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Rapid Detection of *bla*$_{KPC}$ Carbapenemase Genes by Internally Controlled Real-Time PCR Assay Using Bactec Blood Culture Bottles$^{\nabla}$

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Rapid detection of drug-resistant bacteria in clinical samples plays an instrumental role in patients' infection management and in implementing effective infection control policies. In the study described in this report, we validated a multiplex TaqMan real-time quantitative PCR (qPCR) assay for the detection of *bla*$_{KPC}$ genes and the human RNase P gene in Bactec blood culture bottles. The MagNA Pure LC (version 2.0) instrument was utilized to extract nucleic acids from the inoculated broth, while bovine serum albumin (BSA) was utilized as the PCR inhibitor reliever. The multiplex assay, which was specific for the detection of *bla*$_{KPC}$ genes, had a limit of detection of 19 CFU per reaction mixture with human blood-spiked Bactec bottles. Of the 323 Bactec blood culture sets evaluated, the same 55 (17%) blood cultures positive for carbapenem-resistant bacteria by culture were also positive by the validated qPCR assay. Thus, the sensitivity, specificity, positive predictive value, and negative predictive value of the qPCR assay compared to the results of culture were all 100%. *bla*$_{KPC}$ genes were also detected from the same Bactec bottle broth after manual extraction with a QIAamp DNA minikit; however, there was an average 3-threshold-cycle delay in the qPCR readings. With the limited therapeutic options available, the accurate and rapid detection of *bla*$_{KPC}$-possessing bacteria by the described *bla*$_{KPC}$/RNase P assay will be a crucial first step in ensuring optimal clinical outcomes and infection control.

The emergence and rapid spread of *Klebsiella pneumoniae* carbapenemase gene (*bla*$_{KPC}$)-encoding bacteria have exerted unprecedented pressure on physicians and complicated the management of patients' infections. Indeed, high mortality rates, up to 66%, were associated with bacteremia due to *bla*$_{KPC}$-positive bacteria (5, 20). The high mortality rate may be attributed to multiple resistance mechanisms that are associated with *bla*$_{KPC}$-positive bacteria (21). *bla*$_{KPC}$-positive bacteria have been implicated in many cases of hospital-acquired infections, which mandates the rapid detection of these pathogens by the clinical laboratory in order to control their spread (16, 20, 30). The *bla*$_{KPC}$ enzymes are encoded in large plasmids that vary in size and structure. *bla*$_{KPC}$ genes are embedded within a Tn*3*-type transposon, and this leads to the possibility of being highly transmissible (19). To date, 10 different types of *bla*$_{KPC}$ have been reported in various bacterial species.

Detection of the *bla*$_{KPC}$ resistance mechanism has been hampered by the heterogeneous expression of β-lactam resistance (4, 13). Some bacterial clones encoding *bla*$_{KPC}$ may appear to be susceptible to carbapenems by the reference Clinical and Laboratory Standards Institute (CLSI) agar dilution or broth microdilution methods as well as by automated identification systems (4, 27). Automated systems, in particular, have variable sensitivity to detect *bla*$_{KPC}$ carbapenem resistance, ranging from 7 to 87%, and samples often require subsequent testing with confirmatory tests, such as the modified Hodge test, which can predict carbapenemase production in isolates of *Enterobacteriaceae* (27). On the other hand, molecular diagnostic techniques, in particular, real-time quantitative PCR (qPCR), have been shown to be rapid and accurate methods for identifying *bla*$_{KPC}$ genes (8, 12, 25).

Blood cultures are considered to be the "gold standard" for detecting microorganisms in the bloodstream, including those that encode *bla*$_{KPC}$ genes (29). Automated blood culture systems take approximately 1 to 2 days, on average, to signal a positive blood culture and another 1 to 2 days to finalize bacterial identification and antimicrobial testing. With the advent of qPCR, the time to bacterial identification and detection of drug resistance has been reduced to 4 to 6 h after a positive blood culture has turned positive (22, 24, 26, 28). However, the presence of PCR inhibitors in the blood culture bottles has thus far reduced the sensitivity of PCR assays (10). Rapid detection of bacterial resistance mechanisms in blood culture bottles can assist the physician in both patient management and infection control.

