

Identification of Residual DNA Contaminants in Sterile Blood Culture Media

Abstract

Background Bloodstream infection is a leading cause of morbidity and mortality in hospitals, and the rapid detection and identification of microorganisms can lead to faster, more accurate treatment and better prognosis for septic patients. Molecular tools for bacterial identification can provide increased sensitivity and rapid results, but false positives are observed in up to 10% of patient samples. It is unknown whether these false positive results are due to slow-growing, fastidious organisms causing sepsis and/or DNA contamination of un-inoculated blood culture media. The purpose of this study is to examine the presence of residual bacterial DNA in sterile media of blood culture bottles.

Methods Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR™) and real-time PCR (qPCR) were used to assess the presence of DNA in sterile blood culture media from 2 vendors: Beckton-Dickinson (Sparks, MD) and BioMérieux (Durham, NC). Media was collected from un-inoculated BACTEC (n=22) and BacT/Alert SA (n=22) blood culture bottles, PCR inhibitors were removed, and DNA was extracted. qPCR was performed with 16S specific primers, while TEM-PCR was performed for identification of specific bacterial organisms.

Results qPCR analysis of 16S rDNA levels suggested the presence of bacterial DNA in all tested samples. The rates of total positives observed with TEM-PCR in BACTEC and BacT/Alert SA blood culture media were 22.7% and 100% with both sets of media positive for *Enterococcus faecalis* (9.1% and 40.9%), respectively. Evaluation of BACTEC samples also yielded positive results for *Proteus mirabilis* (13.6%) and *Stenotrophomonas maltophilia* (2.3%), while BacT/Alert SA media was positive for *Enterococcus faecium* (100%), *Staphylococcus aureus* (4.5%), and *Staphylococcus epidermidis* (13.6%). TEM-PCR was more sensitive for identification of contaminant DNA than qPCR (41% concordance) due to an initial target enrichment step.

Conclusions The increased sensitivity and timeliness of molecular diagnostic methods to identify bacteria in blood has significant potential to improve outcomes in septic patients. However, the presence of residual DNA in blood culture media produces false positives when multiplex diagnostics are used, suggesting that current blood collection media may be unsuitable for use with molecular diagnostics in clinical practice.

Introduction

- Sepsis is a leading cause of morbidity and mortality (10-40%) in intensive care units.^{1,2} Mortality rate increases 7% every hour a patient waits for treatment.³
- Broad spectrum antibiotics are given to patients suspected of sepsis but contributes to increased antibiotic resistance in hospitals.
- Blood culture is the standard for diagnosing a bloodstream infection but can take up to 72 hours for positive identification.^{4,5} The most frequently identified sepsis agent is *Staphylococcus aureus*.
- Using molecular techniques, such as multiplex PCR, rapid detection of bloodstream infection can reduce patient length-of-stay in the hospital, lower the cost of antimicrobial treatment, and more effectively and efficiently treat sepsis patients.^{6,7}

Materials & Methods

Clinical Study. Positive blood culture samples (n=464) were submitted to Diatherix Laboratories, Inc over a five month period by Integris Southwest Medical Center (Oklahoma City, OK). Bottles were incubated on-site at hospitals 1-72 hours prior to shipment to Diatherix. Samples were tested using the Diatherix Infectious Disease Panel (Table 1).

Blood culture media collection and DNA extraction. Blood culture media was removed from bottles and processed in blood culture preparation tubes containing benzyl alcohol and guanidine HCl-Tris to remove PCR inhibitors. DNA was subsequently extracted from the blood culture media preparations using the MagBind® Bacterial DNA Isolation Kit (Omega Biotek, Norcross, GA). For DNA contaminant analysis, media was removed from sterile, uninoculated blood culture bottles [Figure 1; Becton Dickinson BACTEC™ lots 3023161 (n=11) and 2353062 (n=11) and BioMérieux BacT/Alert® SA lot 1034869 (n=22)].



Figure 1. Representative sterile, uninoculated BioMérieux BacT/Alert SA (left) and Becton Dickinson BACTEC (right) blood culture bottles.

Materials & Methods (continued)

Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR™). Isolated DNA from blood culture media samples and *Staphylococcus aureus* control were amplified using the TEM-PCR Infectious Disease Panel (IDP; US Patent 7,851,148 B2). A single TEM-PCR IDP reaction can detect up to 24 gene targets. Each reaction contains two pairs of nested gene-specific primers (Fo, forward out; Fi, forward in; Ri, reverse in; Ro, reverse out) for each target. Fi and Ri primers are designed with a unique tag sequence that can be recognized by SuperPrimers, also included in the same PCR reaction (Figure 2). An initial target enrichment step occurs prior to typical PCR amplification. PCR products are detected by hybridization to a complimentary detection probe covalently coupled to a microsphere or a glass microarray substrate. Streptavidin-phycoerythrin conjugate (SA-PE) is added to detect positive signal.

