS, with the average time from collection to initial positive blood culture flag, it is clear that efforts to decrease the time to identification still requires improvement in order to prove beneficial to the patient treatment outcome (Table 3).

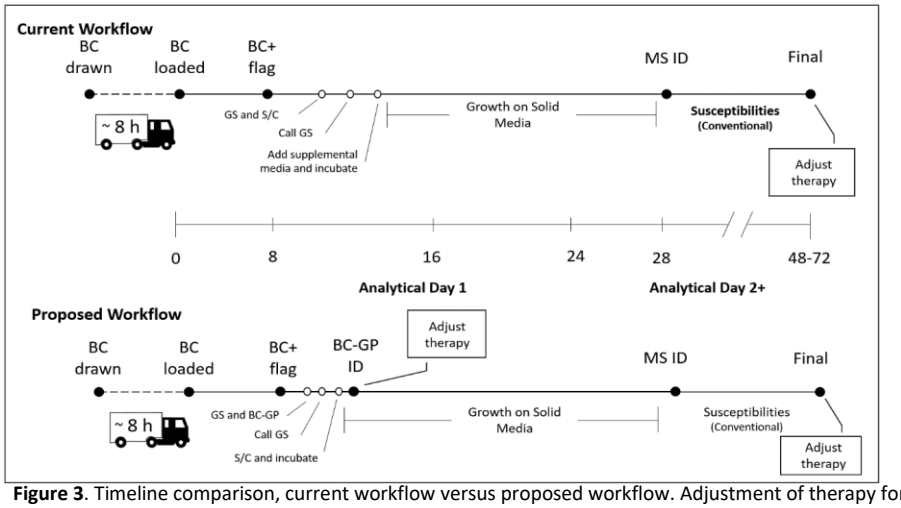

Figure 3. Timeline comparison, current workflow versus proposed workflow. Adjustment of therapy for gram-positive pathogens can be achieved >24 hours sooner by integrating BC-GP assay.

FIGURE 3: TIMELINE COMPARISON

There are other areas, besides the need for rapid identification requiring acknowledgment that time and resources may not be efficiently utilized within the current workflow. For example, literature states the importance of gram stain result notification, regarding it as a "critical value" carrying significant weight to correctly direct appropriate patient care, however, the current pathway places gram stain review second to subculture (26,27). A high-volume reference laboratory may have over six incubation BacT/Alert 3D modules, each with 240 cells for monitoring, thus, the expected throughput of positives per 8-hour shift can be quite high on any given shift. Consideration to the amount of time it takes to organize, document, and subculture a large number of positives before gram stain review should be acknowledged as time directly affecting patient care. The logic behind inoculating solid agar media at the same time as slide preparation is to allow for subculture to be performed as soon as possible in order to maximize incubation time for better culture review on analytical day 2 (Figure 2). This however, is limited because review of blood cultures on analytical day 2 requires supplemental media (i.e. additional selective solid agar media, tube coagulase to rule out *Staphylococcus aureus*, and disk diffusion screen plates) to be included. Furthermore, this additional media to be added is dependent on gram stain morphology. Since supplemental media cannot be added until gram stain review is performed, incubation at the ideal 37°C/CO2 conditions is delayed. This process forces

the bacteriologist in and out of the biohood as follows: 1) To organize biohood with necessary primary media (i.e. blood agar plates, chocolate agar plates, macconkey plates) 2) To label and inoculate solid agar media and gram stain slide 3) To add supplemental media to each respective blood culture and 4) To organize media for incubation after gram stain notification; consequently, allowing room for a decrease in overall work efficiency that may translate over to undesirable mistakes.

