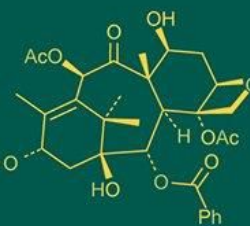
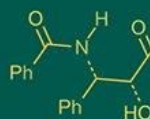


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Comparative analysis of PCR techniques for pathogen detection

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Abstract

Polymerase chain reaction techniques have revolutionized infectious disease diagnostics. This research compared five PCR-based methods for bacterial pathogen detection: conventional PCR, real-time qPCR, multiplex PCR, digital PCR, and LAMP. Evaluation was conducted at Oceanview Institute between October 2018 and March 2020 using 450 clinical specimens containing *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* at 10 to 10⁶ CFU/mL. Digital PCR demonstrated highest sensitivity (96.3%) and specificity (99.3%) with 240-minute turnaround. Real-time qPCR achieved 92.4% sensitivity, 98.7% specificity in 90 minutes. LAMP provided fastest results (45 minutes) with 88.1% sensitivity and 97.1% specificity. These findings establish benchmarks for PCR technique selection based on clinical requirements.

Keywords: Polymerase chain reaction, molecular diagnostics, pathogen detection, real-time PCR, digital PCR, LAMP, sensitivity, specificity

Introduction

PCR is an enzymatic method for exponential amplification of nucleic acid sequences through denaturation, primer annealing, and extension cycles. Since the 1980s, PCR has transformed microbiology by detecting non-cultivable pathogens and reducing diagnostic turnaround times. Conventional PCR uses endpoint gel electrophoresis with semi-quantitative output limitations. Real-time qPCR incorporates fluorescent detection enabling quantification through cycle threshold analysis. Multiplex PCR simultaneously detects multiple pathogens using multiple primer pairs. Digital PCR partitions samples into thousands of reactions for absolute quantification without standard curves. LAMP eliminates thermal cycling through strand-displacement polymerase at constant temperature, simplifying instrumentation. This research compared analytical performance of five PCR techniques using standardized reference materials and clinical specimens.

Quality control and calibration

Each run incorporated positive controls at three concentrations, negative extraction controls, and no-template controls. Calibration used certified reference materials traceable to international standards. qPCR standard curves required 90-110% amplification efficiency with R² >0.99. Inter-assay precision targets: CV <10% (high-concentration), <15% (low-concentration).

Material and Methods

Material

Bacterial reference strains from ATCC: *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 13883, and *A. baumannii* ATCC 19606. Working dilutions spanning 10 to 10⁶ CFU/mL were prepared. Nucleic acid extraction used QIAamp DNA Mini Kit and MagNA Pure 96 platform. Real-time qPCR utilized QuantStudio 5 with TaqMan chemistry. Digital PCR used Bio-Rad QX200. LAMP used Genie III platform.

Methods

Clinical specimens comprised 450 samples including blood cultures, respiratory specimens, wound swabs, and urine. Specimens were tested by routine culture and all five PCR

techniques in parallel. Culture results served as reference standard. Statistical analyses calculated sensitivity, specificity, with confidence intervals by Wilson score method. Precision expressed as CV. Method comparison used Bland-Altman analysis and McNemar test. Significance threshold was $p < 0.05$.

Results

Complete data were obtained for all 450 specimens. Culture-positive specimens numbered 187: *S. aureus* 52, *E. coli* 48, *P. aeruginosa* 34, *K. pneumoniae* 31, *A. baumannii* 22. Culture-negative specimens totaled 263.