In this report we describe the validation of an internally controlled TaqMan qPCR assay for the detection of *bla*$_{KPC}$

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genes and the human RNase P gene from Bactec blood culture bottles. The sequences of the primers and probes used to detect the bla_{KPC} genes were previously described (12). RNase P has been extensively used in molecular diagnostic assays to monitor the efficiency of the DNA extraction and PCR amplification (6, 23). Thus, this assay intended to take advantage of the human DNA sample inoculated in the Bactec bottle as a source of RNase P DNA. Automated DNA extraction with the MagNA Pure LC (version 2.0) instrument (F. Hoffmann-La Roche Ltd., Basel, Switzerland) was evaluated with the qPCR assay. In addition, manual DNA extraction from the bla_{KPC}-positive blood culture bottles with a QIAamp DNA minikit (Qiagen GmbH, Hilden, Germany) was also examined.

MATERIALS AND METHODS

Blood culture samples. The Bactec 9240 blood culture system (Becton Dickinson, BD Biosciences) was utilized at Chaim Sheba Medical Center to detect bacterial blood infections. Of the 18,113 blood sample sets for culture collected between 1 November 2008 and 1 February 2009, 323 (1.8%) sets with Gram-negative rods were flagged positive by the Bactec system. All Gram-negative pathogens were further processed for microbial identification according to routine protocols based on the 2nd edition of the *Clinical Microbiology Procedures Handbook*, and antibiotic susceptibility testing was performed according to CLSI guidelines (7, 15). In addition, 1.5-ml aliquots from the blood culture bottles' broth were removed under sterile conditions, decanted into sterile cryovial tubes, and stored at −20°C for further use.

Detection of carbapenem-resistant bacteria by culture. Standard protocols were followed for culturing and identifying carbapenem-resistant bacteria in blood culture broth (12). Briefly, an aliquot of the blood culture broth was streaked on 5% sheep blood agar and MacConkey agar (Hy-labs, Rehovot, Israel) and incubated for 18 to 24 h at 37°C in the presence of 5% CO_2 or 35°C in room air, respectively. Antimicrobial susceptibility testing was performed by the disk diffusion method on the cultured bacteria according to the CLSI guidelines, and suspected carbapenem-resistant bacteria were confirmed by the modified Hodge test. MICs for the carbapenems imipenem, meropenem, and ertapenem were determined by the Etest (AB Biodisk, Solna, Sweden) methodology (7). The presence of the bla_{KPC} gene in modified Hodge test-positive isolates was confirmed by qPCR as previously described (12).

DNA extraction. (i) DNA extraction from cultured bacterial colonies using QIAamp DNA minikit. Fresh well-isolated colonies were used for DNA extraction using a QIAamp DNA minikit (Qiagen GmbH, Hilden, Germany) according to the protocol suggested by the manufacturer. Briefly, a bacterial suspension equivalent to that of a 2 McFarland standard was prepared in saline, and bacterial DNA was extracted from a 200-μl (1.2×10^8 CFU) suspension. Extracted bacterial DNA was eluted from the columns in 100 μl elution buffer and stored at −20°C.

(ii) DNA extraction from Bactec broth using QIAamp DNA minikit. Well-mixed Bactec bottles' broth (200 μl) was extracted according to the bacterial DNA extraction protocol suggested by the manufacturer. Extracted bacterial DNA was eluted from the columns in 100 μl elution buffer and stored at −20°C.

