Aims An assay based on 16S rDNA PCR technology has been designed to detect a single intact bacterium whilst eliminating free DNA from dead bacteria, thus offering unprecedented sensitivity and scope to the analysis of bacterial carriage in clinical specimens. We hypothesised that application of such an assay to neonatal CSF will enable accurate, fast and inexpensive discrimination of bacteria-free specimens, and will have a small but clinically acceptable false-positive rate.

Methods Design of PCRctic – a novel assay based on 16S rDNA PCR technology utilising ethidium azide for elimination of free bacterial DNA and optimised for neonatal CSF – was presented at this conference in 2016. In this prospective study lasting 12 months, the feasibility of PCRctic was investigated in CSF specimens obtained from newborn babies tested for meningitis. Following interim analysis, sterile snap-top tubes (Eppendorf™) replaced standard universal containers for collection of CSF, and Chloraprep™ replaced Unisept as the choice of antiseptic. Study received National REC and HRA approvals and was funded by the MRC.

Results Fifty-two specimens of CSF were tested before the interim analysis (1st phase) and 21 after (2nd phase). In phase 1, the assay detected bacteria in 19 specimens (36%) and sequencing revealed several organisms of Flavobacteriaceae family (Cloacibacterium, Flavobacterium, Hymenobacter), as well as Ochrobactrum (Brucellaceae), Sneathia amnii (Leptotrichiaceae), Pseudomonas spp, Acinetobacter, Sphingomonadaceae, Oscillatoriales (Cyanobacteria), Ureaplasma urealyticum, Staphylococcus auricularis, Streptococcus spp, Bdellovibrio, Aerococcus christensenii, Methylobacterium, and Pedobacter (Sphingomonadaceae). In phase 2, bacteria were detected in two specimens (9.5%) and sequencing revealed Geobacter in one and mixed spp in the other. No clinical cases of neonatal bacterial meningitis occurred during the study. A positive signal was detected in only one out of 23 negative controls designed to test for environmental contamination (4%), sequencing revealed Bacillus.

Conclusion The assay’s rate of positive results decreased significantly following simple steps to reduce the risk of contamination at the time of CSF collection. Using additional inexpensive measures it may be possible to reduce the rate further and begin to explore the introduction of the assay into practice.