min B12/Folate Dual Radioassay kit (Amersham Corp., Arlington Heights, IL 60005). The other three kits are not yet commercially available: Simultrac boil solid phase and Simultrac no-boil solid phase (Becton Dickinson) and no-boil Dualcount (Diagnostic Products Corp., Los Angeles, CA 90045).

Patients' specimens that had vitamin B12 values either <140 pmol/L (possible vitamin B12 deficiency) or >1500 pmol/L, as analyzed by our present method (Simultrac "True B12," liquid phase), were stored at −40 °C, and we obtained hematological information on these patients. We assayed 27 specimens by all seven methods. Of these 27 patients, one was diagnosed as having chronic myelogenous leukemia, one had myeloproliferative disorder, one had hypocromic microcytic anemia, and the remaining 24 had a hematological profile typical of macrocytic anemia. Results obtained with all the kits agreed well with the hematological information in 26 of the 27 patients.

The case in which these did not agree was that of a 60-year-old native Indian woman, who was admitted to hospital with a history of feeling weak and tired, and with numbness and "pins and needles" sensations in both arms and legs. Her hemoglobin concentration was 78 g/L (normal: 120–160 g/L). The mean corpuscular volume was markedly increased at 145 fl (normal: 79–97 fl). The peripheral blood smear showed marked oval macrocytosis and poikilocytosis of erythrocytes, and a large proportion of the polymorphonuclear leukocytes showed hypersegmented nuclei. Serum lactate dehydrogenase (LDH) activity was increased at 418 U/L (normal: 100–225 U/L); LDH isoenzyme electrophoresis showed increased LDH1 and LDH2. All other routine biochemical values, including iron and total iron-binding capacity, were within the normal reference interval. The serum vitamin B12 value by our current method was <75 pmol/L and the serum folate concentration was 11 nmol/L. A Shillings test was performed. For 57Co with intrinsic factor, the excretion was 0.03 (normal: 0.10 to 0.42); for 57Co without intrinsic factor, the excretion was 0.01 (normal: 0.10 to 0.40). The 57Co/58Co ratio was 2.97, which is characteristic of pernicious anemia. Tests for serum intrinsic factor antibody and parietal cell antibody were positive. After an intramuscular injection of 1000 units of vitamin B12 the patient was discharged.

In a serum specimen obtained from this patient three weeks after discharge, the vitamin B12 concentration was 93 pmol/L by our current method; the serum folate remained unchanged at 11 nmol/L. The serum ferritin concentration was 301 μg/L. No trace of 57Co was detected in this specimen.

We also measured vitamin B12 and folate concentrations in duplicate in this specimen with the kits mentioned earlier. Table 1 summarizes the results. We followed the manufacturer's instructions for each kit. In addition, the specimen was analyzed in duplicate by all methods on another occasion and the paired results by each method were within allowable limits of analytical error.

All values for vitamin B12 obtained on this specimen with kits involving boiling as a denaturation step were below or near the lower limit of normal, whereas those obtained from kits involving alkali denaturation gave vitamin B12 values that were at least twice as high and were within or above the reference interval.

Eight weeks after beginning the vitamin B12 treatment, another specimen was taken. Table 1 also summarizes these results.

Evidently, for certain patients the results obtained with kits involving alkali denaturation differ considerably from those obtained with kits involving boiling denaturation. We recommend caution in evaluating results obtained with different kits for vitamin B12 determinations.

On the basis of this in-house data, Becton Dickinson is modifying their no-boil solid-phase kit.

Trefor Higgins
A. Wu
T. A. Kaspar and Assoc.
10924 107th Ave.
Edmonton, Alberta T5H 0X5
Canada

Concept of "Robustness" for Emergency Test Selection

To the Editor:

A statistical test is defined as "robust" if the α risk (the probability of rejecting the null hypothesis—the hypothesis of no difference or effect—when it is true) has little variation when the conditions for applying the test are not fully met. This definition is also applicable to biochemical tests and may be of value in test selection.

Results of biochemical tests are usually compared with the normal reference interval. In this comparison the α risk is fixed a priori (conventionally equal to 0.05) in such a way that there is an α probability to reject the null hypothesis (the test result is "normal") when it is in fact true. The reference limits used for this comparison must be produced according to defined criteria for specimen collection from reference (well-characterized) individuals (1). These criteria must also be followed for specimen collection from patients (application conditions for the test); if they are not, the α risk varies, usually becoming larger. Consider, for example, the analysis for a constituent that must be performed with serum obtained after a defined interval of fasting. If this time is shorter than defined, the probability of obtaining a result that is out of the reference limits increases and therefore the probability of obtaining a falsely positive result also increases.

