

A simple adherence test for detection of IgM antibodies in typhoid

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Summary. A simple adherence test to detect IgM antibodies in patients with typhoid is described. The test utilises the IgM-“capture” approach, in which the test serum is applied to microtitration plate wells previously coated with anti-human IgM, followed by application of a stained *Salmonella typhi* antigen suspension which shows adherence in positive cases. By this test, 58 (95%) of 61 sera from confirmed cases of typhoid possessed IgM antibodies to the H or O or both antigens of *S. typhi*. In patients for whom a diagnosis of typhoid was based only on a significant Widal-test titre, 31 (41%) of 76 sera had IgM antibodies to the H or O or both antigens of *S. typhi*. Some cross-reactivity of the IgM antibodies was detected, especially with the O antigens of *S. paratyphi* A and B. A total of 82 sera from non-typhoidal fevers (leptospirosis, typhus, dengue fever) showed no reactivity in this test. In normal sera there was no detectable IgM to the O antigen of *S. typhi* and only a small number (3.9%) had low levels of IgM to the H antigen. The significance and potential importance of this simple, sensitive, specific and economical test is discussed.

Introduction

Typhoid remains an important public health problem in many parts of the tropical world, including Malaysia, which had nearly 3000 cases in 1987. Definitive diagnosis of typhoid relies upon successful isolation of the causative organism from patients' specimens such as blood, stool and urine. In addition, especially in situations where relevant facilities for culture are not available, serological tests such as the Widal test are often used as additional diagnostic aids. The many limiting factors affecting the Widal test are well-known—e.g., the stage of illness, the normal population titres in endemic areas, the effects of vaccination, non-specific cross-reactions, anamnestic response, antibiotic therapy and technical factors. These limitations often lead to difficulties in the interpretation of Widal test results. Furthermore, for a meaningful result, the test requires the availability of paired sera; the result on a single specimen is often inconclusive. It has been stated that a rapid immunodiagnostic test for typhoid which is sensitive, specific, simple, rapid and economical does

not yet exist (Edelman and Levine, 1986). Therefore, a simple test capable of reliably detecting IgM antibodies to *Salmonella typhi* would be a useful adjunct to existing tests, as it would presumably indicate recent or ongoing infections. One approach used in the detection of specific IgM antibodies is the IgM-“capture” approach in which anti-human IgM adsorbed to a solid phase is used to bind selectively IgM present in examined sera and thus eliminate competition by other antibody classes in the subsequent testing (Ryan and Kwasnik, 1985). We report here the development of such a test—a simple, specific and economical method to detect IgM antibodies in cases of typhoid, using the IgM-“capture” approach and involving adherence of stained *S. typhi* antigen suspensions in the final stage.

Materials and methods

Sera

Four groups of serum samples were collected from different sets of individuals.

Group 1 consisted of 61 single samples of serum from patients with a clinical diagnosis of typhoid admitted to the University Hospital, Kuala Lumpur. These sera had Widal-test titres of ≥ 640 (for both H and O antigens).

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The diagnosis of typhoid in these patients was confirmed by the isolation of *S. typhi* from blood or faeces, or both. The date of onset of clinical symptoms varied from 3–4 days to 6–7 weeks.

Group 2 consisted of 76 single samples of serum from patients in whom the diagnosis of typhoid was based on a significant titre in the Widal test (≥ 640 to H or O antigen, or both) but without confirmation by isolation of *S. typhi*.

Group 3 consisted of 82 single samples of serum from patients with non-typhoidal fevers common in the study region—leptospirosis (20), typhus (42) and dengue fever (20). Leptospirosis was diagnosed by the microscopic agglutination test (Alexander, 1976), typhus by the Weil-Felix reaction to *Proteus* OXK, OX19 and OX2 antigens (Felix, 1944) and dengue fever by virus isolation (Lam *et al.*, 1986) or haemagglutination inhibition (Clarke and Casals, 1958).

Group 4 consisted of sera from 102 healthy individuals from the medical-student population of the University of Malaya.

Antigens

Antigen suspensions comprised stained, killed H and O antigens from *S. typhi*, *S. paratyphi* A, *S. paratyphi* B and *Proteus* OXK, OX19 and OX2 (Wellcome Reagents Ltd, Kent).

Widal-test

The Widal test on various sera was performed by standard procedures, as described previously (Pang and Puthuchery, 1983).

