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significant B_{12} deficiency is low in the set of sera submitted for assay, the proportion of falsely low values to truly low values will be high, *cf.*, e.g., Elwood et al. (*9*) and Pierce and Hillman (*10*). If the sera come from a clinical setting where the assay is not requested unless there is some reasonable hematological or neurological evidence of B_{12} deficiency, one would expect a much lower fraction of falsely low values.

We conclude that the "improved" vitamin B_{12} assay kits will yield an increased proportion of clinically unexplained low results for serum B_{12}.

It seems prudent for scientific and economic reasons to measure serum vitamin B_{12} only in patients who have hematological or neurological findings that suggest a reasonable probability of vitamin B_{12} deficiency. Measuring serum B_{12} as a screening test in the anemic or the geriatric population will result in a high proportion of low values that cannot be correlated with clinical disease.

References

1. Cooper BA, Whitehead VM. Evidence that some patients with pernicious anemia are not recognized by radiodilution assay for cobalamin in serum. *N Engl J Med* 299, 816–818 (1978).

2. Kolhouse JF, Kondo H, Allen NC, et al. Cobalamin analogues are present in human plasma and can mask cobalamin deficiency because current radioisotope dilution assays are not specific for true cobalamin. *N Engl J Med* 299, 785–792 (1978).

3. Kubasik NP, Ricotta M, Sine HE. Commercially-supplied binders for plasma cobalamin (vitamin B_{12}), analysis—"purified" intrinsic factor, "cobinamide"-blocked R-protein binder, and non-purified intrinsic factor–R-protein binder—compared to microbiological assay. *Clin Chem* 26, 598–600 (1980).

4. Chen IW, Silberstein EB, Maxon HR, et al. Clinical significance of serum vitamin B_{12} measured by radioassay using pure intrinsic factor. *J Nucl Med* 22, 447–451 (1981).

5. Dawson DW, Delamore IW, Fish DI, et al. An evaluation of commercial radioisotope methods for the determination of folate and vitamin B_{12}. *J Clin Pathol* 33, 234–242 (1980).

6. Allen RH. Clinical role and current status of serum cobalamin (vitamin B_{12}) assays. *Ligand Q* 4, 37–44 (1981).

7. Zacharakis R, Muir M, Chanarin I. Comparison of serum vitamin B_{12} estimation by saturation analysis with intrinsic factor and with R-protein as binding agents. *J Clin Pathol* 34, 357–360 (1981).

8. Dawson DW, Fish DI, Frew IDO, et al. Vitamin B_{12} quality control trials. *Clin Lab Haematol* 3, 323–330 (1981)

9. Elwood PC, Shinton NK, Wilson CID, et al. Haemoglobin, vitamin B_{12} and folate levels in elderly. *Br J Haematol* 21, 557–563 (1971).

10. Pierce HI, Hillman RF. The value of the serum vitamin B_{12} level in diagnosing B_{12} deficiency. *Blood* 43, 915–920 (1974).

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Storage of Hydrogen Breath-Test Samples in Plastic Syringes

To the Editor:

The measurement of trace quantities of hydrogen (H_2) in expired air is the basis for various tests of carbohydrate absorption, intestinal transit time, and bacterial overgrowth (*1*). The amount of gas sample injected into the analytical instruments varies from 2 (*2*) to 30 mL (*3*). H_2 being the most diffusable of all gases, the procedures for storing breath samples before analysis are important. Foil gas-envelopes (*4*, *5*), rubber-stoppered glass tubes (*6*, *7*), Teflon-lined glass-tight (Hamilton) syringes (*5*), and disposable plastic syringes (*8*) have been used. In the foil bags, the concentration of H_2 remained stable for 47 days (*5*), and was constant in the Vacutainer® Tubes (Becton Dickinson, Rutherford, NJ 07070) for as long as three weeks (*6*). The advisability of using glass tubes has recently been questioned by Jensen et al. (*9*), who observed contamination of silicone-coated Vacutainer Tubes with volatile reducing gases. This has been confirmed in our laboratory (B Caballero: unpublished observations) Plastic syringes would appear to be a useful alternative when storage of large sample volumes is required, Perman et al. (*8*) having found no loss of H_2 concentration in gas stored in plastic syringes over a 12-h period. Because more breath analysis tests are being used in population surveys and field studies (*10*, *11*), we undertook to examine the limits of storage of gas samples in plastic, disposable 60-mL syringes.

