Starting the evaluation on 22 August 1978, we maintained a room temperature of 22 ± 2 °C until 29 September, at which time the room temperature increased to 32 °C over a weekend and remained high for several days. During this time the enzyme coil rapidly deteriorated. When the temperature was again stabilized on 11 October, we inserted a new coil in the system and continued the evaluation until 18 December. At this time we filled the coil completely with working buffer solution, with no air bubbles, and removed the coil to a refrigerator, where it was stored at 4 °C until 2 January 1979. We then re-inserted the coil in the system and continued testing until 10 January, at which time the evaluation was halted without determining the total capability of the enzyme coil.

The total workload for the second coil was 2441 tests, run during 91 days (43 working days), at 40–70 tests per day, three to five days per week. The coil, manifold system, and all reagents remained in place at room temperature throughout the evaluation except as specified above. Table 1 summarizes our data on control sera. A comparison of values obtained by the two methods, used on 495 patients' sera, had a regression line of \( y = 0.91x + 8.75 \) \( (r = 0.99) \).

Evidently this immobilization approach to enzymatic analyses makes it feasible for the small laboratory to use the advantages of automation for precision and enzyme analysis for specificity. This evaluation does not imply a government endorsement of any commercial product.

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**Table 1. Values Obtained for Control Sera**

<table>
<thead>
<tr>
<th></th>
<th>Control I</th>
<th>Control II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glucose, mg/L</td>
<td></td>
</tr>
<tr>
<td><strong>Coil 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>820</td>
<td>2120</td>
</tr>
<tr>
<td>SD</td>
<td>26</td>
<td>51</td>
</tr>
<tr>
<td>CV, %</td>
<td>3.2</td>
<td>2.4</td>
</tr>
<tr>
<td><strong>Coil 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First day</td>
<td>830</td>
<td>2090</td>
</tr>
<tr>
<td>Last day</td>
<td>870</td>
<td>2180</td>
</tr>
<tr>
<td>Average</td>
<td>820</td>
<td>2110</td>
</tr>
<tr>
<td>SD</td>
<td>21</td>
<td>68</td>
</tr>
<tr>
<td>CV, %</td>
<td>2.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

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**Reference**


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**HBsAg, Anti-HBs, Anti-HBc, and Anti-HAV in Commercial Lyophilized Quality-Control Sera**

To the Editor:

Hepatitis may be caused by several different viruses. Those recognized include hepatitis A virus (HAV); hepatitis B virus (HBV); non-A, non-B virus, which has been referred to as hepatitis C virus (HCV); and perhaps other, yet-undefined viruses (1, 2). Antibodies may arise to the virus or to various antigenic determinants associated with the virus. Antibodies to HAV, HBs, and HBC antigens frequently appear in the blood during convalescence from infection, generally after the corresponding antigen is at a low or nondetectable concentration in the serum. Anti-HAV appears at the time of clinical onset of the disease and the titer rises rapidly. Anti-HBc rises reasonably soon after
exposure to HBV and is detectable in most chronic carriers of hepatitis B surface antigen (HBsAg) and in patients with clinical hepatitis B (2, 3). Anti-HBs appears in serum when the concentration of HBsAg is low or nondetectable. The presence of anti-HBs or anti-HAV may protect a person from re-infection with the particular virus that induced the antibody (4, 5). The presence of an antibody indicates that the individual has been exposed to the virus, and serum containing such antibodies may be infectious if obtained in the early clinical stages (2).

We determined the presence of HBsAg, anti-HBs, and anti-HBc, and anti-HAV in quality-control sera dispensed by several manufacturers. These sera were assayed by radioimmunoassay procedures detailed by Abbott Laboratories, Diagnostic Division, Abbott Park, North Chicago, IL, 60064; Ausrim (HBsAg), Ausab (anti-HBs), CorAB (anti-HBc), and HavAB (anti-HAV).