Standardization is an important step in optimizing any process and removing variability can lead to better efficiency. The current workflow allows employees to choose between two fixing methods before performing a gram stain: heat fix or methanol fix (Fig. 2). Both fixing methods require blood samples inoculated on a glass slide to air dry completely before fixing for optimal results and use of crystal violet, iodine, decolorizer, and safranin immediately after fixing. A simple study done to compare gram stain fixing methods, found methanol fixing to produce better looking stains over those that were heat fixed such that, a higher number of organisms adhered to slides and a decreased probability of gram-positives over-decolorizing were observed with methanol fixation (28). The former posing great risk to patient care resulting in missed organisms upon gram stain review and the latter commonly a source of error with organisms like *Bacillus sp.* and *Clostridium sp.* even amongst experienced bacteriologists (28). It appears counterintuitive to allow variability in a critical step of a workflow process.

The last topic requiring discussion is that of inter-shift work endorsement. The current process requires a last "pull" of positives 2 hours before end of shift to allow for all work up and post-analytical review of LIS documentation to avoid carry over of unfinished processes to the next shift. Flagged positive blood cultures during this last 2-hours of this work up and documentation review time remain in the automated incubator for the next shift to process. The attempt to minimize this "lag" time between shifts is done by scheduling an overlap between the last person that leaves a shift and the first person that arrives for a shift. Since scheduling problems may arise from time

to time, it may be important to devise a set framework for continuous workflow to avoid turn-around delays for clinically significant results.

Modified blood culture work-up method: Verigene implementation

The initiative to integrate rapid blood culture testing was seriously considered at the evaluated high-volume reference laboratory back in 2015. For undisclosed reasons, the implementation was never followed up. Per discussion with upper management overseeing research and development at the reference lab, interest in introducing the Verigene BC-GP assay into positive blood culture workflow processes exists and it is apparent that it will be more seriously considered upon laboratory expansion later this year. It is the goal of this section to introduce a modified blood culture workflow that integrates rapid blood culture testing for gram positive organisms and considers solutions to the aforementioned opportunities for improvement.

The modified workflow process, in contrast to the current method, puts emphasis on gram stain review and call notification over subculture to address the critical component of gram stain evaluation, as it relates to provider notification and initiation of rapid identification assay (Fig. 4). Immediately after gram stain review, BC-GP panels will be set up for all gram-positive cocci in cluster, gram-positive cocci in pairs and/or chains, and any gram-positive rods isolated from aerobic blood culture bottles not previously identified by rapid assay (Fig. 5). During the 2 h BC-GP assay turnaround time, gram stain notification is performed per shift protocol, media is respectively organized/labeled, subculture in biohood is performed, and subcultures are appropriately incubated at optimal 37°C/CO2 conditions (Fig. 4). Since identification to species level will be obtained for the most common gram-positive blood culture pathogens (Table 1) on analytical day 1 (Fig. 4) the need for tube coagulase to rule out *Staphylococcus aureus* and disk diffusion screen plates specific for gram-positive pathogens is eliminated. Additionally, when the bacteriologist is ready to go into the biohood for subculture, all necessary media will be labeled and ready for inoculation.

