

0477

Impact of delays to incubation and storage temperature on blood culture results in tropical countries: A multi-centre study



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Background: Blood cultures are one of the most important specimens processed by microbiology laboratories. Currently, there is insufficient guidance on how to transport blood cultures in low- and middle- income countries with tropical climates where delays may be unavoidable. To fill this knowledge gap, this study investigated the effects of storage for different times and temperatures on the isolation of important bacterial pathogens.

Methods and materials: In three laboratories located in Cambodia, Laos and Thailand, paediatric blood culture bottles were spiked with approximately 3 CFU/ml of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, or *Haemophilus influenzae*. The spiked bottles were stored at 25 °C, in a cool box at ambient temperature or at 40 °C; for 0 h, 6 h, 12 h or 24 h before testing by one of four blood culture systems (two manual and two automated). All bottles were incubated until positive or for at least five days, with all growth identified.

Results: No significant differences in positive bottle yields were detected when bottles were stored for 6 h (240/270, 88.9%), 12 h (233/269, 86.6%), at 25 °C (243/270, 90.0%); or in a cool box (232/269, 86.2%), when compared to no storage (80/90, 88.9%; *p* values > 0.05). However, significantly lower yields compared to no storage were detected when stored for 24 h (212/270, 78.5%; *p* = 0.030) or at 40 °C (210/270, 77.8%; *p* = 0.021); with the lowest yield obtained for bottles stored for 24 h at 40 °C (59/90, 65.6%; *p* < 0.001). The median time to positivity from inoculation increased with increased storage time; rising from 13.0 h (IQR 12.3–16.4) with no storage to 28.1 h (IQR 26.8–30.9) following 24 h of storage (*p* for trend < 0.001).

Conclusion: Blood culture bottles should be incubated with minimal delay to maximise pathogen recovery and timely result reporting, however, this study provides some reassurance that unavoidable delays in incubating blood cultures in tropical climates can be managed to minimise negative impacts. If delays to incubation are unavoidable and are ≥ 12 h, transportation at a

temperature not exceeding 25 °C and blind sub-cultures prior to incubation should be considered.

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0478

Standardization of LAMP Assay for early detection of *Acinetobacter baumannii* and its resistant variants from patients with sepsis



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Background: *Acinetobacter baumannii* (AB) is an important nosocomial pathogen and AB sepsis is commonest infection. The mortality rate is up to 20% (neonates), 43.4% (ICU) and 16.3% (Outside ICU) in adults. Carbapenem resistance (CR) among AB has emerged globally. The risk of mortality increases by 7.6% with every 1 hour delay in optimal therapy. The turnaround time (TAT) for identification and detection of resistance using conventional diagnostic is 24–48 h. There is an urgent need of rapid diagnostics which detects both the pathogen and CR in early phase to initiate early optimal treatment and prevent unnecessary use of last resort antibiotics. Loop Mediated Isothermal Amplification (LAMP) assay for rapid detection of AB and CR directly from blood and blood culture broth from patients with sepsis was standardized in this study.

Methods and materials: LAMP primers were designed for detection of AB targeting ITS 16S–23S rRNA gene and CR targeting commonly prevalent gene, blaOXA-23. An in-house technique for bacterial DNA extraction from blood & culture broth was developed. 1st the LAMP protocol was standardized using ATCC (controls) to optimize the time & temperature of assay. Limit of detection (LOD) of LAMP was determined using ATCC strains for each primer and results were compared with PCR. LAMP assay was then used for detection of AB and CR from direct blood sample and blood culture broth.

Results: The optimal temperature for LAMP assay was 65 °C, and the detection time varied with primers. Using ATCC strains, LOD of LAMP Assay & PCR was 100 pg/μl & 1 ng/μl of DNA concentration and 10⁴ cfu/ml & 10⁶ cfu/ml of colony count respectively. The sensitivity of LAMP assay was 10 and 100 times better than PCR using DNA concentration and colony count respectively. The In-house DNA extraction method successfully detected AB using LAMP directly from blood & culture broth inoculated with *Acinetobacter*. LOD was 10⁶ cfu/ml from direct blood. TAT using LAMP assay was 4–6 h from blood collection to report generation.

Conclusion: The above results showed that, LAMP assay can be used for early detection of AB and its resistant variant from clinical samples of sepsis patients. Depending on the locally prevalent carbapenem resistant genes LAMP assay can be modified.

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