(iii) DNA extraction from blood culture using Roche MagNA Pure LC instrument. A MagNA Pure LC DNA isolation kit III (bacteria, fungi) was used to extract bacterial DNA from well-mixed Bactec culture bottles' broth according to the manufacturer's suggestions. Briefly, external lysis was performed on the maximum aliquot volume allowed (100 μl) to inactivate the bacteria. This was followed by loading of the sample on the automated MagNa Pure LC DNA extractor. Extracted DNA was eluted in 100 μl elution buffer and stored at −20°C.

***bla*$_{KPC}$/RNase P detection by real-time PCR.** The ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA) was used for the amplification and detection of the bla_{KPC} amplicon (246 bp) by TaqMan technology, after modification of the previously developed and validated bla_{KPC} assay (12). The assay was modified by adding the internal control RNase P (catalogue no. 4316844; Applied Biosystems, Foster City, CA) and bovine serum albumin (BSA) Cohn fraction V at ≥96% (agarose gel electrophoresis) (Sigma-Aldrich, St. Louis, MO) to the master mix. Evaluating the sequences of the bla_{KPC} primers and probe in the NCBI database showed no matches to sequences other than those for the bla_{KPC} genes, and indeed, they should detect all 10 types of bla_{KPC}. The sensitivity of the modified TaqMan assay was optimized by evaluating different concentrations of the bla_{KPC} primers (200, 300, 600, and 900 nM) and probes (150, 200, and 300 nM), RNase P primer-probe mix (0.1×, 0.2×, 0.5×, 0.75×, and 1×), and BSA (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, and 0.8%). The concentrations of the primers, probes, and BSA that gave the best detection limits were 300 nM for bla_{KPC} primers, 200 nM for the 6-carboxyfluorescein (FAM)-labeled bla_{KPC} probe, 0.5× for the VIC-labeled RNase P primer-probe mix, and 0.5% for BSA. The 25-μl qPCR mixture contained the qPCR MasterMix plus reaction buffer (Eurogentec, Belgium), 5-carboxy-X-rhodamine succinimidyl ester (ROX) as an internal reference dye, HotGoldStar DNA polymerase, deoxynucleoside triphosphates (including dUTP), and uracil-*N*-glycosylase. qPCR was performed under the following conditions: 2 min at 50°C, 10 min at 95°C, and 50 cycles of 15 s at 95°C and 1 min at 60°C.

Interpretation of results. A blood culture was considered positive for the presence of the bla_{KPC} gene if the FAM signal was below the threshold cycle (C_T) of 40 and the internal control RNase P VIC C_T signal was below 45. Samples positive for the bla_{KPC} signal and negative for RNase P were also considered bla_{KPC} positive. Samples negative for both bla_{KPC} and RNase P would have been deemed uninterpretable, but no such results were encountered during the study.

RESULTS

***bla*$_{KPC}$/RNase P real-time PCR analytical sensitivity.** The analytical sensitivity of the multiplex bla_{KPC}/RNase P qPCR assay was determined after known concentrations of bla_{KPC-3}-positive *K. pneumoniae* (6×10^8 CFU) were serially diluted in saline and 1 ml of the diluted bacteria was spiked in either different naïve Bactec bottles or Bactec bottles that were already spiked with 5 ml human blood. A 100-μl aliquot of the well-mixed Bactec bottles with the different spiked dilutions was extracted by the MagNA Pure LC extractor and tested by qPCR. The analytical sensitivity of the bla_{KPC}/RNase P assay in naïve Bactec bottles was linear over 6 log dilutions (r^2 = 0.9977; slope = −3.53), and the amplification efficiency was 92%. On the other hand, the analytical sensitivity of the bla_{KPC}/RNase P assay in human blood-spiked Bactec bottles was linear over 5 log dilutions (r^2 = 0.989; slope = −3.88), and the amplification efficiency was 80.5%. The detection limits of the bla_{KPC}/RNase P assay in naïve and human blood-spiked Bactec bottles were 2 and 19 CFU per reaction mixture, respectively. The limit-of-detection analysis was repeated in triplicate, with similar results. Beyond the detection limits, the results were not reproducible, most likely due to the well-recognized stochastic properties of qPCR on highly diluted nucleic acids (11).