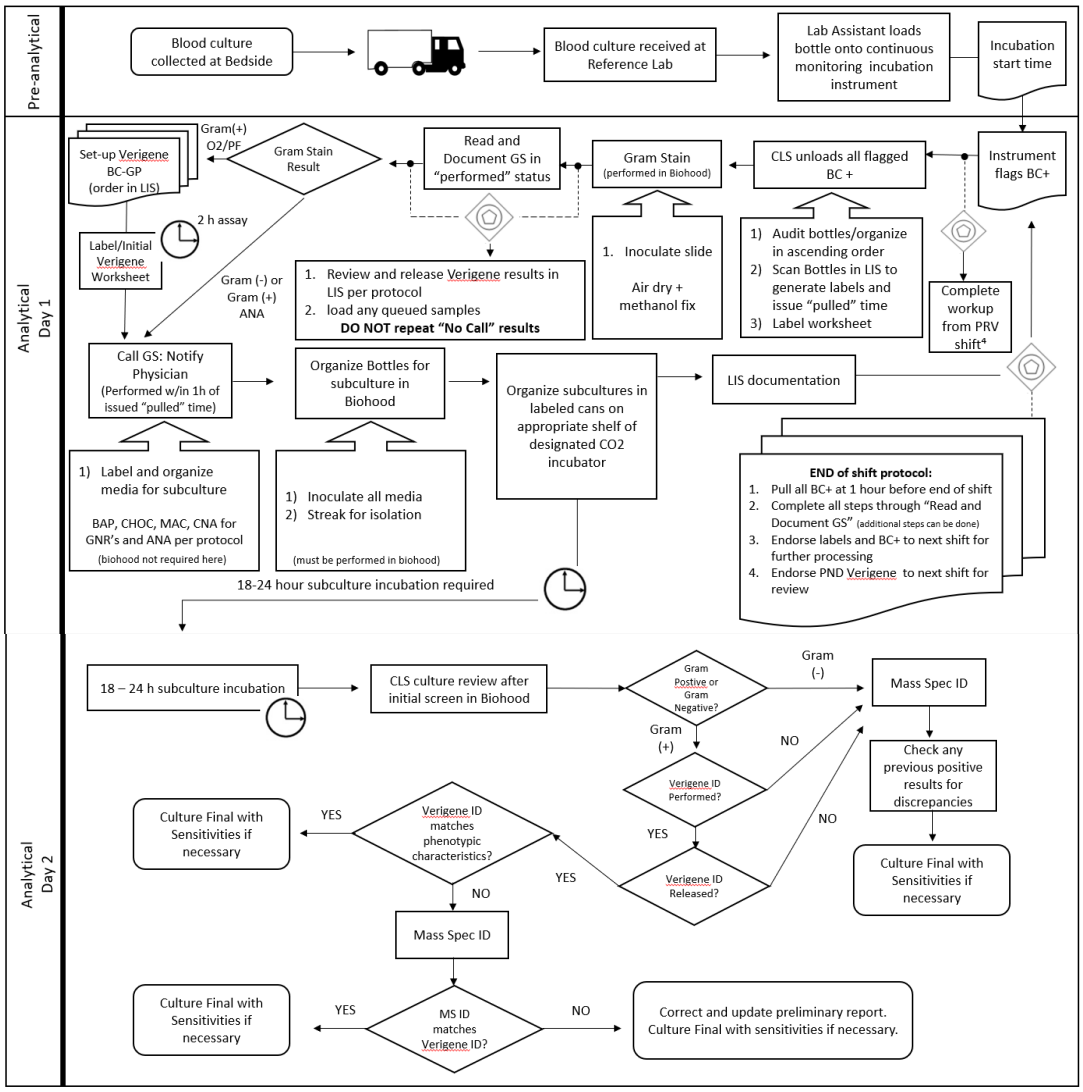

Figure 4. Proposed blood culture workflow. Swim lane process map depicting steps in the three phases of the proposed blood culture workflow. Clock symbol indicates incubation time required. "Pulled" refers to positive blood culture bottle removed from continuous monitoring incubation instrument. Diamond with encircled hexagon inside indicates decision activity dependent on fulfilled situation. Dotted process flow line refers to alternate workflow pathway.

FIGURE 4: PROPOSED WORKFLOW

Upon incubation of first "pull" subcultures, the bacteriologist will ensure Blood Culture Positive Worksheet is filled in (Appendix A) and necessary LIS documentation has been verified before continuing on with the process. Moreover, before gram stain reviews are performed on subsequent "pulls" or shortly thereafter, one of the two scheduled bacteriologists should monitor the Verigene instrument (assigned as an alternate workflow pathway and indicated as a dotted line on Figure 4) for pending BC-

GP results and document any completed results in LIS per information outlined in Figure 6 and Table 4.

