Adenylate kinase activity is calculated as follows:

\[
AK_{\text{acty, U/L}} = \left( A_{\text{test}} - A_{\text{blank}} \right) \times \frac{8}{5} \times \frac{1}{6.22 \times 10^{-3}}
\]

Here \( A \) = the change in \( A_{340} \)min, 8/5 is the dilution factor, and \( 6.22 \times 10^{-3} \) is the \( \epsilon_{340} \) of NADH, 1 \( \mu \)mol/L.

The following tabulation shows the differences between the described procedure (B) and the assay conditions as used by Ronquist and coworkers (A):

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol</td>
<td>mL</td>
<td>mL</td>
</tr>
<tr>
<td>Temp</td>
<td>°C</td>
<td>°C</td>
</tr>
<tr>
<td>Mg(^{2+} ), mmol/L</td>
<td>13</td>
<td>0.5</td>
</tr>
<tr>
<td>Starting reagent</td>
<td>CSF</td>
<td>AMP</td>
</tr>
</tbody>
</table>

The effect of temperature was measured by performing the procedure at 30 °C as well as at 25 °C. At 30 °C the AK activity in a CSF pool was 1.5 times that at 25°C: \( x_{30°C} = 4.26 \) U/L (n = 8, SD = 0.18 U/L) vs \( x_{25°C} = 2.81 \) U/L (n = 8, SD = 0.16 U/L). So by increasing the temperature to 30 °C and using a dilution factor for CSF of 1.6 instead of 6.0, the sensitivity of the procedure as used by Ronquist et al. can be increased 5.6-fold. A final magnesium reagent concentration of 0.5 mmol/L is optimal. CSF contains about 1.2 mmol of Mg\(^{2+} \) per liter (2), so the final Mg\(^{2+} \) concentration in the proposed procedure is the same as that described by Bergmeyer (3). Use of AMP as starting reagent has the advantage that the blank includes reagents and CSF. The day-to-day CVs we estimated for two CSF pools were 11.1% (for \( x = 1.02 \) U/L, n = 18) and 8.6% (for \( x = 2.89 \) U/L, n = 22).

For a group of 15 patients without objective neurological disorders and no abnormalities in conventional CSF analysis, the AK activity ranged from 0.17 to 1.16 U/L (mean, 0.57 U/L).

**References**

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**Extended Linearity of the Sigma Procedure for Ethanol Determination**

**To the Editor:**

Alcohol:NAD\(^{+} \) oxidoreductase (EC 1.1.1.1), also known as alcohol dehydrogenase (ADH), is widely used in the clinical laboratory (1, 2) to catalyze the reaction

\[
\text{Ethanol} + \text{NAD}^{+} \xrightarrow{\text{ADH}} \text{acetaldehyde} + \text{NADH} + H^{+}
\]

The concentration of ethanol is proportional to the increased absorbance at 340 nm, related to the production of NADH. This determination can be made with a kit (no. 332-UV) manufactured by Sigma Chemical Co., St. Louis, MO 63178. Each reaction vial, which contains 150 U of lyophilized ADH and 1.8 \( \mu \)mol of NAD\(^{+} \), is reconstituted with 3.0 mL of glycine buffer (0.5 mol/L, pH 9.0). The manufacturer’s suggested method (3) is to dilute samples fivefold with the glycine buffer and then add 100 \( \mu \)L of this to a freshly reconstituted reaction vial.

An 800 mg/L calibrator is included in the kit to calculate the unknown ethanol concentration according to Beer’s law. This calculation presents problems, however, because the absorbance plot at 340 nm is nonlinear over the range 0–3000 mg/L (Figure 1). With use of only one standard, the values for samples with ethanol concentrations exceeding 1000 mg/L would be underestimated. Typically, a 2000 mg/L specimen would be reported about 20% too low. Sigma recommends use of a calibration curve; however, their standard curve extends only to 1800 mg/L. For ethanol concentrations exceeding 1600 mg/L a second dilution is required, making the test more labor intensive and increasing the chances for error.

By increasing the enzyme-to-substrate ratio, the linearity can be extended. We have modified the procedure so that no sample dilution is required and the calibration curve is linear over 0–3000 mg/L (Figure 1). We add 10 \( \mu \)L of standard, control, or serum to a freshly reconstituted ADH/NAD\(^{+} \) reaction vial, then measure absorbance, which is maximum after 10 min of incubation at 22–30 °C. By extending the linearity of the standard curve to 3000 mg/L, a single calibrator suffices as a standard and the unknown concentration \( C_{u} \) can be calculated as:

\[
C_{u} = \frac{A_{340, u}}{A_{340, std}} \times C_{std}
\]

The coefficient of variation averaged 3.3% over six days with 16 replicates at each concentration. Therefore, a single calibration plot could be used successfully for several days. However, we recommend including a plot of a new standard curve with each run to ensure accuracy.

**References**
3. Technical bulletin no. 332-UV. Ethyl alcohol, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 (Jan. 1983).

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**Choriongonadotropin: Now You See It—Now You Don’t**

**To the Editor:**

Upon reviewing the last set of results from the College of American Pathologists (CAP) Ligand Assay Survey Series (1), we chanced upon a "remarkable" set of "scientific" data. We refer specifically to the detection and measurement of human choriongonadotropin (hCG) in specimens K-4, K-5, and K-6. The 865 participant laboratories, representing more than 20 different kits, reported hCG concentrations for all three specimens. The means, SD, and other statistical parameters describing hCG in these spec-