Adherence test to detect IgM

The adherence test to detect IgM to *S. typhi* was performed by adding 100 μ l of rabbit anti-human IgM (μ chain-specific, Dako Immunochemicals, Copenhagen, Denmark) at a dilution of 400 in borate-saline buffer (pH 9.0) to wells of U-bottom microtitration plates (Immulon 2, Dynatech Laboratories, Alexandria, VA, USA). The dilution of 400 was decided after testing the antisera at various dilutions from 100 to 1600. After overnight incubation at 4°C, coated plates were washed three times with distilled water containing Tween 20 (0.05%) and tapped dry. Plates prepared in this manner can be stored at 4°C for up to 4 months. Patients' sera were then serially diluted in the anti-human IgM-coated plates in normal saline, starting with an initial dilution of 80 (100 μ l final volume/well). Plates were then incubated for 2 h at 37°C, washed three times with distilled water and tapped dry. Then 50 μ l of antigen suspension (diluted approximately 1 in 16 in normal saline) was added to the wells and the plates were incubated further at 37°C for 4 h (for O antigen) and overnight (for H antigen). Plates were then read by means of a microtitre mirror (Cooke Engineering Co., Alexandria, VA, USA). A positive result was shown

by formation of an adherence pattern, whereas a "button" indicated the absence of IgM antibody.

IgM abolition by 2-mercaptoethanol treatment

For removal of IgM from selected sera, 20 μ l of 2-mercaptoethanol (2-ME; Sigma Chemical Co., St Louis, MO, USA) was mixed with an equal volume of the serum and the mixture was incubated for 1 h at 37°C and then tested for IgM as described above.

IgM removal by immunobeads

Samples (100 μ l) from selected sera were pre-incubated with an equal volume of anti-human IgM-coated immunobeads (BioRad Laboratories, Richmond, CA, USA), at a concentration of 1 mg/ml in borate-saline buffer, at 4°C for 1 h. Sera were then tested in the usual manner.

Results

Of the 61 Group-1 sera from confirmed cases of typhoid, as indicated by isolation of *S. typhi*, 58 (95%) had IgM antibodies to the H or O, or both, antigens of *S. typhi* by our test (table I). Within this group, 51 (84%) had IgM antibodies to both H and O antigens, six (10%) to only the H antigen and one (2%) to only the O antigen. Of the 58 IgM-positive sera in this group, more than 80% had IgM titres of ≥ 1280 (table II). In a random sample of ten of

Table I. Levels of IgM antibody to *S. typhi* in various groups of sera

Group	Category	Number of sera in group	Number IgM positive*
1	Typhoid (confirmed)	61	58 (95%)
2	Typhoid (serology only)	76	31 (41%)
3	Other fevers	82	0
4	Normal	102	4 (3.9%)

* Positive to *S. typhi* H or O antigens, or both, at a titre of ≥ 80 .

Table II. Titres of IgM antibodies to *S. typhi* H and O antigens in the 58 positive Group-1 sera by the adherence test

Titre range	Number of sera reacting to	
	H antigen	O antigen
80–640	8 (14%)	10 (17%)
1280–5120	40 (69%)	34 (59%)
$\geq 10\ 240$	10 (17%)	14 (24%)

these IgM-positive sera—nine with titres of 1280– $\geq 10\,240$ (O and H antigens) and one of 320 (O), 640 H—treatment for removal of IgM by 2-ME resulted in marked falls in the O and H titres, measured by our method, to undetectable levels, except for one serum, which nevertheless showed a very large reduction, from $\geq 10\,240$ to 640 (O), 320 (H). Similar results were obtained by treatment of the same set of sera with anti-human IgM-coated immunobeads, as used in earlier dengue virus IgM studies (Gunasegaran *et al.*, 1986). These 10 sera all gave negative results in the latex agglutination test for rheumatoid factors (Winchester, 1976). Of 42 sera from Group 1 showing IgM to *S. typhi* H antigen, two (5%) cross-reacted with the H antigen of *S. paratyphi* A and one (2%) cross-reacted with the H antigen of *S. paratyphi* B. All three cross-reacting sera had higher IgM titres to *S. typhi* than to *S. paratyphi* A or B. Of 39 Group-1 sera showing reactivity to *S. typhi* O antigens, 13 (33%) showed various degrees of cross-reactivity to the O antigens of *S. paratyphi* A and B and, of these, five showed a high degree of cross-reactivity (titres $\geq 10\,240$) to the O antigen of *S. paratyphi* A or B.

In Group 2, for which a diagnosis of typhoid was based solely on a significant Widal-test titre, 31 (41%) out of 76 sera tested had IgM antibodies to H or O, or both, antigens of *S. typhi* (table I). In Group 3, none of the sera from patients with leptospirosis (20), or typhus (42) or dengue fever (20) reacted with *S. typhi* in this test (table I). Similarly, none of the 102 normal sera tested (Group 4) showed detectable levels of IgM antibody to the O antigen of *S. typhi* and only four (3.9%) had a measurable titre against the H antigen (table I).