The proposed process map includes two additional steps in analytical day 1, not present in the current process map, in an effort to address the need for continuous workflow and promote a structured endorsement of work between shifts. Additionally, changes to analytical day 2 were required to account for rapid identification performed. At the beginning of a shift, the scheduled bacteriologists must acknowledge receipt of any work from the previous shift and will assume responsibility to complete any additional tasks required as outlined on "End of shift protocol" (Figure 4). Conversely, one hour before end of shift all flagged blood culture positives must be "pulled", reviewed, documented on Inter-Shift Worksheet (Appendix B) and verified in LIS. Both additional steps detailing inter-shift work endorsement are assigned as an alternate workflow pathway and indicated as a dotted line on Figure 4. Rapid blood culture testing performed on analytical day 1 has an impact on workflow for analytical day 2. Figure 6 shows the decision algorithm required to evaluate BC-GP released results. Any questionable results or non-phenotypic matches should be confirmed by MALDI-TOF MS.

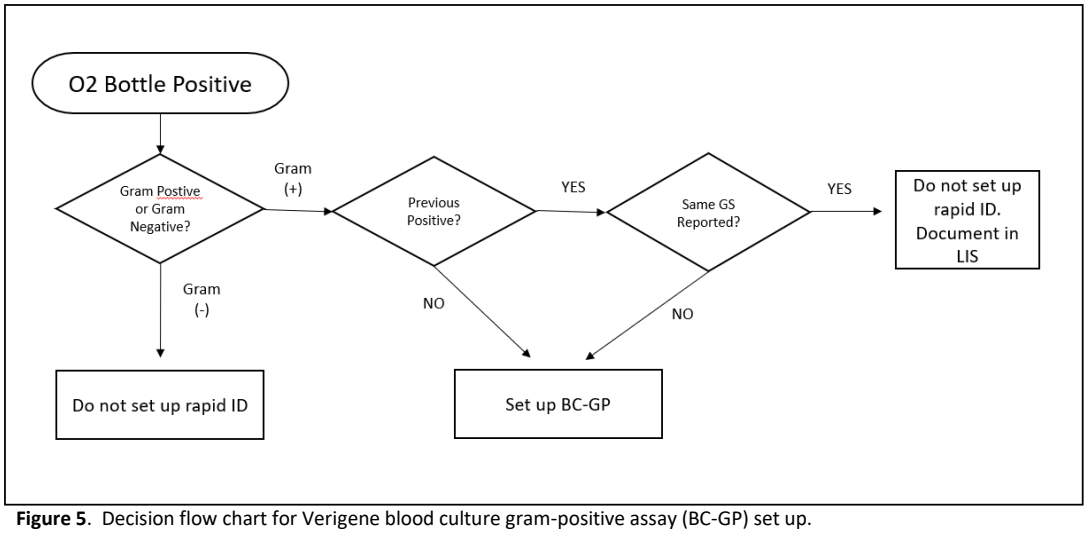

Figure 5. Decision flow chart for Verigene blood culture gram-positive assay (BC-GP) set up.

FIGURE 5: DECISION CHART FOR BC-GP SET UP

TABLE 4: CALLS FOR VALID RESULTS

TABLE 4. Calls for valid results reported as "Detected" when assay controls also "Detected". Adapted from Verigene Package Insert.

Test Result Reported as "Detected"			Reportable Organism/Gene
Genus	**Species**	**Resistance Marker**	
Staphylococcus	-	-	Presumptive *Staphylococcus spp.* [1]
Staphylococcus	*S. aureus*	-	Presumptive *Staphylococcus aureus*
Staphylococcus	*S. aureus*	*mecA*	Presumptive *Staphylococcus aureus, mecA*
Staphylococcus	*S. epidermidis*	-	Presumptive *Staphylococcus epidermidis*
Staphylococcus	*S. epidermidis*	*mecA*	Presumptive *Staphylococcus epidermidis, mecA*
Staphylococcus	*S. lugdunensis*	-	Presumptive *Staphylococcus lugdunensis*
Streptococcus	-	-	Presumptive *Streptococcus spp.* [2]
Streptococcus	*S. pneumoniae*	-	Presumptive *Streptococcus pneumoniae*
Streptococcus	*S. pyogenes*	-	Presumptive *Streptococcus pyogenes*
Streptococcus	*S. agalactiae*	-	Presumptive *Streptococcus agalactiae*
Streptococcus	*S. anginosus group*	-	Presumptive *Streptococcus anginosus group* [3]
-	*E.faecalis*	-	Presumptive *Enterococcus faecalis*
-	*E.faecalis*	*vanA*	Presumptive *Enterococcus faecalis,vanA*
-	*E.faecalis*	*vanB*	Presumptive *Enterococcus faecalis,vanB*
-	*E. faecium*	-	Presumptive *Enterococcus faecium*
-	*E. faecium*	*vanA*	Presumptive *Enterococcus faecium,vanA*
-	*E. faecium*	*vanB*	Presumptive *Enterococcus faecium,vanB*
Listeria spp.	-	-	Presumptive *Listeria spp.* [4]

