Reviewer’s report

**Title:** Comparison of Microbiological Culture and Real-time PCR for the Detection of Invasive Salmonella serovars in Blood and Bone Marrow Specimens from Enteric fever Patients

**Version:** 1  **Date:** 19 August 2009

**Reviewer:** Harsh Vardhan V Batra

**Reviewer’s report:**

This manuscript describes a real time multiplex PCR assay to detect Salmonella serovar Typhi and serovar Paratyphi A from blood and bone marrow samples. The real time multiplex PCR assay target 131 bp segment of STY0201 and 104 bp segment of SSPA2308 for typhi and paratyphi serovars respectively. The authors have done substantial amount of work to validate the PCR assay by spiking blood samples with different bacterial dilutions.

**Major compulsory revision**

The glaring conclusion of the present work is the low sensitivity (42% for typhi and 39% for paratyphi A). Quite a number of reports describing highly sensitive PCR assays are available. To cite a few, Sanchez-Jimenez et al, J. Med Microbiol (2004), 53, 875–878 have reported PCR assays which were more sensitive than the culture method. Similarly, PCR assays developed by Levvy et al J. Clin Microbiol 2008: 46, 1861-1866 also have 100% sensitivity and specificity. The authors further mention in the discussion section that PCR assay may be an alternative in situations where blood culture is not routinely performed. But with this low level of sensitivity, the present PCR assay cannot be an alternative for blood culture.

The real time PCR assay has been evaluated in situations where the clinical enteric fever in patients is due to salmonella serovars of Typhi and Paratyphi A. Salmonella serovars other than these two serovars involved in systemic infection need to be tested; atleast at organism level these be included to work out specificity. Otherwise the assay would not be even suggestive that enteric fever could be due to some other Salmonella serovar. The investigators probably need to reassess the real time PCR on to more appropriate clinical samples that should include the day of pyrexia samples before the initiation of rational antibiotic therapy. The spectrum of bacterial strains used for specificity testing should be increased by incorporating more strains of serovars like Paratyphi B, C, choleraesuis etc.

In all the PCR assays the E. coli strain VU1 has been spiked into the blood inoculations and other bacterial dilutions for the purpose of internal amplification control (IAC). The normal practice is to incorporate a synthetic IAC construct in the PCR assays rather than spiking the E. coli strain harbouring the IAC construct. Real time PCR assays standardised with suitable concentration of
primers and probe for the IAC also would improve the robustness of the assay. The gene sequence targeted in the PCR assays should be sequenced and submitted in the data base.

The geneeral conclusion derived by the investigators suggesting that the reported PCR do not have a high level of sensitivity when compared to culture cannot be accepted with the data presented. Even in our group we always find nearly 100% sensitivity of the reported PCR's with culture and antigen detection.

The utility of this real time PCR assay can be improved by incorporating in the multiplex PCR assay a suitable gene target which is generic for salmonella serovars responsible for the enteric fever. The sensitivity could be improved by standardizing suitable DNA purification methods and improved sampling methods.

Minor essential revision
1. Materials and methods section:1st para, The 7th line to be rephrased so that it does not begin with numeral.
2. Page 9; fourth line to be corrected gramatically.
3. Abstract line No 4 need to be gramatically corrected.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests' below