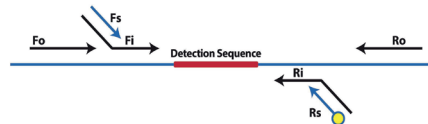


Figure 2. TEM-PCR™ Scheme. Low concentration nested gene-specific primers are (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) designed to enrich the targets during the initial PCR cycles. Later in the procedure, a pair of universal SuperPrimers (Fs and Rs) is used to amplify all targets. The Rs primer is labeled with biotin for subsequent detection.

Table 1. Organisms and antibiotic resistance targets detected by the Diatherix Laboratories, Inc TEM-PCR Infectious Disease Panel (IDP).

Organism/Antibiotic Resistance	Target Name
<i>Acinetobacter baumannii</i>	ABM
<i>Enterobacter aerogenes</i>	EAER
<i>Enterobacter cloacae</i>	ENCL
<i>Enterococcus faecalis</i>	EFLS
<i>Enterococcus faecium</i>	EFCM
<i>Escherichia coli</i>	ECO
<i>Klebsiella pneumoniae</i>	KPN
<i>Proteus mirabilis</i>	PMA
<i>Pseudomonas aeruginosa</i>	PMA
<i>Serratia marcescens</i>	SMA
<i>Staphylococcus aureus</i>	NUC
<i>Staphylococcus epidermidis</i>	EPI
<i>Staphylococcus coagulase-negative</i>	TUF
<i>Staphylococcus aureus</i> , methicillin resistant	MECA
<i>Streptococcus pyogenes</i>	SPY
<i>Stenotrophomonas maltophilia</i>	XMA
Aminoglycoside resistance	AACA
Erythromycin/Clindamycin resistance	ERMA/ERMC
Panton-Valentine Leukocidin Toxin	PVL
Tetracycline resistance	TETK/TETM
Vancomycin resistance	VANA/VANB

Real-time PCR (qPCR). Gene sequences for 16S, EFLS, EFCM, PROM, XMA, EPI, NUC, and TUF were amplified for each sterile, uninoculated blood culture media sample and PCR positive controls for each target. SYBR Green qPCR reactions (20µL) were setup using 4µL template and 200nM forward and reverse primers. Primer sequences used in this evaluation for 16S rDNA include forward 5'-ACTCCTACGGGAGGCAGCAGT-3' and reverse 5'-GTATTACCGCGGCTCTGGA-3'. PCR analyses were performed on the BioRad CFX96™ thermal cycler (Hercules, CA).

Results

Table 2. Bacterial target identification in positive blood cultures (n=464) using the TEM-PCR Infectious Disease Panel (IDP).

Target	# Positive	Target	# Positive	Target	# Positive	Target	# Positive
TUF	2 (0.4%)	ERMA	6 (1.3%)	ABM	1 (0.2%)	KPN	2 (0.4%)
EPI	4 (0.9%)	ERMC	1 (0.2%)	EAER	0 (0%)	PMA	1 (0.2%)
NUC	15 (3.2%)	TETK	0 (0%)	ECO	8 (1.7%)	PROM	5 (1.1%)
MECA	23 (5%)	TETM	9 (1.9%)	EFCM	1 (0.2%)	SMA	0 (0%)
PVL	7 (1.5%)	VANA	0 (0%)	EFLS	62 (13.4%)	XMA	0 (0%)
AACA	1 (0.2%)	VANB	0 (0%)	ENCL	8 (1.7%)	SPY	0 (0%)

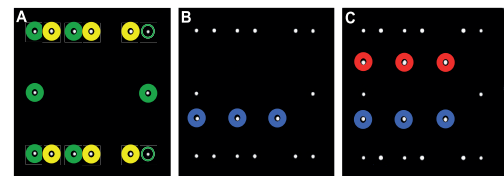


Figure 3. Enterococcus faecalis and Enterococcus faecium are detected in sterile, uninoculated blood culture media with TEM-PCR. Media from sterile, uninoculated blood culture bottles [BD BACTEC (n=22) and BioMérieux BacT/Alert SA (n=22)] were collected and processed to remove PCR inhibitors. DNA was extracted from processed media and examined with the TEM-PCR IDP. PCR products are detected using complimentary detection probes covalently bound to microarrays. SA-PE is added to detect positive signal. Microarray images are representative of samples with no detectable signal (A), EFLS single positive (B), and EFLS/EFCM double positive (C). Reference markers, green; internal control, yellow; EFLS, blue; EFCM, red.