[1] Includes: *S. arlettae, S. auricularis, S. capitis, S. caprae, S. chromogenes, S. cohnii, S. haemolyticus, S.hominis, S. intermedius, S. muscae, S. pasteuri, S. saccharolyticus, S. saprophyticus, S. schleiferi, S. sciuri, S.simulans, S. warneri, and S. xylosus*

[2] *S. bovis, S. dysgalactiae, S. dysgalactiae subsp equisimilis, S. equi, S. equinus, S. gallolyricus, S. gallolyticus pasteurianus, S. gordonii, S. infantarius subsp. coli, S. infantarius subsp. infantarius, S. infantis, S. mitis, S. mitis/oralis, S. mutans, S. oralis, S. parasanguinis, S. peroris, S. salivarius, S. sanguinis, and S.thoraltensis*

[3] *S. anginosus, S. constellatus, and S. intermedius*

[4] *L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri, and L. welshimer*

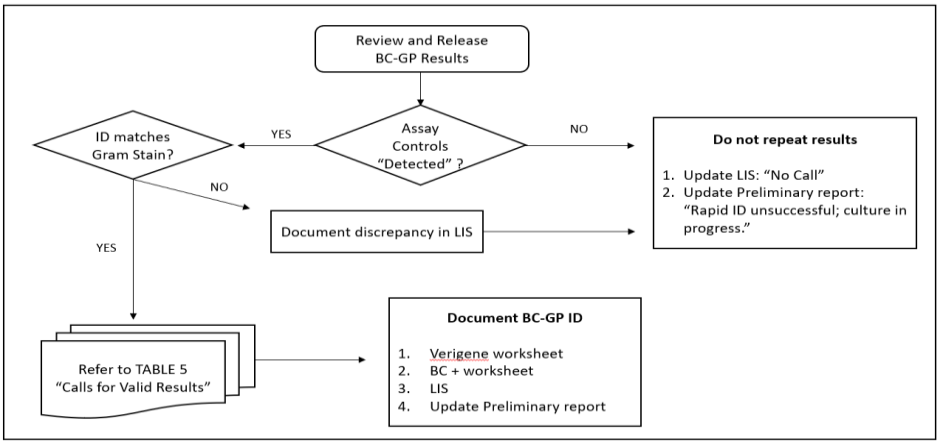

FIGURE 6: DECISION CHART FOR RELEASING RESULTS

Anticipated workflow benefits and challenges

Essentially, the BC-GP ID assay is not meant to take the place of any of the current steps of the workflow process thus as expected, when comparing process maps for the current and proposed method, the proposed method appears more cumbersome. Benefits to workflow processes are not as obvious as one might presume but they do exist.

Although it is clear that additional steps are required to set up a supplementary method of identification on analytical day 1, hands on time for the Verigene BC-GP is less than 5 minutes (16,17). Moreover, the proposed workflow, outlines rapid ID to be limited to aerobic specimens without documented previous history hence anticipating a minor interruption of overall workflow (Fig. 5). These additional steps required in the analytical phase of day 1 will prove beneficial to the workflow in the analytical phase of day 2 in that, isolates with rapid ID results should require minimal phenotypic bacteriologist review before susceptibility workup can be initiated. A retrospective look at organism identification, for 108 randomly selected non-duplicate positive blood samples, demonstrated that over 80% of organisms identified by conventional method could have benefited from rapid identification on analytical day 1 (Table5).

