Enzyme-Linked Immunosorbent Assay for Detection and Quantitation of Capsular Antigen of 
*Haemophilus influenzae* Type b

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Received for publication 18 August 1978

An enzyme-linked immunosorbent assay was developed to detect the presence of the ribose-ribitol phosphate capsular antigen of *Haemophilus influenzae* type b in laboratory and clinical specimens. The assay is simple, sensitive, specific, and quantitative and should prove to be of value in the diagnosis and management of *H. influenzae* infections.

*Haemophilus influenzae* type b is a principal cause of bacterial septicemia and meningitis in children. Counterimmunoelectrophoresis (CIE) has been used for the detection of *H. influenzae* type b antigen (7) and has offered rapid diagnosis of *H. influenzae* type b infections. However, CIE is limited by subjective interpretation of the presence or absence of an often faint precipitin band, which may lead to misinterpretation of results and limits the accuracy of antigen quantitation.

This investigation was undertaken to determine whether the enzyme-linked immunosorbent assay (ELISA) technique could be used to detect and quantify the ribose-ribitol phosphate (RRP) capsular polysaccharide antigen of *H. influenzae* type b in purified preparations of the antigen and in the body fluids of patients and laboratory animals with *H. influenzae* type b disease.

Purified RRP and purified capsular antigens from *Escherichia coli* strains N67, K1, and K100 (Easter), *Neisseria meningitidis* type C (3), and hyperimmune burro anti-RRP antiserum (6) were kindly supplied by John B. Robbins, Bureau of Biologics, Food and Drug Administration, Bethesda, Md. Purified capsular antigens (9) from *Streptococcus pneumoniae* types 1, 2, 3, 4, 14, 19, and 25, were obtained from Gerald Schiffman, Department of Microbiology, State University of New York, Downstate Medical Center, Brooklyn, N. Y.

Anti-RRP antibody was conjugated to alkaline phosphatase by the method of Engvall and Perlmann (1).

The assay used was a modification of that of Voller et al. (8). Polystyrene microtiter wells (Dynatech, Alexandria, Va.) were filled with 0.2 ml of a 1:5,000 dilution of anti-RRP antiserum in coating buffer, covered with strips of transparent plastic tape, and incubated overnight at 4°C. The wells were shaken out, washed three times with phosphate-buffered saline-Tween, and filled with 0.2 ml of either a known concentration of soluble antigen or an unknown sample. The wells were taped, incubated at 20°C for 150 min, shaken out, washed three times with phosphate-buffered saline-Tween, and filled with 0.2 ml of a 1:20 dilution of antibody-enzyme conjugate. The wells were again taped, incubated at 20°C for 150 min, shaken out, washed four times with phosphate-buffered saline-Tween, filled with 0.2 ml of p-nitrophenylphosphate in diethanolamine buffer, and incubated at 20°C for 30 min. The contents of each well were mixed, aspirated, and diluted in 2.0 ml of distilled water, and the absorbance at 400 nm was determined with a Beckman spectrophotometer (Beckman Instruments, Fullerton, Calif.). Results were expressed as absorbance of sample minus absorbance of antigen-negative control sample to correct for nonspecific adherence of antibody-enzyme conjugate to the well. Quantitation of RRP in unknown samples was performed by assaying serial fivefold dilutions of the samples and calculating the result from a standard curve performed with each experiment.

CIE was performed by the method of Shackelford et al. (7). By using anti-RRP burro antiserum, the technique allowed detection of concentrations of RRP ≥1 ng/ml. *H. influenzae* type b bacteremia was established in 10-day-old rats by intranasal inoculation by a method previously described (5). Ani-
mals were bled by decapitation 24 h after inoculation, quantitative blood cultures were performed, and the serum was frozen at \(-70^\circ\)C for later analysis. Samples of cerebrospinal fluid from children admitted to the Johns Hopkins Hospital with bacteriologically proven \(H.\ influenzae\) type b meningitis were obtained at the time of admission and similarly frozen.

