IAP Task Force Report: Diagnosis of Enteric Fever in Children

Wirting Committee

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Enteric fever, despite its presence for a long time, very little progress has been made in the diagnosis of the disease. Diagnosis is still made either clinically or by Widal test and very rarely by blood culture. Blood culture is not only the gold standard for diagnosis but also suggests the sensitivity pattern of the organism.

Till 1990's enteric fever was managed by cheap effective first line drugs. With the emergence of multi drug resistant typhoid the treatment has become complex with several antibiotics being used. This has further added to the problem of resistance with some strains showing resistance to both fluoroquinolones and cephalosporins. Indian Academy of Pediatrics has set up a National Task Force under IAP Action Plan 2006 to form a uniform guideline on management of enteric fever,

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particularly antibiotics to be used, which can be followed by all members of IAP in general. The list of the members who took part in the workshop is given in Annexure I.

The correct and rapid diagnosis of enteric fever is of paramount importance for instituting appropriate therapy and also for avoiding unnecessary therapy.

Complete Blood Count (CBC)

For practical purposes the CBC in enteric fever is unremarkable. The hemoglobin is normal in the initial stages but drops with progressing illness. Severe anemia is unusual and should make one suspect intestinal hemorrhage or hemolysis or an alternative diagnosis like malaria. The WBC count is normal in most cases and leukocytosis makes the diagnosis less probable. Leukopenia perceived to be an important feature of typhoid fever and has been reported in only 20-25% cases[1]. The differential count is usually unremarkable except for eosinopenia. Eosinopenia often absolute may be present in 70-80% cases[2,3]. Presence of absolute eosinopenia offers a clue to diagnosis but does not differentiate enteric fever from other acute bacterial or viral infections. Conversely, a normal eosinophil count does make typhoid fever a less likely possibility. Platelet counts are normal to begin with and fall in some cases by the second week of illness. Overall prevalence of thrombocytopenia is around 10-15%[4].

Cultures

Blood Culture

Blood cultures are the gold standard diagnostic method for diagnosis of enteric fever[5]. The

sensitivity of blood culture is highest in the first week of the illness and reduces with advancing illness(6). Overall sensitivity is around 50% but drops considerably with prior antibiotic therapy(6). Failure to isolate the organism may be caused by several factors which includes inadequate laboratory media, the volume of blood taken for culture, the presence of antibiotics and the time of collection. For blood culture it is essential to inoculate media at the time of drawing blood.

Salmonella can be easily cultured in most microbiologic laboratories with use of routine culture media (Hartley's media, Blood agar and MacConkey agar). Automated blood culture systems such as BACTEC certainly enhance the recovery rate. Sufficient amount of blood should be collected for culture as the median bacterial count in the peripheral blood is only 0.3 CFU/ml (inter quartile range 0.1 to 10; range, 0.1 to 399)(7). At least 10 mL of blood in adults and 5 mL in children should be collected. Dilution should be appropriate in order to adequately neutralize the bactericidal effect of serum and a ratio of 1:5 to 1:10 of blood to broth is recommended. Clot cultures, wherein the inhibitory effect of serum is obviated have not been found to be of superior sensitivity as compared to blood cultures in several clinical studies(8-10). In the laboratory, blood culture bottles should be incubated at 37°C and checked for turbidity, gas formation and other evidence of growth after 1, 2, 3 and 7 days. For days 1, 2 and 3 only bottles showing signs of positive growth are cultured on agar plates. On day 7 all bottles should be sub-cultured before being discarded as negative.

There are considerable advantages of routine blood cultures in investigation of suspected enteric fever. Not only are they 100% specific, but they also provide information on the antimicrobial sensitivity of the isolate. This is vital in today's scenario of multidrug resistance. Moreover, alternative methods for diagnosis particularly serology are rather unsatisfactory as will be discussed later. Blood cultures may actually turn out to be cheaper and very cost effective in the long run, as positive cultures unequivocally establish the diagnosis of enteric fever and all other investigations for PUO can be safely deferred.