TABLE 5: MS ID FOR N=108

TABLE 5. Number of organism MS ID results for 108 randomly chosen nonduplicate blood cultures positives between December 2017 and February 2018 listed next to BC-GP and Filmarray targets for comparison.

Entire cohort (*n* =108) Gram Positives (*n* =56[a]) Gram Negatives (*n* =42) Other (*n* =10[b])	No. Organims for each Target ID by MS	% GP potential benefit from rapid ID method (*n* =56)	% Organisms potential benefit from rapid ID method (*n* =108)
BC-GP Gram Positive Bacterial Targets			
Staphylococcus spp.	9	16	
Staphylococcus aureus	16[d]	29	
Staphylococcus epidermidis	7	13	
Staphylococcus lugdunensis	1	2	
Streptococcus spp.	3	5	
Streptococcus pneumoniae	3	5	**82**
Streptococcus pyogenes	1	2	
Streptococcus agalactiae	3	5	
Streptococcus anginosus group	0	0	
Enterococcus faecalis	1[e]	2	
Enterococcus faecium	6[e]	11	
Listeria spp.	0	0	
Total:	**50**	**89**	
Filmarray Gram Positive Bacterial Targets			
Staphylococcus spp.	17	30	
Staphylococcus aureus	16[d]	29	
Streptococcus spp.	3	5	
Streptococcus agalactiae	3	5	**82**
Streptococcus pyogenes	1	2	
Streptococcus pneumoniae	3	5	
Enterococcus spp.	7[e]	13	
Listeria monocytogenes	0	0	
Total:	**50**	**89**	

[a]Total number of gram positives in the entire cohort, includes 2 Micrococcus sp., 2 Corynebacteria sp., and 2 Aerococcocus sp. not identified by either assay

[b]Other includes polymicrobial cultures, anaerobic organisms, yeast isolates, and nonviable organisms

[d]2 of 16 Staphylococcus aureus isolates were finalized as MRSA by conventional susceptibility methods

[e]No vancomycin resistant Enterococcus sp. were confirmed by conventional susceptibility methods

When considering the total volume of blood culture positives that a high-volume reference laboratory may incur, this percentage can directly translate over to bacteriologist workup time which in turn translates over to laboratory cost.

In further analyzing the workflow processes of the proposed plan one can identify other areas where bacteriologist time and efficiency can be improved. One area worth mentioning is the twofold benefit of focusing on gram stain review immediately after a blood culture positive is flagged; to the benefit of patient care, an immediate gram stain review can lead to faster turn-around times of clinically significant information reaching the clinician (Figure 3) and to the benefit of the bacteriologist, blood culture gram stain information available at the time of initial subculture allows for a streamlined process when gathering and organizing appropriate subculture media.

The task of including additional media based on gram stain review is made more efficient in the proposed method by eliminating the need for tube coagulase inoculation and plating of disk diffusion blood agar media. This media is added to help screen for the most common gram-positive pathogens i.e. tube coagulase for *Staphylococcus aureus*, Bacitricin (A disk) for *Streptococcus pyogenes*, Optochin (P disk) for *Streptococcus pneumoniae*. Identification to genus and species level by BC-GP assay will divert bacteriologist time from gathering, organizing, and sub-culturing additional media to setting up a rapid identification assay whose results, available in 2 hours, can be more clinically relevant than that of a documented tube coagulase result 18 - 24 h after positivity (Figure 3). The cost of eliminating tube coagulase and disk diffusion test set up may not be significant enough to offset the overall laboratory cost of implementing a rapid molecular identification test but when the clinical and economic impact is analyzed from the perspective of patient care the potential for cost savings can be significant.

