An immunoblotting procedure comprising O = 9,12 and H = d antigens as an alternative to the Widal agglutination assay

H Chart, L R Ward, B Rowe

Abstract
Aims—To compare the established Widal agglutination assay with an immunoblotting procedure.
Methods—110 sera were used to compare the established Widal agglutination assay with an immunoblotting procedure incorporating lipopolysaccharide (LPS) (O = 9,12) and flagellar (H = d) antigens.
Results—Antibodies to the LPS antigens were detected in 18 sera by the Widal assay and in 37 by immunoblotting. Antibodies to the flagellar antigens were detected in 27 sera by Widal assay and in 25 by immunoblotting.
Conclusions—An immunoblotting procedure incorporating O = 9,12 LPS and H = d flagellar antigens was rapid and more sensitive than the established Widal agglutination assay for providing evidence of infection with S typhi.
(J Clin Pathol 1998;51:854–856)

Keywords: immunoblotting; Widal reaction; S typhi

In the United Kingdom, most cases of typhoid occur in travellers returning from areas where this disease is endemic. The diagnosis of typhoid can be confirmed by culturing Salmonella typhi from blood or faeces; however, in the absence of culturable S typhi, serological methods offer an alternative approach to provide evidence of infection. Patients infected with S typhi have been known to produce serum antibodies to this organism since the early studies of Widal, per formed over a century ago. The Widal test was based on the ability of patients’ antibodies to agglutinate S typhi bacteria, and provided a valuable adjunct to established bacteriology. Variation in results obtained by different laboratories2 initiated attempts to standardise the Widal agglutination assay,3 and with the use of Dreyer’s “standard” bacterial suspensions, a definitive Widal assay was issued by the Laboratory of Enteric Pathogens (LEP) for routine typhoid serology and screens sera for antibodies to S typhi in clinical cases, carriers, and healthy persons. In the present study, 110 sera received by the LEP for routine typhoid serology were used to establish whether SDS-PAGE immunoblotting would be a suitable test to replace the established Widal agglutination assay.

Methods
BACTERIA
Salmonella enteritidis strain P132344 (O = 1,9,12; H = g,m) and S meunchen strain JT54 (O = 6,8; H = d) were used to prepare lipopolysaccharide and flagella antigens.
Table 1 Comparison of immunoblotting with the Widal agglutination test when screening 110 sera for antibodies to lipopolysaccharide (LPS) antigens (O=9,12) and flagellar antigens (H=d)

<table>
<thead>
<tr>
<th>Immunoblotting</th>
<th>Widal agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS Flagellar</td>
<td>LPS Flagellar</td>
</tr>
<tr>
<td>No of positive sera</td>
<td>37</td>
</tr>
</tbody>
</table>

SERA
Overall 110 sera had been referred to the Laboratory of Enteric Pathogens for routine Widal serology. Sera were predominantly from patients suspected of having been infected with S typhi, but also included six sera from patients with reactive arthritis, five apparently healthy persons involved with catering or water industries, and three people vaccinated against typhoid. All sera were stored at −30°C until used.

LIPopolysaccharide/Flagella
LPS was prepared from S enteritidis using proteinase-K digestion (Sigma) and hot phenol extraction of bacterial outer membranes. Flagella were partially purified from S meunchen by heat extraction (60°C, 30 minutes). Aliquots of preparations of LPS and flagella were stored at −30°C until required.

SDS-PAGE/IMMUNOBLOTTING
SDS-PAGE and immunoblotting was performed using a mini gel system. One microgram of LPS or 10 µg flagellar protein were loaded per lane of a gel comprising a 4.5% stacking gel and a 12.5% separation gel. Following electrophoresis, replicate profiles of LPS and flagellar protein were either stained for assessment of purity and profile resolution, or used for immunoblotting and reaction with human sera. For immunoblotting, profiles were transferred onto nitrocellulose membranes and reacted with sera (30 µl per lane), and bound antibodies detected with a goat antihuman polyvalent antibody conjugated with alkaline phosphatase (Sigma). Antibody–antibody–conjugate complexes were detected with an enzyme substrate comprising nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma). Immunoblotting reactions were graded between 0 and 3, with 0 indicating a complete absence of antibody binding and 3 where antibody binding was readily observed. Only sera giving an immunoblot reaction of 2 or 3 was considered antibody positive for this study.

WIDAL SEROLOGY
Widal serology was performed based on the standard procedure described by Felix and Gardner. In the LEP an agglutinating antibody titre of > 1:100 is considered as significant for antibodies to LPS and flagellar antigens.

Results
Of the 110 sera examined, 42 did not have antibodies to either LPS or flagellar antigens, by either of the methods under investigation. Antibodies to LPS and flagellar antigens were detected by immunoblotting in 37 and 25 sera, respectively (table 1; fig 1). Eleven sera contained antibodies to both LPS and flagellar antigens, 26 had antibodies to LPS only, and 14 had antibodies to flagella only.

The Widal agglutination assay detected 18 and 27 sera with antibodies to LPS and flagella antigens, respectively (table 1). Four sera had antibodies to both LPS and flagellar antigens, 16 had antibodies to LPS only, and 23 had antibodies to flagella only.

The five sera from water workers were shown not to contain antibodies to LPS or flagella antigens by both tests. The three sera from people who had been vaccinated against typhoid were found to contain antibodies to LPS (2) and flagellar antigens (3) by immunoblotting, but the Widal assay detected only antiflagellar antibodies in two of these sera. Sera with high levels of antibodies by Widal test but antibody negative by immunoblotting were not detected.

Discussion
In this study we used 110 sera submitted to the LEP for routine Widal serology, to compare the established Widal agglutination assay with an immunoblotting procedure incorporating LPS (O = 9,12) and partially purified flagellar proteins (H = d). The immunoblot procedure detected over twice as many sera with antibodies to O = 9,12 LPS as compared with the Widal agglutination assay. Both procedures detected a similar number of sera with antibodies to flagellar antigens. The Widal agglutination assay detected four sera with antibodies to both LPS and flagellar antigens; however, immunoblotting detected 11 patients with serum antibodies to both of these antigens.

Antibodies to the O = 9,12 LPS only were detected in 16 and 26 sera by the Widal and immunoblot assays, respectively. It has been shown that patients infected with S enteritis also produce antibodies to the 9,12 LPS antigens; therefore, detecting antibodies to LPS alone would not distinguish patients infected with S typhi from those infected with S enteritis. Also, studies from this laboratory have shown that approximately one third of patients known to have been infected with S typhi may not produce antibodies to flagella antigens during infection. These facts emphasise the importance of considering patients’ symptoms, any history of foreign travel, and records of previous typhoid vaccinations when interpreting the results of serological tests.

From this study we conclude that an immunoblotting procedure incorporating O = 9,12 LPS and H = d flagellar antigens is more sensitive than the established Widal agglutination assay for providing evidence of infection with S typhi. Although the immunoblot test requires slightly more elaborate laboratory equipment than the Widal agglutination assay, the apparatus required for SDS-PAGE and immunoblotting has been miniaturised extensively and simplified considerably. The immunoblot can be performed in a single working day, in contrast to the Widal agglutination assay which
takes two days. We suggest that the immunoblot we describe should be considered as a viable alternative to the long established Widal agglutination assay.

An immunoblotting procedure comprising O = 9,12 and H = d antigens as an alternative to the Widal agglutination assay.
H Chart, L R Ward and B Rowe

doi: 10.1136/jcp.51.11.854

Updated information and services can be found at:
http://jcp.bmj.com/content/51/11/854

These include:

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/