In both Ausrim and Ausab, a “sandwich” principle is used, a solid-phase radioimmunoassay technique for measuring HBs antigen and antibody, respectively, in serum. CorAB and HavAB utilize the principle of competitive binding of the respective antibody in serum with the radioactive-tagged antibody to the antigen-coated solid phase. The 125I tracer was counted in a model 5385 gamma counter (Packard Instrument Co., Inc., Downers Grove, IL 60515).

Table 1 summarizes our findings. Twenty of 23 (86.6%) of the sera were positive for anti-HBc, 16 of 23 (69.6%) were positive for anti-HBs, and 21 of 23 (91.3%) were positive for anti-HAV. One serum was positive for HBsAg, anti-HBc, and anti-HAV, and could be presumed to be infective for HBV. Only one of these control sera was negative for all four; the source of this preparation was bovine serum albumin. Perhaps one approach to preparing a laboratory control material free of hepatitis-associated constituents is to utilize animal serum sources.

In a study by Hoofnagle et al. (6) four cases of type B hepatitis (HBV) were reported after transfusion with a single unit of anti-HBc-positive blood. They state, “It is possible that some chronic carriers circulate HBsAg (HBV) at too low a level to be detected even by the most sensitive, currently available methods. These low-level carriers should have anti-HBc in high titer and might also be infectious.” This may also be true of quality-assurance materials similarly positive for anti-HBc.

In conclusion, we believe that controls labeled “negative for HBsAg by RIA” may lull the laboratory into a false sense of security. The testing for HBsAg exposure...
in quality-control material does not preclude the possibility that these sera may contain other hepatitis-related constituents. We suggest that quality-control assurance sera should be considered, handled, and disposed of as though they were infective until the risk of infectivity is determined by direct virology experimentation.

We greatly appreciate the technical assistance of Miss Heidi Hunter and Miss Cynthia McClure in performing the laboratory tests.

References

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Free Thyroxine Assay and Thyroxine Index Compared

To the Editor:
We recently evaluated the new IMMO Phase® Free-Thyroxine test system (Corning Medical, Medfield, MA 02052) and compared data on 88 patients with their free thyroxine indices (T7) calculated from thyroxine (T4) and triiodothyronine (T3) resin-uptake values obtained by using reagents from Beckman Instruments, Inc., Fullerton, CA 92634 and Abbott Laboratories, Diagnostics Division, North Chicago, IL 60064.

In the Corning Free T4 assay an immobilized antibody is used in a two-tube assay. In one tube, the immobilized antibody binds endogenous free T4 and added radiolabeled T4; in the second, the total endogenous T4 is allowed to bind, in the presence of radiolabeled T4, to the antibody in an endpoint reaction. Endogenous total T4 is released from its binding proteins by thimerosal. A ratio of values obtained from both tubes gives the proportion of the total reaction that has taken place in the tube that allows endogenous free T4 to bind. The rate at which T4 is attached to the immobilized antibody is proportional to the amount of T4 in the presence of a constant amount of antibody. The free T4 is calculated:

Relative rate, % = (bound free T4, cpm)/(bound total T4, cpm) = (free T4)

The normal range for the free T4 = 8 to 23 ng/L.

The Beckman T7 (free thyroxine index) was calculated as the product of the Beckman T4 radioimmunoassay, which involves a solid-phase second-antibody separation step and a T3 uptake procedure with use of a solid-phase absorbent. The Abbott T7 was calculated from the Abbott T4 radioimmunoassay procedure with its polystyrene glycol mode of separation and the resin sponge technique for the T3 uptake procedure.

We studied 88 patients: 47 euthyroid, 21 hypothyroid, and 20 hyperthyroid, as categorized from their clinical history and laboratory results for thyroid hormone.

As Figure 1 shows, the Corning Free T4 result for the 88 patients compared favorably (correlation coefficient r = 0.92) with the Beckman free thyroxine

Figure 1.

Figure 2.