The proposed workflow is not without its challenges. For example, the topic of continuous workflow, set forth, is not easy to address in large departments with high specimen volume. To avoid continuous workflow from being met with resistance by

staff members, the hand-off should be standardized. The main goal of introducing an end of shift protocol is to set a framework with specific expectations that each shift must abide to in order for continuous workflow to be effective. Furthermore, in an effort to standardize the hand-off of pending work, documentation and accountability of performed tasks are required through the introduction of worksheets presented in Appendix B,C as well as in pre-integrated LIS scripted workup. Any hand-off isolates requiring completion of additional tasks by the incoming shift should be clearly documented both on paper and in LIS. As previously mentioned, the evaluated reference laboratory gives scheduling priority to the blood culture positive task allowing for an overlap of bacteriologist between shifts however, scheduling conflicts are common and can lead to turn-around time delays. A detailed look at the time between positive flag time and pulled time during shift changes may be necessary to prove the need for adopting a continuous workflow method. A study performed in an effort to decrease the time to pathogen identification at a clinical microbiology lab in Houston Methodist Hospital, found that improving positive blood culture "pull" time can significantly decrease total processing time (27). Minor changes to the blood culture work up process at Houston Methodist Hospital lab improved the blood culture "pull" times from 38 m to 8 m (27). It may be arguable that 30 minutes is not significant enough to impact patient care but, when mortality rate is increased by turn-around time delays and time for definitive pathogen identification is prolonged by the unavoidable pre-analytical aspect of processing a blood culture specimen at a reference laboratory, the impact can be regarded as more substantial.

Limitations to the BC-GP assay causing discrepancies in reportable results must be addressed as additional challenges (Table 2). The proposed workflow process has integrated decision algorithms for both analytical day 1 and analytical day 2 that direct the bacteriologist to reject or confirm BC-GP assay results respectively depending on internal control results and phenotypic characteristics (Fig. 5, Fig. 3). LIS reporting considerations must be taken to acknowledge the risk of false-positive results due to cross-reactivity between identifiable targets as well as due to its documented poor

performance in identifying organisms in polymicrobial cultures (17). A report of, "Presumptive (insert organism and resistance marker if any) identification obtained by rapid ID method; culture results to follow.", will be issued on the analytical day 1 of work up contingent on internal control results. Including "Presumptive" in the initial preliminary report will allow for confirmation of results on analytical day 2. Furthermore, as detailed in Figure 5, if controls are "not detected" or a "no call" result is obtained, repeat testing will not be performed and a result of "Rapid ID unsuccessful; culture in progress." will be reported.

Conclusion and outlook

It is impossible to foresee all workflow challenges that may arise when implementing a new platform thus, the proposed workflow for the integration of the Verigene BC-GP assay into the established blood-culture work up method at the evaluated high-volume reference laboratory, is set forth as an initial framework to build upon. Even if plans to integrate the Verigene assay are not fulfilled, there are a number of benefits to visually mapping out the current blood culture positive pathway as it has been done here. Observations to specific potential problem areas were made in this process analysis and suggestions for improvement presented can be a topic for further discussion at department meetings.

My recommendation for the high-volume reference laboratory under evaluation is to try the proposed gram stain suggestions and presented continuous workflow steps before moving forward with integrating a rapid blood culture identification test. This may prove beneficial in the long run such that one test of change carried out in small scale can help anticipate potential problems and will allow staff to more easily adapt to upcoming changes. A reasonable approach would be to direct one of two scheduled bacteriologists to adhere to the proposed continuous workflow process while the other follows established protocol to allow comparisons to be made side by side.

The opportunity to integrate molecular diagnostics into microbiology testing, at the evaluated high-volume reference laboratory, comes with the need for expansion as

testing volumes continue to increase. With this opportunity to integrate exciting new platforms also comes the opportunity to evaluate workflow processes with the end goal of optimizing a pathway that is known to have a big impact on patient care. Clinical microbiology laboratories must not forget the role they play in the larger scheme of patient care and look for ways to evolve with the advancement of molecular technology.

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APPENDIX A. BLOOD CULTURE POSITVE WORKSHEET

Blood Culture Positive Worksheet

GS Control Slide: ☐ **Acceptable** ☐ **Unacceptable**

Acceptable gram stain control results indicated by observed SA (ATCC25923) = Purple Cocci and EC (ATCC25922) = Pink Rods
Document discrepancies as unacceptable and indicate corrective action below.