The precision of the RNase P internal control assay was determined on the DNA extracted (n = 16) from the blood-spiked Bactec bottles. The mean RNase P C_T results of all the dilutions was 28.5, while the standard deviation (SD) and coefficient of variation (CV) were 0.7 and 2.6%, respectively.

***bla*$_{KPC}$/RNase P real-time PCR cross-reactivity.** The bla_{KPC}/RNase P qPCR assay did not cross-react with bacterial DNA of several strains extracted by the QIAamp DNA minikit (Table 1). In addition, testing of spiked blood culture bottles with bla_{NDM} and modified Hodge test-positive clinical isolates (*Escherichia coli* and *Providencia rettgeri*) did not give a positive signal with the bla_{KPC}/RNase P qPCR assay.

Detection of carbapenem-resistant bacteria by culture and qPCR after MagNA Pure LC DNA extraction. Of the 323 Bactec blood culture bottles evaluated, bacteria resistant to the carbapenems imipenem, meropenem, and ertapenem were isolated and confirmed to be positive by the modified Hodge test in 55 (17%) bottles. *Klebsiella pneumoniae*, *E. coli*, and *Enterobacter* species were isolated from 53 (96.4%) bottles, 1 (1.8%)

TABLE 1. Cross-reactivity of the multiplex bla_{KPC}/RNase P qPCR assay

Bacterial strain	C_T reading	bla_{KPC}/RNase P qPCR result
K. pneumoniae ATCC 13883	Undetermined[a]	Negative
K. pneumoniae ATCC 700603 (ESBL[b])	Undetermined	Negative
E. coli ATCC 25922	Undetermined	Negative
E. coli ATCC 35218	Undetermined	Negative
K. pneumoniae (AmpC)	Undetermined	Negative
E. coli (AmpC)	Undetermined	Negative
Acinetobacter baumannii	Undetermined	Negative
Citrobacter koseri	Undetermined	Negative
Enterobacter species	Undetermined	Negative
Serratia marcescens	Undetermined	Negative
Salmonella species	Undetermined	Negative
Salmonella paratyphi type A	Undetermined	Negative
Shigella species	Undetermined	Negative
Stenotrophomonas maltophilia	Undetermined	Negative
Proteus mirabilis	Undetermined	Negative
Pseudomonas aeruginosa (MDR[c])	Undetermined	Negative
Morganella morganii	Undetermined	Negative
Providencia species	Undetermined	Negative
Aeromonas hydrophila (MDR)	Undetermined	Negative
Staphylococcus aureus (methicillin resistant)	Undetermined	Negative
Staphylococcus aureus (methicillin susceptible)	Undetermined	Negative
Streptococcus agalactiae	Undetermined	Negative
Enterococcus species	Undetermined	Negative
Chryseobacterium species	Undetermined	Negative
Bacteroides species	Undetermined	Negative
E. coli (bla_{NDM})	Undetermined	Negative
Providencia rettgeri (bla_{NDM})	Undetermined	Negative
Candida albicans ATCC 14053	Undetermined	Negative
Candida parapsilosis ATCC 22019	Undetermined	Negative

[a] Undetermined, the sample's fluorescence reading was below the threshold cutoff value of 50 cycles.
[b] ESBL, extended-spectrum β-lactamase producer.
[c] MDR, multidrug resistant.

bottle, and 1 (1.8%) bottle, respectively. Testing of undiluted extracted DNA from the Bactec bottles by qPCR in the presence of 0.5% BSA revealed that the same 55 samples were positive for bla_{KPC} genes. Thus, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the qPCR assay were 100% (Table 2). The mean C_T value of the bla_{KPC} qPCR was 18.8, and the C_T range was from 15.9 to 26.6. The RNase P internal control gave a C_T of value less than 45 for 322 (99.7%) of the undiluted extracted DNAs; the mean C_T value of all samples was 27.6. Only one sample which was positive for bla_{KPC} genes gave a C_T value of 50. Upon stratification of the RNase P results by C_T value, 59 (18.3%) gave C_T values of <25, 243 (75.2%) gave C_T values of 25 to <35, 20 (6.2%) gave C_T values of 35 to <45, and 1 (0.3%) gave a C_T value of >45.

