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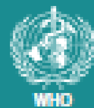
Background document: The diagnosis, treatment and prevention of typhoid fever

Communicable Disease Surveillance and Response
Vaccines and Biologicals



World Health Organization

IDI. Tubex® test

The Tubex® test is simple (essentially a one-step test) and rapid (taking approximately two minutes). It exploits the simplicity and user-friendliness of the Widal and the slide latex agglutination tests but uses the separation of coloured particles in solution to improve resolution and sensitivity. Specificity is improved by means of an inhibition assay format and by detecting antibodies to a single antigen in *S. typhi* only. The O9 antigen used in the test is extremely specific because its immunodominant epitope is a very rare dideoxyhexose sugar that occurs in nature. This antigen has been found in serogroup D *salmonellae* but not in other microorganisms. The closest to it is the tyvelose antigen found in *Trichinella spiralis* but antibodies to these two antigens do not cross-react with each other. A positive result given by Tubex® invariably suggests a *Salmonella* infection, although the test cannot tell which group D *Salmonella* is responsible. Infections caused by other serotypes, including *S. paratyphi* A, give negative results.

Immunogenically, the O9 antigen is immunodominant and robust. Unlike the capsular (Vi) and flagellar antigens that are thymus-independent type II in nature and poorly immunogenic in infants, the O9 antigen (or LPS in general) is thymus-independent type I, immunogenic in infants, and a potent B cell mitogen. It can stimulate B cells without the help of T cells (unlike protein antigens) and, consequently, anti-O9 responses are rapid. This is important teleologically, as they form the first line of host defence. For reasons yet to be elucidated, Tubex® detects IgM antibodies but not IgG. This makes it invaluable as an aid in the diagnosis of current infections.

The test pack includes: 1) sets of specially-designed V-shaped tubes that allow six samples per set to be examined simultaneously; 2) reagent A, comprising magnetic particles coated with *S. typhi* LPS; 3) reagent B, comprising blue-coloured latex particles coated with a monoclonal antibody specific for the O9 antigen. The reagents are stable for over a year at 4°C, and for at least some weeks at ambient temperature.

A drop of test serum is mixed for about one minute with a drop of reagent A in the tube. Two drops of reagent B are then added and the contents are mixed thoroughly for 1–2 minutes. The set of tubes is then placed on a magnet-embedded stand, across which they are slid several times. The result, which can be read immediately or up to many hours later, is based on the colour of the reaction mixture. A range of colours involving varying proportions of redness and blueness can be expected, and a colour chart is provided for the purpose of scoring. Red indicates negativity while increasing blueness denotes increasing positivity.

The rationale of the test is as follows. If the serum is negative for O9 antibodies the antibody-coated indicator particles bind to the antigen-coated magnetic beads. When a magnet is applied, the magnetic particles settle to the bottom of the tube together with any blue indicator particles associated with these. Consequently a background red colour is left in the solution. This background colour is actually exploited to camouflage the sample colour of haemolysed sera. If, on the other hand, the patient's serum contains O9 antibodies, these bind to the magnetic particles and prevent the indicator particles from binding to them. The indicator particles thus remain suspended and the resultant colour of the solution is blue.

Tubex® has not been evaluated extensively but several trials are being planned. In a preliminary study involving stored sera the test performed better than the Widal test in both sensitivity and specificity (24).

Typhidot® test

This test makes use of the 50 kD antigen to detect specific IgM and IgG antibodies to *S. typhi* (25). It has undergone full-scale multinational clinical evaluation of its diagnostic value (26, 27, 28). This dot EIA test offers simplicity, speed, specificity (75%), economy, early diagnosis, sensitivity (95%) 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 increases. Since IgG can persist for more than two years after typhoid infection (29) the detection of specific IgG cannot 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 latter cannot be detected and its effect is masked. A possible strategy for solving these problems is to enable the detection of IgM by ensuring that it is unmasked (30). In order to increase diagnostic accuracy in these situations the original Typhidot® test was modified by inactivating total IgG in the serum sample. Studies with the modified test, Typhidot-M®, have shown that inactivation of IgG removes competitive binding and allows access of the antigen to the specific IgM when it is present. The detection of specific IgM within three hours suggests acute typhoid infection. Evaluations of Typhidot® and Typhidot-M® in clinical settings showed that they performed better than the Widal test and the culture method (30).

In laboratory diagnoses of typhoid fever the method used as the gold standard should approach 100% in sensitivity, specificity and positive and negative predictive values. Evaluation studies have shown that Typhidot-M® is superior to the culture method (28). Although culture remains the gold standard it cannot match Typhidot-M® in sensitivity (>93%), negative predictive value and speed (28). Typhidot-M® can replace the Widal test when used in conjunction with the culture method for the rapid and accurate diagnosis of typhoid fever. The high negative predictive value of the test suggests that Typhidot-M® would be useful in areas of high endemicity.

IgM dipstick test

The typhoid IgM dipstick assay is designed for the serodiagnosis of typhoid fever through the detection of *S. typhi*-specific IgM antibodies in serum or whole blood samples.

The assay consists of a dipstick, a lyophilized non-enzymatic detection reagent, liquid to reconstitute the detection reagent, liquid to wet the test strip of the dipstick before incubation with serum and detection reagent, and test tubes. The components are stable for two years if stored in the temperature range 4-25°C in a dry place and protected from direct exposure to sunlight.

The assay is based on the binding of *S. typhi*-specific IgM antibodies to *S. typhi* LPS antigen and the staining of bound antibodies by an anti-human IgM antibody conjugated to colloidal dye particles. The white test strip of the dipstick contains the antigen immobilized in a distinct line. The strip also has a control line with anti-human IgM antibodies.

The assay is performed by incubation of the wetted test strip in a mixture of serum and detection reagent, the serum being diluted at 1:50 in the detection reagent. Whole blood may be tested at a 1:25 dilution in detection reagent. The incubation period is three hours at room temperature. When incubation is complete the test strip is rinsed thoroughly with water and then allowed to dry. The result is read by visual inspection of the test strip for staining of the antigen and control lines. The test result is scored negative if no staining of the antigen line occurs and is graded 1+, 2+, 3+ or 4+ if there is weak, moderate strong or very strong staining as indicated by comparison with a coloured reference strip. The control line should stain in all runs.

Evaluations of the dipstick test in laboratory-based studies in Indonesia (31, 32), Kenya (33), Viet Nam (33) and Egypt (34) have shown consistent results. These studies indicated sensitivities of 65% to 77% for samples collected at the time of first consultation from culture-confirmed patients and specificities of 95% to 100%. The results of culture and serological investigation may be influenced by various factors, among them the time of sample collection and the use of antibiotics before consultation and sample collection. In a study conducted in Makassar, Indonesia, the sensitivity of the blood culture method was estimated to be 66%, and that of the dipstick test calculated for the combined group of culture-confirmed and culture-negative patients with a final clinical diagnosis of typhoid fever was 48%. The sensitivity ranged from 29% for samples collected during the first week of illness to 96% for samples collected at a later stage. Tests on follow-up samples showed seroconversion in the majority of the dipstick-negative typhoid patients.

The dipstick test provides a rapid and simple alternative for the diagnosis of typhoid fever, particularly in situations where culture facilities are not available. The assay can be performed by people without formal training and in the absence of specialized equipment. Electricity is not required, as the components can be stored without cooling. The results of the dipstick test can be obtained on the day when patients present. This makes prompt treatment possible. Specific antibodies usually only appear a week after the onset of symptoms and signs. This should kept in mind when a negative serological test result is being interpreted.