Bone marrow culture

Salmonella typhi is an intracellular pathogen in the reticuloendothelial cells of the body including the bone marrow(5). Studies have revealed that the median bacteremia in the bone marrow is 9 CFU/mL (IQR 1 to 85; range 0.1 to 15,805) compared to 0.3 CFU/mL (IQR 0.1 to 10; range 0.1 to 399) in blood. This bone marrow: peripheral blood ratio which is around 4.8 (IQR 1 to 27.5) in the first week of the illness increases to 158 (IQR 60 to 397) during the third week owing to disappearance of bacteria from the peripheral blood(7). The overall sensitivity of bone marrow cultures ranges from 80-95% and is good even in late disease and despite prior antibiotic therapy(5,11-13).

The invasive nature of bone marrow aspiration deters from its use as a first line investigation for diagnosis of typhoid fever. It is however a very useful and valid investigation in evaluation of PUO wherein the marrow should be inoculated in the culture bottle at the bedside.

Stool, urine and other cultures

Stool specimen should be collected in a sterile wide mouthed container. Specimens should preferably be processed within 2 hours after collection. If there is a delay the specimen should be stored in a refrigerator at 4°C or in a cool box with freezer packs. The sensitivity of stool culture depends on the amount of feces cultured, and the positivity rate increases with the duration of the illness. Rectal swabs should

be avoided as these are less successful. Stool cultures are positive in 30% of patients with acute enteric fever(5). For the detection of carriers, several samples should be examined because of irregular shedding of salmonella. Urine cultures are not recommended for diagnosis in view of poor sensitivity(5,14). Other methods such as duodenal string and skin snip culture of rose spots have been reported to be more efficacious than blood cultures but are mainly of academic importance(14-16).

Antimicrobial sensitivity testing

The crucial issue here pertains to fluoro-quinolone susceptibility testing. Fluoro-quinolones were introduced in 1989 and during the past decade there has been a progressive increase in the MICs of ciprofloxacin in *Salmonella typhi* and *paratyphi*(5). Since the current MIC's are still below the National Committee for Clinical Laboratory Standards (NCCLS) susceptibility breakpoint, laboratory reports will continue to report *Salmonella typhi/paratyphi* as ciprofloxacin/ofloxacin sensitive(17). However, use of fluoroquino-lones in this scenario is associated with a high incidence of clinical failure(5,17). It has also been demonstrated that resistance to nalidixic acid is a surrogate marker for high cipro-floxacin MIC's, predicts fluoroquinolone failure and can hence be used to guide antibiotic therapy (*i.e*, if culture results show resistance to nalidixic acid irrespective of the results of ciprofloxacin/ofloxacin sensitivity, quinolones should not be used or if used high doses should be given)(18). Since MIC testing is not within the scope of most laboratories, nalidixic acid susceptibility testing is mandatory to help guide choice of antibiotics.

Serologic tests

Widal test

This test first described by F Widal in 1896, detects agglutinating antibodies against the O and H antigens of *Salmonella typhi* and H antigens of *paratyphi* A and B(6,19). The "O" antigen is the somatic antigen of *Salmonella typhi* and is shared by *Salmonella paratyphi A, paratyphi B*, other Salmonella species and other members of the Enterobacteriaceae family(20). Antibodies against the O antigen are predominantly IgM, rise early in the illness and disappear early(20). The H antigens are flagellar antigens of *Salmonella typhi, paratyphi A* and *paratyphi B*. Antibodies to H antigens are both IgM and IgG, rise late in the illness and persist for a longer time(19,20). Usually, O antibodies appear on day 6-8 and H antibodies on days 10-12 after the onset of disease. The test is usually performed on an acute serum (at first contact with the patient). A convalescent serum should preferably also be collected so that paired titration's can be performed.