Similar results were obtained upon testing of 1:10-diluted MagNA Pure LC-extracted DNA. All 55 culture-positive samples were found to be positive by qPCR; thus, the sensitivity, specificity, PPV, and NPV of the qPCR assay were 100% (Table 2). The mean C_T value of the bla_{KPC} results was 21.1, and the range was from 17.9 to 29.9. The internal control RNase P, which gave an average C_T value of 31.5, was positive in 318 (98.5%) of 323 samples tested. Upon stratification of the RNase P results by C_T value, 1 (0.3%) gave a C_T value of <25, 278 (86.1%) gave C_T values of 25 to <35), 37 (11.5%) gave C_T values of 35 to <45, 2 (0.6%) gave C_T values of >45, and 5 (1.6%) were negative.

The clinical sensitivity and specificity of the qPCR assay were not affected by the presence of mixed pathogens in the positive Bactec blood culture bottles. Overall, a single organism was isolated from 283 (87.6%) of the Bactec bottles evaluated, while 2 or 3 organisms were detected in 38 (11.8%) and 2 (0.6%) of the blood culture bottles, respectively. Isolated organisms in mixed infections included *Acinetobacter baumannii*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Aeromonas* species, *Bacillus* species, *Bacteroides* species, *Chryseobacterium* species, *Clostridium* species, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Providencia* species, *Staphylococcus aureus*, *Enterococcus* species, *Candida* species, *Salmonella paratyphi* type A, *Serratia marcescens*, *Shewanella* species, *Stenotrophomonas maltophilia*, and *Streptococcus* species.

Manual extraction of the 55 bla_{KPC}-positive Bactec broth samples by the QIAamp DNA minikit (Qiagen GmbH, Hilden, Germany) and testing by the bla_{KPC}/RNase P assay gave similar results. All 55 (100%) broth samples positive for carbapenem-resistant bacteria by culture and qPCR after automated

TABLE 2. Comparison of detection of carbapenem-resistant bacteria in Bactec bottle broth by culture and detection of bla_{KPC}/RNase P genes by qPCR after extraction with the Roche MagNA Pure LC automated extractor

bla_{KPC} DNA dilution and qPCR result	No. of samples with the following culture result for CRE[a]:		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Avg C_T value		
	Positive	Negative					bla_{KPC}	RNase P	Negative samples
Undiluted									
Positive	55	0	100[b]	100[c]	100	100	18.8 (15.9–26.6)	27.6 (21–41.8)	
Negative	0	268							Undetermined[d]
Diluted 1:10									
Positive	55	0	100[b]	100[c]	100	100	21.1 (17.9–29.9)	31.5 (24.4–>45)	
Negative	0	268							Undetermined

[a] CRE, carbapenem-resistant *Enterobacteriaceae*.
[b] 95% confidence interval = 93.45 to 100%.
[c] 95% confidence interval = 98.59 to 100%.
[d] Undetermined, the sample's fluorescence reading was below the threshold cutoff value of 50 cycles.

