Development of Real Time Polymerase Chain Reaction for the Detection of 
Salmonella in Stool Specimens

Angeziwa Chunga¹,⁴, Tonney Nyirenda¹,², Ndaru Kaluwa¹, Chisomo Msefula¹,², Sandra Molina⁴ and Melita Gordon¹,³

¹Malawi Liverpool Wellcome Trust Clinical Research Programme, Malawi; ²Department of Pathology, College of Medicine, Malawi; ³University of Liverpool, UK; ⁴London School of Hygiene and Tropical Medicine, UK

Background: Salmonellae are among the leading cause of bacteraemia and death in sub-Saharan African children. The burden of Salmonella in Africa and the link between Salmonella exposure within the gastrointestinal tract and blood stream is poorly understood in part due to lack of reliable diagnostic test for detection of Salmonella. Stool culture, which is the gold standard for Salmonella detection, is less sensitive and time consuming. In this study, we aimed at validating Quantitative Real Time-Polymerase Chain Reaction (RT-PCR) test for the detection of Salmonella in stool specimens from a cohort of Salmonella asymptomatic children.

Methods: RT-PCR tests using primers from Tetrathionate (TTR) respiration gene and Salmonella Invasion gene A (InvA). TTR and InvA RT-PCR assays were tested for inclusivity using different Salmonella strains and exclusivity was tested using different gram positive and negative non Salmonella bacteria. PCR efficiency and limits of detection were determined using Salmonella Typhimurium D23580 reference strain. The primers were also validated against stool culture for Salmonella. Salmonella exposure events in 409 stool samples collected from a cohort of healthy children aged 6-18 months was also determined and sensitivity and specificity rates of the assays were calculated.

Results: Both TTR and InvA RT-PCR demonstrated 100% inclusivity and between 87% and 94% exclusivity rates. Both assays had superior limits of detection of up to 1 CFU/ml when sub cultured in selenite F broth with 98% PCR efficiency. Sensitivity and specificity of TTR was 73.91%, 91.3% and 96.89%, 95.08% and for InvA it was 78.26%, 82.61% and 92.49%, 90.41% for neat and selenite sub cultured stool samples respectively.

Conclusion: TTR and InvA RT-PCR assays demonstrated superior performance than stool culture. Selenite sub culturing of the samples improves performance and reduces cross reactivity. The two primers can be used together as a diagnostic tool for surveillance studies.