A biochemical test may be defined as "robust" if no error of interpretation of results is induced when the prescribed standards for specimen collection are not met. This concept of robustness of a biochemical test should be especially applicable in the selection of tests to be used in the emergency laboratory, because such tests frequently must be

---

Table 1. Comparison of Results Obtained with Different Kits for Vitamin B12 and Folate Concentrations Three Weeks and Eight Weeks after Commencing Vitamin B12 Treatment

<table>
<thead>
<tr>
<th>Vitamin B12, pmol/L</th>
<th>Mean</th>
<th>Lower limit of normal*</th>
<th>Folate, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boil kits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simultrac liquid phase</td>
<td>93</td>
<td>77</td>
<td>140</td>
</tr>
<tr>
<td>Simultrac no-boil solid phase</td>
<td>142</td>
<td>—</td>
<td>184</td>
</tr>
<tr>
<td>Quantaphase</td>
<td>152</td>
<td>—</td>
<td>184</td>
</tr>
<tr>
<td>No-boil kits (alkali denaturation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combestat II</td>
<td>350</td>
<td>169</td>
<td>160</td>
</tr>
<tr>
<td>Simultrac no-boil solid phase</td>
<td>1798</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>No-boil Dualcount</td>
<td>302</td>
<td>—</td>
<td>184</td>
</tr>
<tr>
<td>Dual radioassay (Amersham)</td>
<td>805</td>
<td>865</td>
<td>133</td>
</tr>
</tbody>
</table>

* As stated by suppliers.
performed on samples collected without much regard for the defined criteria. For example, an unconscious patient arriving at the hospital can not state if he or she has eaten or exercised recently. Thus, emergency tests should be as robust as possible. If several tests for a given analyte are available for use in an emergency situation, the concept of robustness should be added to the usual concepts (sensitivity, specificity, practicability, etc.) used in test selection.

Reference


Javier Fuentes
Servicio de Bioquímica
C.S. Príncipes de España
Feixa Llarga s/n
L'Hospital de Llobregat
Barcelona, Spain

Serum Lactate Dehydrogenase Isoenzyme-1: Effect of Time of Sampling and Total Serum LD Activity on Diagnostic Efficacy

To the Editor:

Several papers assessing the diagnostic efficacy of the "Isomune LD-1" kit (Roche Diagnostics, Nutley, NJ 07110) have focused on the correlation with electrophoretic lactate dehydrogenase isoenzyme 1 (LD-1) (1–3), determined cutoff values (1–3), and established its superiority over the diagnostic use of an LD-1/LD-2 ratio exceeding 1 (2–5). We examined the variability of LD-1 isoenzyme determination and its efficiency as a function of sampling time after admission for suspected infarction. In this analysis we used two variables: total LD activity and the percent of it represented by the LD-1 fraction, for measurements taken during nonoverlapping time intervals after admission.

We studied 49 patients admitted for evaluation of recent myocardial infarction (MI), drawing serum samples as previously described (2). The diagnosis was established by a combination of clinical symptoms, electrocardiographic changes, and enzyme changes consisting of the presence of CK-MB and of an LD-1/LD-2 ratio exceeding 1. When the biochemical evidence was not clear, the discharge diagnosis was relied on for the classification of patients. When the electrocardiographic evidence was not clear, the clinicians used the enzyme findings in establishing a discharge diagnosis. The data are unbiased to the extent that the LD-1 data were not used for the clinical diagnoses, but bias is present insofar the diagnosis was not always exclusive of biochemical evidence requiring isoenzyme determination. The total LD activity and LD-1 activity after immunological fractionation were measured at 37 °C with an aca (DuPont Instrument Division, Wilmington, DE 19898). For immunochromatography we used the "Isomune LD-1" kit.

Figure 1 illustrates separation of MI and non-MI patients by use of the bivariate distribution of total LD activity and percent LD-1 for four nonoverlapping time intervals indicated. There is a significant overlap of the data for MI and non-MI patients classified at the time of admission (A) by using total LD activity and percent LD-1. Many patients with MI showed no increase in LD activity and <30% LD-1 activity. We consider LD-1 activity exceeding 27% to be significant (2). On the other hand, a few patients without MI showed more than 27% LD-1, with normal values for total LD activity. Figure 1B, C, and D—similar plots of data on the same patients, obtained 12, 24, and 48 h after admission—show the better resolution of MI and non-MI groups that results from the increase both in total serum LD activity and percent LD-1 fraction.

Figure 2 shows a series of receiver operator characteristic curves constructed by varying the cutoff values for LD-1 activity to determine the effect on sensitivity and specificity of the test at the time of admission and 12, 24, and 48 h later. It is apparent that there is 50% specificity and 50% sensitivity with a diagnostic efficiency of 69% if LD-1 is assayed at the time of admission (with a cutoff for LD-1 activ-