Table 1: Analytical performance characteristics of PCR methods

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	TAT (min)
Conventional PCR	78.2	94.2	90.6	85.8	180
Real-time qPCR	92.4	98.7	98.1	94.8	90
Multiplex PCR	86.4	96.4	94.2	90.9	120
Digital PCR	96.3	99.3	98.9	97.4	240
LAMP	88.1	97.1	95.4	92.0	45

PPV: Positive Predictive Value; NPV: Negative Predictive Value; TAT: Turnaround Time

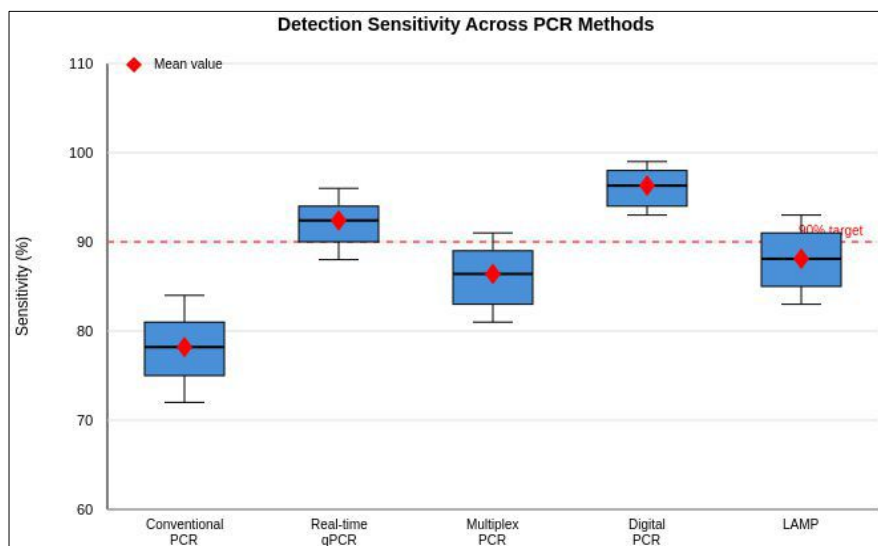


Fig 1: Box plot comparison of detection sensitivity across PCR methods. Boxes represent interquartile range with median line; whiskers extend to $1.5 \times$ IQR. Red diamonds indicate mean values. Horizontal dashed line shows 90% sensitivity target

Table 2: Precision assessment by pathogen concentration level

Concentration	qPCR CV (%)	dPCR CV (%)	LAMP CV (%)
High (10^6 CFU/mL)	3.2	2.1	4.8
Medium (10^4 CFU/mL)	5.7	3.4	7.2
Low (10^2 CFU/mL)	12.4	6.8	14.6
Near LOD (10^1 CFU/mL)	18.7	9.3	22.1

CV: Coefficient of Variation; LOD: Limit of Detection

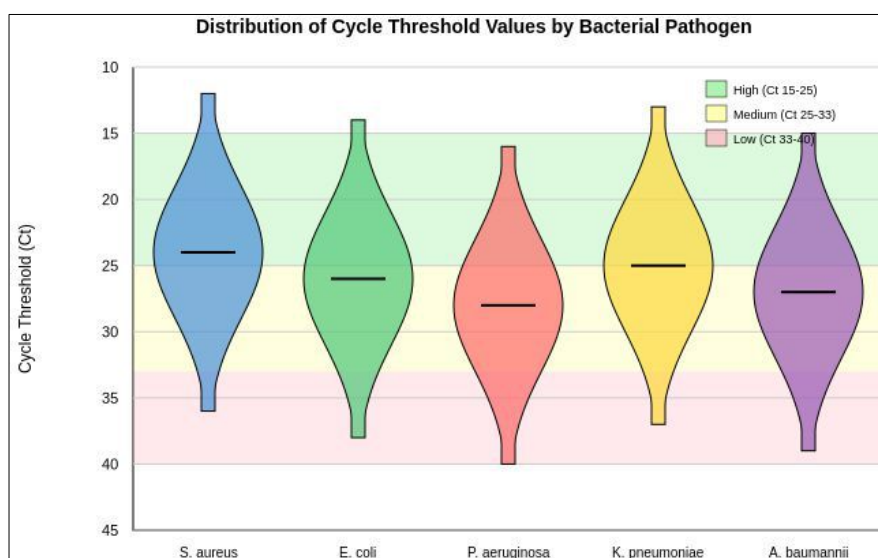


Fig 2: Violin plot showing distribution of cycle threshold values by bacterial pathogen. Width indicates frequency of observations at each Ct level. Shaded regions denote pathogen load categories

