New Enzyme-Linked Immunosorbent Assay for Detection of Antibodies against Hepatitis Delta Virus Using a Hepatitis Delta Antigen Derived from a Taiwanese Clone and Comparison to the Abbott Radioimmunoassay

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An anti-hepatitis delta (HD) enzyme-linked immunosorbent assay (ELISA) using a specific recombinant hepatitis delta antigen derived from a local dominant hepatitis delta virus (hepatitis D virus; HDV) strain in Taiwan has been established. The detection efficiency of this assay was comparable to that of the commercially available Abbott anti-HD radioimmunoassay (RIA) and could be useful in routine laboratory diagnoses of HDV infection.

Areas where hepatitis delta virus (hepatitis D virus; HDV) is endemic have been reported in Asia (23). In Taiwan, the prevalence of HDV infection in patients with chronic liver disease is between 5 and 12% (3). Analysis of the HDV nucleotide sequence reveals at least eight genotypes with unexplained variations and specific geographical distributions (5, 10, 23). The composition of HDV genotypes is particularly complex in Taiwan compared to that in other afflicted areas (25). However, the predominant genotype found in Taiwan is genotype II, which accounts for approximately 40% of the HDV infections in Taiwan (2, 13, 20, 22, 25), and its complete nucleotide sequence and amino acid sequence are approximately 69 to 78% related to those of type I and type III HDV (12).

Considering that concurrent infection by hepatitis B virus (HBV) and HDV not only causes more severe liver disease than infection with HBV alone but also influences an individual’s response to therapy, every individual who is hepatitis B surface antigen (HBsAg) positive should be tested for HDV infection at least once (7, 10, 16, 23). To date, various serological enzyme-linked immunosorbent assays (ELISAs) have been developed for the diagnosis of HDV infection, in which HD antigen (HDAg) mainly came from liver tissues of HDV-infected animals or serum of HDV-infected individuals, and this can pose barriers to effective quality control (6, 9). In the present study, we have established an ELISA for the detection of anti-HD in human serum utilizing a specific recombinant HDAg protein cloned from the local dominant HDV strain, and we have evaluated this new assay by comparing it with a commercially available Abbott radioimmunoassay (RIA).

The HDAg gene fragment of 971 bp, which included the coding region of the small-form HDAg of HDV, comprised of amino acids 1 to 127, was isolated using primers 5'-CGCCTAGCATATGATGAGCCAATCCGAGTCGAG-3' and 5'-CCGGATCCCTACGGGAATCCCTGTTTCCT-3' (12). Escherichia coli [BL21(DE3)pLysS] harboring the expression plasmid pET15b-SMII was grown in Luria-Bertani (LB) medium, and expression of the rHDAg was induced with isopropyl β-D-1-thiogalactopyranoside (0.4 mM). As shown in Fig. 1, the expected 23-kDa rHDAg protein was purified from soluble E. coli lysates using a HiTrap chelating HP column (Pharmacia, Sunnyvale, CA). Coats of purified rHDAg proteins (0.05 μg) were then applied to each well of a microtiter plate (Costar, Corning, NY), and the free binding sites were blocked with blocking buffer (10 mM potassium phosphate buffer...

FIG 1 Expression and purification of recombinant HDAg. Lane 1, crude extract from E. coli [BL21(DE3)pLysS] transfected with the expression plasmid (pET15b-SMII); lane 2, purified rHDAg protein. The arrow marks the position of the rHDAg protein fragment (23 kDa). M, molecular size ladder.

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The prevalent local strain. The rHDAg gene used in this study was the RNA sequence of the HDV genome is highly variable (10), it was 592 bp (11). No HDV RNA was detected in any of the three specimens.

- **Positive**: 110
- **Negative**: 3
- **Total no.**: 113

<table>
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<tr>
<th>Anti-HD ELISA result</th>
<th>No. of anti-HD RIA results</th>
<th>Positive</th>
<th>Negative</th>
<th>Total no.</th>
</tr>
</thead>
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<td>Positive</td>
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<tr>
<td>Negative</td>
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<td>3</td>
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<tr>
<td>Total no.</td>
<td></td>
<td>113</td>
<td>107</td>
<td>220</td>
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</tbody>
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*The sensitivity and specificity of the anti-HD ELISA were 97.3% and 100%, respectively.*


24. Reference deleted.