Conventionally, a positive Widal test result implies demonstration of rising titers in paired blood samples 10-14 days apart(19). Unfortunately, this criterion is purely of academic interest. Decisions about antibiotic therapy cannot wait for results from two samples. Moreover, antibiotics may dampen the immune response and prevent a rise in titers even in truly infected individuals. Therapeutic decisions have to be generally based on results of a single acute sample. In endemic areas, baseline anti O and anti H antibodies are present in the population owing to repeated subclinical infections with *Salmonella typhi/paratyphi*, infections with other Entero-bacteriaceae and other tropical diseases such as dengue and malaria(19-21). These antibody titers vary with age, socio economic strata, urban or rural areas and prior immunization with the TAB vaccine. Establishing appro-priate cut offs for distinguishing acute from past infections is thus important for the

population where the test is applied. In one study from Central India, anti O and anti H titer of more than 1:80 was seen in 14% and 8% respectively of a study sample of 1200 healthy blood donors(22).

While interpreting the results of the WIDAL test, both H and O antibodies have to be taken into account. There is controversy about the predictive value of O and H antibodies for diagnosis of enteric fever. Certain authorities claim that O antibodies have superior specificity and positive predictive value (PPV) because these antibodies decline early after an acute infection(23). Other studies report a poorer positive predictive value of O antibodies probably due to rise of these antibodies in other salmonella species, gram-negative infections, in unrelated infection and following TAB vaccination(21). For practical purpose and for optimal result this test should be done after 5-7 days of fever by tube method and level of both H and O antibodies of 1 in 160 dilution (four fold rise) should be taken as cut off value for diagnosis. H antibodies once positive can remain positive for a long time.

The Widal test as a diagnostic modality has suboptimal sensitivity and specificity(19-21). It can be negative in up to 30% of culture proven cases of typhoid fever. Sub optimal sensitivity results from negativity in early infection, prior antibiotic therapy and failure to mount an immune response by certain individuals(19). Poor specificity, an even greater problem and is a consequence of pre-existing baseline antibodies in endemic areas, cross reactivity with other Gram-negative infections and non-typhoidal salmonella, anamnestic reactions in unrelated infections and prior TAB or oral typhoid vaccination. The purity and standardization of antigens used for the WIDAL test is a major problem and often results in poor specificity and poor reproduci-bility of test results(19). The slide Widal test

should also be discouraged owing to high rate of false positives(20).

Not withstanding these problems, the WIDAL test may be the only test available in certain resource poor set ups for diagnosis of enteric. In Vietnam, using a cutoff of >1/200 for the O agglutinin or >1/100 for H agglutinin test performed on acute-phase serum the Widal test could correctly diagnose 74% of blood culture positive typhoid fever, however 14% results would be false positive and 10% false negative(21). Hence, it is important to realize the limitations of the Widal test and interpret the results carefully in light of endemic titers so that both over diagnosis and under diagnosis of typhoid fever and the resulting consequences are avoided(24).

Other serologic tests

In view of the limitations of the Widal test and need for a cheap and rapid diagnostic method, several attempts to develop alternative serologic tests have been made. These include rapid dipstick assays, dot enzyme immuno-assays and agglutination inhibition tests(25-27).

Enzyme Immunoassay (EIA) test or Typhidot® *test:* A dot enzyme immunoassay that detects IgG and IgM antibodies against a 50 KD outer membrane protein distinct from the somatic (O), flagellar (H) or capsular (Vi) antigen of *Salmonella typhi* is commercially available as Typhidot®(27). The sensitivity and specificity of this test has been reported to vary from 70-100% and 43-90% respectively(28-33). This dot EIA test offers simplicity, speed, early diagnosis and high negative and positive predictive values. The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. In areas of high endemicity where the rate of typhoid transmission is high the

detection of specific IgG increase. Since IgG can persist for more that 2 years after typhoid infection(34) the detection of specific IgG can not differentiate between acute and convalescent cases. Furthermore, false positive results attributable to previous infection may occur. On the other hand IgG positivity may also occur in the event of current reinfection. In cases of reinfection there is a secondary immune response with a significant boosting of IgG over IgM, such that the later can not be detected and its effect masked. A possible strategy for solving this problem is to enable the detection of IgM by ensuring that it is unmasked(35). The original Typhidot® test was modified by inactivating the total IgG in the serum samples. Studies with modified test, Typhidot® M, have shown that inactivation of IgG removes competitive binding and allows the access of the antigen to the specific IgM when it is present.

The Typhidot® M that detects only IgM antibodies of *Salmonella typhi* has been reported to be slightly more specific in a couple of studies(26,33).