DNA extraction were also positive for the bla_{KPC} genes following manual DNA extraction. The mean bla_{KPC} C_T value was 22.2. Stratifying the bla_{KPC} C_T values revealed that 48 (87.3%) blood culture bottles gave C_T values of <25 and 7 (12.7%) had C_T values of between 25 and <35. Stratifying the C_T values of the 7 samples that gave C_T values of between 25 and 35 revealed that 7 samples gave C_T values below 30, while one sample gave a C_T value of 33. The internal control RNase P was positive in all 55 (100%) of the positive bottles. Upon stratification of the RNase P results by C_T value, 11 (20%) gave C_T values of <25, 42 (76.4%) gave C_T values of 25 to <35, and 2 (3.6%) gave C_T values of 35 to <45.

Detection of bla_{KPC}/RNase P by qPCR in the absence of BSA. Testing extracted DNA from the 55 bla_{KPC}-positive Bactec blood culture bottles by bla_{KPC}/RNase P qPCR assay in the absence of BSA revealed that only 7 (12.7%) blood culture bottles were positive for the bla_{KPC} genes. The mean C_T value of the bla_{KPC} was 29, and the C_T range was from 21 to 37.4. The internal control RNase P was positive in only 18 (32.7%) of the 55 positive blood culture bottles, and the C_T values were between 30.2 and 49.1. Diluting the extracted DNA (1:10) relieved some of the inhibitors present in the extracted DNA. Indeed, of the 55 bla_{KPC}-positive blood culture bottles, 43 (78.2%) gave a positive signal. The mean C_T value of the bla_{KPC} qPCR was 26.3, and the C_T range was from 18.8 to 51.6. Upon stratification of the bla_{KPC} results by C_T value, 29 (52.7%) gave C_T values of <25, 8 (14.5%) gave C_T values of 25 to <35, 4 (7.3%) gave C_T values of 35 to <45, 2 (3.6%) gave C_T values of >45, and 12 (21.8%) were negative. The internal control RNase P was positive in 52 (94.5%) samples, and the C_T values were between 25.1 and 50.7. Breaking down the RNase P internal control results by C_T value revealed that none (0%) gave a C_T value of <25, 35 (81.8%) gave C_T values of 25 to <35, 4 (7.3%) gave C_T values of 35 to <45, 3 (5.5%) gave C_T values of >45, and 3 (5.5%) were negative. Diluting the bacterial DNA samples 1:100 and 1:1,000 did not further improve the result relative to that with the 1:10 dilution (data not shown).

DISCUSSION

Here we report on the development and validation of a TaqMan multiplex qPCR assay for the rapid detection of bla_{KPC} genes from Bactec bottles. The assay, which includes human gene RNase P as an internal control, has been optimized to circumvent the inhibitors present in the broth of Bactec bottles and to be completed in less than 4 h. This assay also showed that once BSA fraction V (96%) was utilized as an inhibitor reliever, no manipulation of the samples was required before or after manual or automated extraction, as previously reported (10, 14).

The multiplex bla_{KPC}/RNase P assay is especially valuable in institutions that experience a high incidence of bacteremias due to bla_{KPC}-positive bacteria. The implementation of this assay at Chaim Sheba Medical Center in the peak of the bla_{KPC}-positive bacteria outbreak would have helped in starting the appropriate antimicrobial therapy 24 to 48 h earlier than it was when culture results became available. Given the limited therapeutic options available, the accurate detection of bla_{KPC}-possessing bacteria will ensure optimal therapy, and it will be a crucial first step in controlling their spread.

The analytical sensitivity of the assay was 2 CFU/reaction mixture in bacterium-spiked naïve Bactec bottles and 19 CFU/reaction mixture in blood-spiked Bactec bottles. The analytical sensitivity in naïve Bactec bottles was similar to the analytical sensitivity of the singleplex bla_{KPC} assay reported earlier (12). The assay is sensitive for concentrations 1 log unit below the limit of detection of the Bactec system (at least 10^4 CFU/ml); thus, it will detect the bla_{KPC}-positive bacteria if they are present in the bottles (26). The analytical sensitivity of the multiplex bla_{KPC}/RNase P assay was comparable to sensitivities achieved for detection of 10 CFU/reaction mixture of both *E. coli* and *Klebsiella pneumoniae* in blood culture bottles (10, 17). However; the earlier studies did not determine the analytical sensitivity in human blood-spiked Bactec bottles.