Solutions of purified RRP antigen ranging in concentration from 100 pg to 10 \(\mu g/ml\) were prepared and assayed simultaneously. As depicted in Fig. 1, the dose-response curve was the same whether the RRP was dissolved in serum or buffer. The relationship between absorbance and antigen concentration in this semilogarithmic plot was linear over the range 1 to 30 ng/ml, and this relationship was reproducible in all experiments. Thus, in later quantitation experiments, serial fivefold dilutions of samples with unknown RRP concentrations yielded in all cases at least one sample value within the linear portion of a standard plot.

RRP and purified capsular polysaccharides from common bacterial pathogens were assayed simultaneously at 100 ng/ml. The absorbances of tubes containing capsular antigens of \(S.\ pneumoniae\) types 1, 2, 3, 4, 14, 19, and 25; \(N.\ meningitidis\) group C; and \(E.\ coli\) K1, N67, and K100 were all less than 10% of that of RRP. It is of note that the capsular antigen of \(E.\ coli\) K100 is strongly cross-reactive with the same RRP burro antiserum in CIE in our laboratory.

Sera from infant rats with experimentally induced \(H.\ influenzae\) type b septicemia and cerebrospinal fluid from bacteriologically proven human cases of \(H.\ influenzae\) type b meningitis were assayed by the ELISA technique. In addition, all samples were tested for precipitin band formation on CIE, and serum samples were cultured quantitatively to determine the concentration of viable \(H.\ influenzae\) type b. As shown in Table 1, RRP was detected in seven of nine samples tested and in all samples positive by CIE. Antigen concentration ranged from 70 ng to 1.68 \(\mu g/ml\). There was a rough correlation between the concentration of viable bacteria in the sera and the concentration of RRP detected by ELISA.

The RRP assay described here extends the usefulness of ELISA to include detection of a bacterial polysaccharide antigen. The RRP-ELISA technique is a simple test which can be performed in most laboratories, with readily available materials and without the radioisotopes or scintillation counter required for radioimmunoassay. The assay is at least as sensitive as CIE but gives an exact end point. Furt

![Graph showing dose-response relationship for RRP-ELISA](http://iai.asm.org/)

**FIG. 1.** Dose-response relationship for RRP-ELISA showing absorbance at 400 nm as a function of the concentration of RRP in nanograms per milliliter in standard test solutions.

<table>
<thead>
<tr>
<th>Specimen source</th>
<th>Conc of bacteria(a)</th>
<th>Results of CIE</th>
<th>Conc of RRP by ELISA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1.0 (\times) 10(^2)</td>
<td>Negative</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Serum</td>
<td>4.1 (\times) 10(^3)</td>
<td>Negative</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Serum</td>
<td>1.0 (\times) 10(^4)</td>
<td>Positive</td>
<td>80</td>
</tr>
<tr>
<td>Serum</td>
<td>3.0 (\times) 10(^4)</td>
<td>Positive</td>
<td>200</td>
</tr>
<tr>
<td>Serum</td>
<td>6.0 (\times) 10(^4)</td>
<td>Positive</td>
<td>1,680</td>
</tr>
<tr>
<td>Serum</td>
<td>1.7 (\times) 10(^5)</td>
<td>Positive</td>
<td>630</td>
</tr>
<tr>
<td>CSF(b) (D.N.)</td>
<td>ND</td>
<td>Positive</td>
<td>70</td>
</tr>
<tr>
<td>CSF (J.D.)</td>
<td>ND</td>
<td>Positive</td>
<td>100</td>
</tr>
<tr>
<td>CSF (K.L.)</td>
<td>ND</td>
<td>Positive</td>
<td>1,100</td>
</tr>
</tbody>
</table>

\(a\) Colony-forming units per milliliter.  
\(b\) CSF, Cerebrospinal fluid.

In addition to the detection and quantitation of \(H.\ influenzae\) type b RRP by ELISA in rat serum and human cerebrospinal fluid, the RRP-ELISA technique was used to determine the concentration of antigen in serum and human cerebrospinal fluid, and to determine the concentration of antigen in serum and human cerebrospinal fluid.

This investigation was supported by Public Health Service grants AI-00461 and AI-11637 from the National Institute of Allergy and Infectious Diseases and NS-12554 from the National Institute of Neurological Diseases and Stroke and by the Hospital for Consumptives of Maryland (Eudowood), Baltimore, Md. J. A. W. is an Investigator of the Howard Hughes Medical Institute.
LITERATURE CITED