IDL Tubex® test: The Tubex® test is easy to perform and takes approximately 2 minutes time(36). The test is based on detecting antibodies to a single antigen in *S. typhi* only. The 09 antigen used in this test is very specific found in only sero group D salmonellae. A positive result always suggest a salmonellae infection but not which group D salmonella is responsible. Infection by other serotypes like *S. paratyphi* A give negative result. This test detects IgM antibodies but not IgG which is further helpful in the diagnosis of current infections.

IgM dipstick test(26): The test is based on the binding of *S. typhi* specific IgM antibodies to *S. typhi* lipopolysaccharide (LPS) antigen and the staining of the bound antibodies by an antihuman IgM antibody conjugated to colloidal dye particles. This test will be useful in places where culture facilities are not available as it can be performed without formal training and in the absence of specialized equipments. One should keep in mind that specific antibodies appear a week after the onset of symptoms so the sensitivity of this test increases with time.

Antigen detection tests: Enzyme immuno-assay's, counterimmune electrophoresis and co-agglutination tests to detect serum or urinary somatic/flagellar/Vi antigens of *Salmonella typhi* have been evaluated(37-40). Sensitivity of Vi antigen has been found to be superior to somatic and flagellar antigen and has been reported as ranging from 50-100% in different studies(37-40). Similarly, specificity estimates have been reported to vary from 25-90% (37-40). The suboptimal and variable sensitivity and specificity estimates, inability to detect *Salmonella paratyphi* infection and Vi antigen negative strains of *S typhi* are serious limitations of the Vi antigen detection tests.

Molecular methods

The limitations of cultures and serologic tests advocate for development of alternative diagnostic strategies. PCR as a diagnostic modality for typhoid fever was first evaluated in 1993 when Song, *et al.* successfully amplified the flagellin gene of *S. typhi* in all cases of culture proven typhoid fever and from none of the healthy controls(41). Moreover, some patients with culture negative typhoid fever were PCR positive suggesting that PCR diagnosis of typhoid may have superior sensitivity than cultures. Over the next 10 years a handful of studies have reported PCR methods targeting the flagellin gene, somatic gene, Vi antigen gene, 5S-23S spacer region of the ribosomal RNA gene, invA gene and hilA gene of *Salmonella typhi* for diagnosis of

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Messages

- Blood culture is the gold standard for diagnosis of typhoid fever. Blood culture can turn out to be cheaper and cost effective in the long run as positive culture unequivocally establishes the diagnosis and also gives the sensitivity pattern.

- Nalidixic acid sensitivity is a surrogate marker of fluoroquinolone sensitivity and nalidixic acid susceptibility testing is mandatory to help to guide the choice of antibiotics.

- Sensitivity of marrow culture is 80-95 % even in late disease and prior to antibiotic therapy. Marrow should be inoculated in the culture bottle at bed side.

- Widal test has poor sensitivity due to its negativity in early infection, prior antibiotic therapy may influence it and certain individuals fail to mount an immune response to the disease. It should be done after 5-7 days of fever by tube method and level of 1 in 160 for both H and O antibodies are usually taken as diagnostic. Slide Widal test should be discouraged due to high rate of false positivity.

- Typhidot test detects IgG and IgM against outer membrane protein of *S. typhi.* As IgG can persist over a long time it is difficult to distinguish between acute infection and convalescent cases. This test has been improved in modified typhidot M test which detects only IgM antibodies.

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typhoid fever(42-50). These studies have reported excellent sensitivity and specificity when compared to positive (blood culture proven) and healthy controls. The turn-around time for diagnosis has been less than 24 hours.

These reports should be viewed within the context of certain limitations. Clinical utility of PCR tests has been inadequately evaluated. Performance of the test in individuals with febrile illnesses other than typhoid, in those with past history of typhoid, carriers of *S typhi*, and those vaccinated with typhoid vaccine is not known. Patients with a clinical diagnosis of typhoid fever who are culture negative but PCR positive may in fact be false positives. Comparison of PCR to bone marrow cultures as a gold standard may be a superior way of evaluating the sensitivity and specificity of these tests, but has not been done. The tests claim to detect as few as 10 organisms, but it should be remembered that in typhoid the median bacteremia is 0.3 CFU/ml of blood(7). Using small volumes of blood for DNA extraction may significantly lower the sensitivity of these tests. The cost and requirement for sophisticated instruments is also a potential drawback of molecular methods.

