PCR Diagnosis of Typhoid Fever

This is in response to practical concerns from Dr. Frankel (1) about our nested PCR to detect *Salmonella typhi* DNA in the blood of patients with typhoid fever. As shown in our paper (4), nested PCR with two sets of primers, which we used, detected amplification products of both *S. typhi* and *S. muenchen* in the specificity test with DNAs from various *Salmonella* species. This was due to the nonspecificity of the second set of primers (ST 3 and ST 4) that was used in the second round of PCR, as Dr. Frankel pointed out. I would like to second Dr. Frankel's suggestion to use a new ST 1 primer in the first and second rounds of the reaction, which could circumvent this positive cross-reaction between *S. typhi* and *S. muenchen*. The sensitivity of the nested PCR using these new primers, however, should be verified for the clinical specimens, particularly in culture-negative cases. The nested PCR with two sets of primers reported in our paper has been used as a routine diagnostic test for culture-negative suspected typhoid cases in our hospital. As expected, this technique has proved to be very useful in clinical practice. It could minimize the unnecessary use of empirical antibiotics for presumed typhoid fever and could simplify the diagnostic procedures for acute febrile illnesses without an obvious focus of infection. To date, the sensitivity and specificity of this technique with clinical specimens are 93 and 100%, respectively. With the increasing use of this technique as a clinical test, we are concerned about the prevention of carryover contamination as well as simplification of the PCR procedure. The nested PCR technique was sensitive enough for us to avoid the use of Southern blot hybridization, but it is subjected to a certain risk of carryover contamination. Various measures to prevent carryover contamination, including the use of isopsoralen and UV or uracil *N*-glycosylase, should be applied to minimize the chance of a false-positive reaction (3), and the development of a chemiluminescence technique after a single round of the reaction instead of the nested reaction is warranted (2).

REFERENCES

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