The analytical sensitivity of the assay was associated with excellent clinical sensitivity. All 55 bla_{KPC}-positive samples which were detected by culture were also found to be positive by the qPCR after extraction by the MagNA Pure LC (version 2.0) automated system, and no discrepant samples had to be evaluated. Thus, the sensitivity, specificity, NPV, and PPV of the qPCR assay were 100%. All bla_{KPC} C_T values were below 30, and the internal control gave positive C_T values of <45 for all but one sample that was bla_{KPC} positive. Thus, reports were issued for all 323 samples evaluated, and none were reported to be uninterpretable. In the advent that the sample was negative for both bla_{KPC} and RNase P, the result for the sample would have been reported as uninterpretable. Similar results were obtained upon dilution of the DNA 1:10, and since no further improvement in C_T values of the positive samples was noted after 1:100 and 1:1,000 dilutions, the chemistry of the developed assay appears to have relieved the effect of the majority of the inhibitors present in the sample. The excellent and precise sensitivity and specificity of this assay allow the straightforward implementation in laboratories with a molecular setup. Additionally, the absence of background fluorescence signals allows the clinical laboratory personnel to easily and rapidly interpret the results of the samples analyzed.

The major inhibitor in blood culture bottles documented to affect the capacity of PCR assays is the anticoagulant sodium polyanetholesulfonate (SPS) (9). SPS is thought to copurify with the extracted DNA. Relief of the qPCR inhibitor SPS appears to be effectively accomplished by the V fraction of 96% BSA, as previously reported (3), and the utilization of a special extraction protocol, using an organic extraction procedure with benzyl alcohol to relieve the inhibitory effect of SPS, as previously reported, was not necessary (9). Utilization of 0.5% BSA in TaqMan qPCR assays was previously reported to relieve the inhibitors present in the Bactec bottles positive for *Candida* species (18).

It is well documented that PCR efficiency is affected by the heme and IgG components of the blood in the Bactec bottles (1–3). This explains the drop of the qPCR amplification efficiency in the described bla_{KPC}/RNase P assay from 92% in the naïve bottles to 80.5% in the blood-spiked bottles. However, the drop in the PCR amplification efficiency and analytical sensitivity by 1 log unit did not affect the clinical sensitivity of the assay, as 100% correlation was noted between the gold standard, culture, and the qPCR. In addition, all the positive

blood culture bottles gave strong positive results, with C_T values below 27.

For medical institutions with low incidences of *bla*$_{KPC}$-positive bacteria or where automated extractors are not available, manual extraction of bacterial DNA can be used. All 55 samples found to be *bla*$_{KPC}$ positive after MagNA Pure LC automated extraction were also positive after manual extraction with the QIAamp DNA minikit. However, there was an increase in the mean C_T value of the positive samples compared to that with automated extraction, from 18.6 to 22.2. The 1-log-unit difference could be ascribed to the higher efficiency of automated extraction than manual extraction. Indeed, earlier studies have also shown than MagNa Pure LC automated DNA extraction was more efficient than manual extraction, in particular, QIAamp blood DNA extraction (31). We were unable to evaluate the semiautomated extractor system, the bioMérieux NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France), as the resin beads present in the Bactec bottles clogged the system's tubing.

The global spread of *bla*$_{KPC}$-positive drug-resistant bacteria is alarming and mandates that clinical microbiologists have the capabilities to detect these pathogens in a reliable and timely manner. Utilization of the *bla*$_{KPC}$/RNase P assay as described here can reduce the time to detection of positive blood cultures, thus helping physicians in patient management. Moreover, this assay can be used as a model for the development of other molecular assays applicable to Bactec bottles.

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