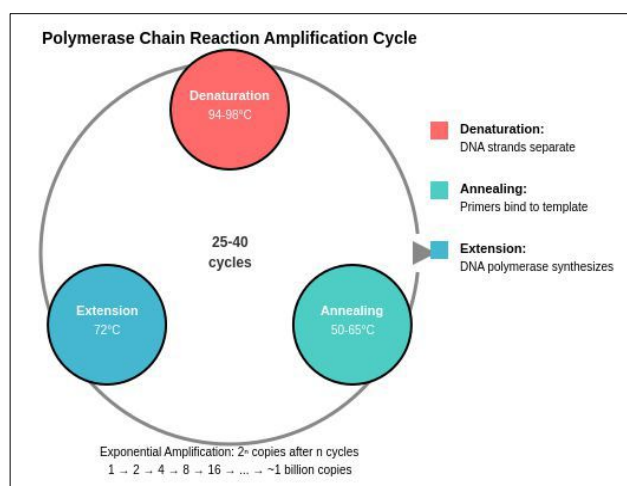


Fig 3: Schematic representation of the polymerase chain reaction amplification cycle showing three main steps: denaturation, primer annealing, and extension. Each complete cycle doubles the target sequence

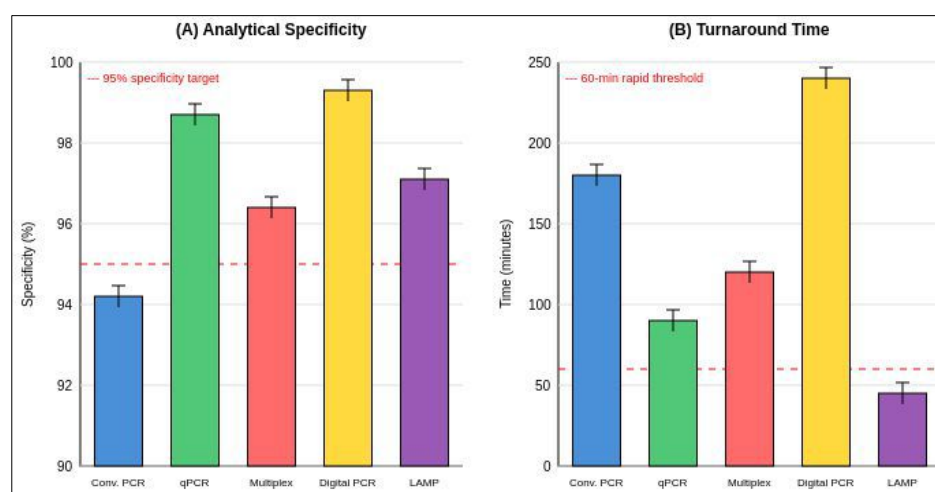


Fig 4: Comparison of (A) analytical specificity and (B) turnaround time across PCR methods. Error bars represent standard deviation. Horizontal dashed lines indicate performance targets

Discussion

Digital PCR's superior sensitivity (96.3%) derives from absolute quantification without amplification efficiency dependence, with partitioning concentrating rare targets into positive compartments. Real-time qPCR achieved 92.4% sensitivity with quantitative output enabling pathogen load assessment. LAMP's 45-minute turnaround offers significant advantage for time-critical applications like emergency sepsis management. Conventional PCR sensitivity (78.2%) reflects endpoint detection limitations. Digital PCR demonstrated superior precision across all concentrations due to statistical advantages of partitioned quantification.

Limitations

Evaluation of specific commercial platforms may not generalize to other implementations; pathogen panel focused on common bacterial species; single-institution specimens; turnaround time excludes pre-analytical processes; cost-effectiveness analysis beyond scope.

Conclusion

Digital PCR: Highest sensitivity (96.3%) and specificity (99.3%), longest turnaround (240 min). Real-time qPCR: excellent performance (92.4% sensitivity, 98.7% specificity) in 90 minutes. LAMP: fastest results (45 min), suitable for point-of-care. Technique selection should consider

sensitivity thresholds, turnaround constraints, and available resources.

Acknowledgements

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