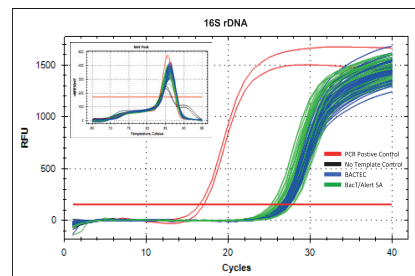


Figure 4. 16S rDNA is present in sterile, uninoculated blood culture media. Media from sterile, uninoculated blood culture bottles [BD BACTEC (n=22, blue) and BioMérieux BacT/Alert SA (n=22, green)] were collected and processed to remove PCR inhibitors. DNA was extracted from processed media, and SYBR qPCR was performed using 4µL template and universal 16S rDNA primers [200nM].

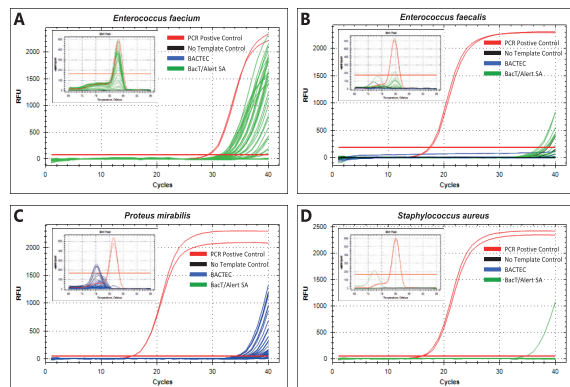


Figure 5. Enterococcus faecium and Enterococcus faecalis can be detected in sterile, uninoculated blood culture media with qPCR. Media from sterile, uninoculated blood culture bottles [BD BACTEC (n=22, blue) and BioMérieux BacT/Alert SA (n=22, green)] were collected and processed to remove PCR inhibitors. DNA was extracted from processed media, and SYBR qPCR was performed using 4µL template and primers [200nM] to detect (A) *Enterococcus faecium*, (B) *Enterococcus faecalis*, (C) *Proteus mirabilis*, and (D) *Staphylococcus aureus*. Results for *Stenotrophomonas maltophilia* and *Staphylococcus epidermidis* are not shown.

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Results (continued)

Table 3. Bacterial target identification using TEM-PCR and qPCR in sterile, uninoculated blood culture media collected from Beckton Dickinson BACTEC (n=22) and BioMérieux BacT/Alert SA (n=22) bottles.

Target	TEM-PCR		qPCR	
	BACTEC	BacT/Alert	BACTEC	BacT/Alert
16S	NA*	NA*	22 (100%)	22 (100%)
EFCM	0 (0%)	22 (100%)	NA*	14 (63.6%)
EFLS	2 (9.1%)	9 (40.9%)	0 (0%)	1 (4.5%)
PROM	3 (13.6%)	0 (0%)	0 (0%)	NA*
XMA	1 (4.5%)	0 (0%)	0 (0%)	NA*
EPI	0 (0%)	3 (13.6%)	NA*	0 (0%)
NUC	0 (0%)	1 (4.5%)	NA*	0 (0%)
TUF	0 (0%)	2 (9.1%)	NA*	0 (0%)

*NA - not tested.

Discussion

- Testing of positive blood culture bottles using the TEM-PCR IDP identified that *Enterococcus faecalis* was the predominant detected bacterium. These results contradicted previous findings that *Staphylococcus aureus* is the most commonly detected bacterium associated with sepsis.
- Evaluation of sterile, uninoculated media from blood culture bottles revealed the presence of contaminant DNA from *Enterococcus faecium*, *Enterococcus faecalis*, *Proteus mirabilis*, *Stenotrophomonas maltophilia*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. No growth was observed after subsequent incubation of blood culture bottles at 37°C, suggesting that bacterial DNA contamination is not eliminated during blood culture bottle manufacturing.
- Molecular diagnostics for the identification of bloodstream infections can provide faster results for better patient treatment and outcome. Target enriched multiplex PCR provides more sensitive detection compared with other molecular diagnostics, such as real-time PCR.

Conclusions

- Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR) provides more rapid and sensitive detection of pathogens compared with other molecular diagnostics.
- Blood culture bottles are unsuitable for clinical molecular diagnostics due to presence of bacterial DNA contaminants.

Acknowledgements

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