Discussion

An important approach to the rapid diagnosis of infectious diseases is the detection of specific IgM antibodies, the presence of which is usually indicative of a recent or ongoing infection (Ryan and Kwasnik, 1985). Other studies have shown a significant increase in IgM levels (as measured by single radial diffusion) in patients with typhoid, at all stages of the illness, from the first week onwards (Kumar *et al.*, 1974). The IgM-adherence method that we have described provides a simple, sensitive, specific and economical test to detect IgM class antibodies in typhoid. The test is economical in using readily available and inexpensive materials and reagents and in its lack of dependence on sophisticated instrumentation. The test was able to detect IgM in 95% of confirmed typhoid cases and no false-positives were detected in sera from other,

non-typhoidal fevers common in the region, *viz.* typhus, leptospirosis and dengue fever. In comparison, other studies based on an ELISA approach detected IgM to the LPS antigen of *S. typhi* in 81.8% (Srivastava and Srivastava, 1986) and 93.1% (Nardiello *et al.*, 1984) of proven cases of typhoid. In retrospect, it would have been useful to have included the ELISA approach in the present study for the purpose of comparison and this should probably be considered in any future similar investigations. Among the three typhoid patients whose sera showed no detectable IgM in the present study, one had been treated with chloramphenicol and other was admitted 2 months after the onset of symptoms, by which time the IgM levels may have dropped to an undetectable level. In relation to this, it is known that early chemotherapy can suppress antibody formation in typhoid cases (Watson, 1957) and that IgM levels may have subsided after 45–90 days (Nardiello *et al.*, 1984). If these two cases are excluded from the group, 58 of the remaining 59 sera (98%) had detectable IgM by this test. As expected, the present study showed cross-reactivity of the IgM antibodies, especially with the O antigens of *S. typhi*-related organisms (*S. paratyphi* A and B) due to the sharing of antigenic component 12 (Christie, 1980).

The present study also suggests that the IgM-adherence test is likely to be helpful in interpreting a single, high-titre Widal test result when isolation of the causative organism is unsuccessful or not attempted. The presence of IgM antibodies in 41% of the sera in this group would provide further diagnostic support for a diagnosis of typhoid in such cases. However, explanations are needed for the remaining, IgM-negative sera in this category. It could be questioned whether they are real cases of typhoid, but if so, it is possible that the IgM level in these sera may have been below the sensitivity limit of the IgM-adherence test. This seems unlikely, in view of the fact that IgM was detectable in 95–98% of confirmed typhoid cases and that the patients' characteristics for the two groups (*i.e.*, age, date of onset, etc.) were similar. The more likely possibility is that these patients were suffering from other febrile illnesses and that the elevated Widal-test titres were thus a result of an anamnestic response. Wicks *et al.* (1974) have suggested that high Widal titres at the early stages of typhoid in an endemic area represented an anamnestic response. It has been suggested that anti-H antibody, in particular, is anamnesticly responsive and easily stimulated by non-specific stimuli (Christie, 1980). Our results with these sera, which showed high titres in the Widal test but which were negative

by isolation, also caution against making a diagnosis of typhoid based on a single, elevated titre in the Widal test.

It seems likely that our test for detecting IgM to *S. typhi* should prove an important additional method in the laboratory diagnosis of typhoid. In relation to other methods previously used for this purpose, such as ELISA (Nardiello *et al.*, 1984; Srivastava and Srivastava, 1986), crossed-immunoelectrophoresis (Tsang and Chau, 1981) and radio-immunoassay (Tsang *et al.*, 1981), the IgM-adherence test is superior in being economical and simple to perform and in utilising non-hazardous materials and not requiring sophisticated instruments. Furthermore, it has the important advantage of being applicable even when only a single serum specimen is available. In our experience, about 70–80% of sera submitted for typhoid diagnosis are single specimens. Furthermore, in endemic areas such as Malaysia, antibody titres may rise very rapidly because of previous exposure and therefore demonstration of rising titres between two speci-

mens may be difficult (Wicks *et al.*, 1974). Thus, in the present study, high titres were already detectable in some single sera only 3–4 days after the onset of symptoms. IgM titres were measured only to the H and O antigens because background Vi agglutinins are known to be present in the normal populations of endemic areas (Report, 1961). Therefore, measurement of these antibodies is of limited value both in the diagnosis of typhoid and in determination of the carrier state. Within the diagnostic laboratory, the IgM-adherence test could be used directly as a sole serological test or, alternatively, as a confirmatory test following screening of sera by the Widal test. The latter approach would be particularly useful in smaller laboratories, for example those attached to district hospitals, where bacteriological isolation and identification facilities may not be available.

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