End of Shift Workflow Reminder

1. Last pull required 1 hour before end of shift
2. Document last pull on Inter-shift Blood Culture Positive Worksheet
3. Complete workup through GS "performed" status
4. Endorse slides, Inter-shift Worksheet, and Pending BC-GP panels to next shift

Date: _____ **Shift:** ☐ Day ☐ Swing ☐ GY **CLS:** _____ **Page No./ Total Pages:** _____

Patient Label Name, MR#,ACC#	#POS/ Total	Bottle Type and Gram Stain Morphology		Call Notification Per Shift Protocol		BC-GP Set-up ✓	BC-GP Result	LIS entry end of shift check ✓
		O2		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O3		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O3		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O4		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O3		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O4		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			

Corrective Action: Date/Initals:

APPENDIX B. INTER-SHIFT BLOOD CULTURE WORKSHEET

Inter-shift Blood Culture Worksheet:

☐ Day Shift → Swing Shift
☐ Swing Shift → Graveyard
☐ Graveyard → Day Shift

GS Control Slide: ☐ Acceptable ☐ Unacceptable

Acceptable gram stain control results indicated by observed SA (ATCC25923) = Purple Cocci and EC (ATCC25922) = Pink Rods
Document discrepancies as unacceptable and indicate corrective action below.

Inter-shift Workflow Reminder

1. Ending shift must document inter-shift transfer in LIS
2. Both shifts must initial Inter-shift Worksheet
3. Starting shift responsible for pending BC-GP panels
4. Starting shift responsible for completing blood culture positives documented on Inter-shift Worksheet

Date:_____ **CLS:**_____ → **CLS:** _____ **Page No./ Total Pages:**_____

Patient Label Name, MR#,ACC#	#POS/ Total	Bottle Type and Gram Stain Morphology		Call Notification Per Shift Protocol		BC-GP Set-up ✓	BC-GP Result	LIS entry end of shift check ✓
		O2		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O3		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O3		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O4		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O3		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			
		O4		MD/RN/AACC/Call Time	PRVPOS			
		ANA			EXPIRED			
		PEDI			NOS			

Corrective Action: Date/Initials:

APPENDIX C. VERIGENE BC-GP WORKSHEET

Verigene BC-GP Worksheet

*If assay controls not "detected" indicate the following:
INT CTL 1= sample prep failure; INT CTL 2= hybrdization failure; INT CTL= failure both levels; OUT=any other alerts

Verigene Module	Patient Label Name, MR#, ACC#	Controls* "Detected" ✓	BC-GP Results	Verigene Module	Patient Label Name, MR#, ACC#	Controls* "Detected" ✓	BC-GP Results
Module 1			Results Verified ☐	Module 1			Results Verified ☐
CLS		CLS		CLS		CLS	
Module 2			Results Verified ☐	Module 2			Results Verified ☐
CLS		CLS		CLS		CLS	
Module 3			Results Verified ☐	Module 3			Results Verified ☐
CLS		CLS		CLS		CLS	
Module 1			Results Verified ☐	Module 1			Results Verified ☐
CLS		CLS		CLS		CLS	
Module 2			Results Verified ☐	Module 2			Results Verified ☐
CLS		CLS		CLS		CLS	
Module 3			Results Verified ☐	Module 3			Results Verified ☐
CLS		CLS		CLS		CLS	

Worksheet Start Date: Worksheet File Date:

APPENDIX D. CERTIFICATION OF APPROVAL

I certify that I have reviewed *Implementation Plan to Integrate the Nanosphere Verigene Assay in a High-volume Reference Laboratory and the Anticipated Workflow Benefits to Adopting Rapid Blood Culture Testing*, and I approve this completed project to be submitted in partial fulfillment of the requirements for the CLS 5700 Course Master's Research Project.

Keith Ng MPH, CLS, MT (ASCP)
Director of Laboratory Services
TPMG Regional Laboratories