Conclusions

The complete blood count is the logical first investigation. Presence of a normal or low leukocyte count with eosinopenia points to possible enteric fever. It also helps in evaluation of alternative diagnoses such as malaria, dengue and other bacteremias. Blood culture remains the most effective investigation for diagnosis of enteric till date. They should be sent early in the course of the illness and prior to starting antibiotic therapy. Susceptibility testing for nalidixic acid should be routinely done for all isolates to aid choice of antibiotics. Bone marrow culture is a highly sensitive diagnostic test even in late stages of the illness and with prior antibiotic therapy. It should be performed in all patients with prolonged pyrexia if routine investigations

have failed to establish a diagnosis. The Widal test has several limitations and should be requested for in the second week of the illness and its results interpreted with caution.

REFERENCES

1. Abdool Gaffar MS, Seedat YK, Coovadia YM, Khan Q. The white cell count in typhoid fever. Trop Geogr Med 1992; 44: 23-27.

2. Deshmukh CT, Nadkarni UB, Karande SC. An analysis of children with typhoid fever admitted in 1991. J Postgrad Med 1994; 40: 204-207.

3. Pandey KK, Srinivasan S, Mahadevan S, Nalini P, Rao RS. Typhoid fever below five years. Indian Pediatr 1990; 27: 153-156.

4. Chiu CH, Tsai JR, Ou JT, Lin TY. Typhoid fever in children: a fourteen-year experience. Acta Pediatr Taiwan 2000; 41: 28-32.

5. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid Fever. N Engl J Med 2002; 347: 1770-8172.

6. Ananthanarayan R, Panikar CKJ. Textbook of Microbiology. Chennai: Orient Longman 1999; 244-249.

7. Wain J, Pham VB, Ha V, *et al.* Quantitation of bacteria in bone marrow from patients with typhoid fever: relationship between counts and clinical features. J Clin Microbiol 2001; 39: 1571-1576.

8. Hoffman SL, Edman DC, Punjabi NH, Lesmana M, Cholid A, Sundah S, Harahap J. Bone marrow aspirate culture superior to strepto-kinase clot culture and 8 mL 1:10 blood-to-broth ratio blood culture for diagnosis of typhoid fever. Am J Trop Med Hyg 1986; 35: 836-839.

9. Simanjuntak CH, Hoffman SL, Darmowigoto R, Lesmana M, Soeprawoto, Edman DC. Streptokinase clot culture compared with whole blood culture for isolation of *Salmonella typhi* and *S. paratyphi* A from patients with enteric fever. Trans R Soc Trop Med Hyg 1988; 82: 340-341.

10. Escamilla J, Florez-Ugarte H, Kilpatrick ME. Evaluation of blood clot cultures for isolation of *Salmonella typhi, Salmonella paratyphi*-A and *Brucella melitensis.* J Clin Microbiol 1986; 24: 388-390.

11. Farooqui BJ, Khurshid M, Ashfaq MK, Khan MA. Comparative yield of *Salmonella typhi* from blood and bone marrow cultures in patients with fever of unknown origin. J Clin Pathol 1991; 44 : 258-259.

12. Duthie R, French GL. Comparison of methods for the diagnosis of typhoid fever. J Clin Pathol 1990; 43: 863-865.

13. Akoh JA. Relative sensitivity of blood and bone marrow cultures in typhoid fever. Trop Doct 1991; 21: 174-176.

14. Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of Salmonella typhi in typhoid fever. Lancet 1975; 31; 1:1211-1213.

15. Vallenas C, Hernandez H, Kay B, Black R, Gotuzzo E. Efficacy of bone marrow, blood, stool and duodenal contents cultures for bacteriologic confirmation of typhoid fever in children.Pediatr Infect Dis 1985; 4: 496-498.

