

The Summer Undergraduate Research Fellowship (SURF) Symposium
4 August 2016
Purdue University, West Lafayette, Indiana, USA

Lysis and Amplification of Neonatal Sepsis Causing Pathogens

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ABSTRACT

Neonatal sepsis, resulting from a bloodstream infection within the first few weeks of life, is the leading cause of newborn deaths worldwide. The gold standard of neonatal sepsis diagnosis requires a blood culture to identify the infecting bacteria, however require days of incubation, expensive equipment, and expertise. Any delay in diagnosis is critical, as the condition can be treated easily if appropriate antibiotics are administered promptly. A low-cost, rapid, and sensitive diagnostic test would enable more timely treatment and lead to better patient outcomes with fewer required resources. Point-of-care, nucleic acid amplification assays are a promising alternative to blood culture that quickly deliver sensitive results with minimal sample volume. However, these require isolated and purified template DNA from the pathogenic bacteria, a task that is difficult to achieve in a field setting. This study sought to develop a simple one-step lysis and amplification protocol for three common bacterial causes of neonatal sepsis, *Streptococcus agalactiae*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The combined efficacy of enzymes, proteinase K and achromopeptidase (ACP), and heat to lyse each of these bacteria for direct DNA amplification was examined. Results showed that all three strains could be effectively lysed by applying 1 U of ACP and incubating for 10 minutes at 37° C. No adverse effects were seen in amplification reactions containing ACP if the compound was inactivated prior to amplification at 85° C for 2 minutes. The demonstrated effectiveness of ACP in rapid bacterial lysis validates its usefulness in a point-of-care device for neonatal sepsis.

KEYWORDS

Lysis, Neonatal Sepsis, Achromopeptidase, point-of-care, Loop-mediated Isothermal Amplification