16. Benavente L, Gotuzzo E, Guerra J, Grados O, Guerra H, Bravo N. Diagnosis of typhoid fever using a string capsule device. Trans R Soc Trop Med Hyg 1984; 78: 404-406.

17. Crump JA, Barrett TJ, Nelson JT, Angulo FJ. Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype typhi and for non-typhi salmonellae. Clin Infect Dis 2003; 37: 75-81.

18. Kapil A, Renuka, Das B. Nalidixic acid susceptibility test to screen ciprofloxacin resistance in *Salmonella typhi*. Indian J Med Res 2002; 115: 49-54.

19. Olopoenia LA, King AL. Widal agglutination test–100 years later: Still plagued by contro-versy. Postgrad Med J 2000; 76: 80-84.

20. Rodrigues C. The Widal test more than 100 years old: abused but still used. J Assoc Physicians India 2003; 51: 7-8.

21. Parry CM, Hoa NT, Diep TS, Wain J, Chinh NT, Vinh H, *et al*. Value of a single-tube Widal test in diagnosis of typhoid fever in Vietnam.

J Clin Microbiol 1999; 37: 2882-2886.

22. Shukla S, Patel B, Chitnis DS. 100 years of WIDAL test and its reappraisal in an endemic area. Indian J Med Res 1997; 105: 53-57.

23. Schoeder SA. Interpretation of serologic tests for typhoid fever. J Am Med Assoc 1968; 206: 839-840.

24. Nsutebu EF, Ndumbe PM, Koulla S. The increase in occurrence of typhoid fever in Cameroon: overdiagnosis due to misuse of the Widal test? Trans R Soc Trop Med Hyg 2002; 96: 64-67.

25. Jesudason M, Esther E, Mathai E. Typhidot test to detect IgG & IgM antibodies in typhoid fever. Indian J Med Res 2002; 116:70-72.

26. Hatta M, Goris MG, Heerkens E, Gooskens J, Smits HL. Simple dipstick assay for the detection of *Salmonella typhi*-specific IgM antibodies and the evolution of the immune response in patients with typhoid fever. Am J Trop Med Hyg 2002; 66: 416-421.

27. Gasem MH, Smits HL, Goris MG, Dolmans WM. Evaluation of a simple and rapid dipstick assay for the diagnosis of typhoid fever in Indonesia. J Med Microbiol 2002; 51: 173-177.

28. Khan E, Azam I, Ahmed S, Hassan R. Diagnosis of typhoid fever by Dot enzyme immunoassay in an endemic region. J Pak Med Assoc 2002; 52: 415-417.

29. Cardona-Castro N, Agudelo-Florez P. Immunoenzymatic dot-blot test for the diagnosis of enteric fever caused by *Salmonella typhi* in an endemic area. Clin Microbiol Infect 1998; 4: 64-69.

30. Handojo I, Dewi R. The diagnostic value of the ELISA-Ty test for the detection of typhoid fever in children. Southeast Asian J Trop Med Pub Hlth 2000; 31: 702-707.

31. Bhutta ZA, Mansurali N. Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: A prospective comparative evaluation of two dot-enzyme immunoassays and the Widal test. Am J Trop Med Hyg 1999; 61: 654-657.

32. Jackson AA, Ismail A, Ibrahim TA, Kader ZS, Nawi NM. Retrospective review of dot enzyme immunoassay test for typhoid fever in an endemic area. Southeast Asian J Trop Med Pub Hlth 1995; 26: 625-630.

33. Choo KE, Davis TM, Ismail A, Tuan Ibrahim TA, Ghazali WN. Rapid and reliable serological diagnosis of enteric fever: comparative sensitivity and specificity of Typhidot and Typhidot-M tests in febrile Malaysian children. Acta Tropica 1999; 72: 175-183.

34. Saha SK, Talukdar SY, Islam M, Saha S. A highly ceftriaxone resistant *Salmonella typhi* in Bangladesh. The Ped Infect Dis J 1999; 18 : 297-303.

35. Bhutta ZA. Impact of age and drug resistance on mortality in typhoid fever. Arch Dis Child 1996; 75 : 214-217.

36. Lim PL, Tam FC, Cheong YM, Jegathesan M. One-step 2-minute test to detect typhoid-specific antibodies based on particle separation in tubes. J Clin Microbiol 1998; 36: 2271-2278.

37. Rao PS, Prasad SV, Arunkumar G, Shivananda PG. *Salmonella typhi* Vi antigen co-agglutination test for the rapid diagnosis of typhoid fever. Indian J Med Sci 1999; 53: 7-9.

38. Sharma M, Datta U, Roy P, Verma S, Sehgal S. Low sensitivity of counter-current immuno-electrophoresis for serodiagnosis of typhoid fever. J Med Microbiol 1997; 46: 1039-1042.

39. Pandya M, Pillai P, Deb M. Rapid diagnosis of typhoid fever by detection of Barber protein and Vi antigen of *Salmonella serotype typhi*. J Med Microbiol 1995; 43:185-188.

40. Chaicumpa W, Ruangkunaporn Y, Burr D, Chongsa-Nguan M, Echeverria P. Diagnosis of typhoid fever by detection of *Salmonella typhi* antigen in urine. J Clin Microbiol 1992; 30: 2513-2515.

41. Song JH, Cho H, Park MY, Na DS, Moon HB, Pai CH. Detection of *Salmonella typhi* in the blood of patients with typhoid fever by polymerase chain reaction. J Clin Microbiol 1993; 31:1439-1443.

42. Sanchez-Jimenez MM, Cardona-Castro N. Validation of a PCR for diagnosis of typhoid fever and salmonellosis by amplification of the

hilA gene in clinical samples from Colombian patients. J Med Microbiol 2004; 53: 875-878.

43. Cocolin L, Manzano M, Astori G, Botta GA, Cantoni C, Comi G. A highly sensitive and fast non-radioactive method for the detection of polymerase chain reaction products from Salmonella serovars, such as *Salmonella typhi*, in blood specimens. *FEMS* Immunol Med Microbiol 1998; 22: 233-239.

44. Chaudhry R, Laxmi BV, Nisar N, Ray K, Kumar D. Standardisation of polymerase chain reaction for the detection of *Salmonella typhi* in typhoid fever. J Clin Pathol 1997; 50: 437-439.

45. Zhu Q, Lim CK, Chan YN. Detection of *Salmonella typhi* by polymerase chain reaction. J Appl Bacteriol 1996; 80: 244-251.

46. Hashimoto Y, Itho Y, Fujinaga Y, Khan AQ, Sultana F, Miyake M, *et al.* Ezaki T. Development of nested PCR based on the ViaB sequence to detect *Salmonella typhi*. J Clin Microbiol 1995; 33: 775-777.

47. Hirose K, Itoh K, Nakajima H, Kurazono T, Yamaguchi M, Moriya K, *et al.* Selective amplification of tyv (rfbE), prt (rfbS), viaB, and fliC genes by multiplex PCR for identification of *Salmonella enterica serovars typhi* and *Paratyphi A*. J Clin Microbiol 2002; 40: 633-636.

48. Haque A, Ahmed N, Peerzada A, Raza A, Bashir S, Abbas G. Utility of PCR in diagnosis of problematic cases of typhoid. Jpn J Infect Dis 2001; 54: 237-239.

49. Massi MN, Shirakawa T, Gotoh A, Bishnu A, Hatta M, Kawabata M. Quantitative detection of *Salmonella enterica serovar typhi* from blood of suspected typhoid fever patients by real-time PCR. Int J Med Microbiol 2005; 295:117-120.

50. Prakash P, Mishra OP, Singh AK, Gulati AK, Nath G. Evaluation of nested PCR in diagnosis of typhoid fever. J Clin Microbiol 2005; 43: 431-432.

Annexure I

List of participants of the workshop organized by IAP Task Force on Guidelines for Diagnosis and Management of Enteric Fever in Children under IAP Action Plan 2006.

Chairperson :	Dr. Nitin K. Shah
Convenor :	Dr. Ritabrata Kundu
Members :	Dr. Ajay Kalra
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	Dr. Ashok Kapse
	Dr. Deepak Ugra
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