The syringes had a luer-lock adapter (Monoject®; Sherwood Medical, St. Louis, MO 63103), to which was tightly screwed a plastic three-way stopcock (Medex, Inc., Hilliard, OH 43026). We used two gas mixtures: 100 μL of H_2 per liter of N_2 (Scotty Gas II; Supelco, Bellefonte, PA 16823), and 97 μL of H_2 per liter of room air (L'l Squirt; Ideal Gas Products, Edison, NJ 08818). The H_2 concentration in gas samples was determined with a MicroLyzer Model 12 gas chromatograph (Quintron Instruments, Milwaukee, WI 53215), calibrated with the respective standard gas (*3*).

To determine the stability of gas content, we filled four syringes to a 60-mL volume with the 100 μL/L gas standard and four additional syringes with 30 mL of the same reference standard diluted to 60 mL with room air. Twelve hours later, we mixed the undiluted gas standard with an equal volume of room air, and analyzed the gas content from all of the syringes. The mean (± SD) H_2 content of the prediluted and stored samples was 50 ± 1.4 μL/L, of the stored and subsequently diluted samples 51 ± 1.0 μL/L. These observations confirm the findings of Perman et al. (*8*) for 12-h storage.

We next placed duplicate samples of the undiluted 100 μL/L gas standard and of serial dilutions of the standard with room air to form 50, 25, and 12.5 μL/L concentrations in syringes. One set of the four syringes were analyzed after 24 h and the other after 48 h. The 100 μL/L standard decreased to 97 and 94 μL/L after the respective intervals. At the two greatest dilutions, a change of only 1 μL/L was detectable at each interval. Finally, we determined the effect of five days of storage. The 97 μL/L gas standard and a breath sample containing 21 μL of H_2 per liter were each placed into 20 syringes, and four syringes of each mixture were chosen without conscious bias for analysis at each 24-h interval. With both gases, H_2 concentrations decreased by about 4% per day (Table 1).

In field studies, or clinical settings in which the test is performed away from an analytical laboratory, it may not be

Table 1. Stability of Hydrogen Concentration in Samples Stored in Plastic Syringes

Storage, h	Measured H_2 concn, μL/L[a]			
	Std. gas mixture		Expired air	
0	97	(100)	21.5 ± 0.5	(100)
24	97.0 ± 0.5	(97)	21.2 ± 0.5	(100)
48	88.2 ± 0.9	(91)	20.2 ± 0.5	(94)
72	83.2 ± 0.5	(86)	19.7 ± 0.5	(92)
96	79.7 ± 0.5	(82)	18.0 ± 0.8	(84)
120	77.7 ± 0.5	(80)	17.0 ± 0.5	(79)

[a] Mean ± SD of four samples. Percentage of initial concentration is given in parentheses.

possible to analyze breath samples immediately. At sea level in a temperate environment, the loss of gas samples stored in plastic syringes fitted with three-way stopcocks is negligible at 12 h, about 1 μL/L per day for each 25 μL/L of initial concentration. For the breath-test diagnosis based on a criterion of a predetermined increase in breath H_2 concentration above baseline, e.g., $\leq$ 20 μL/L, the decline in diagnostic sensitivity over 48 h would be minimal. If more sophisticated quantification of H_2 excretion, such as integration under the discontinuous curve of H_2 concentration (5), is desired, nomograms can be constructed to correct samples to their initial concentrations, bearing in mind that the rate of H_2 diffusion in a given locality would be influenced by ambient temperature and altitude.

We conclude that plastic syringes fitted with three-way stopcocks are not hermetically "gas-tight," but they do maintain the concentration of H_2 sufficiently constant to represent practical vessels for the storage of breath-test samples for periods of several days before analysis.

References

1. Solomons NW. The hydrogen breath test and gastrointestinal disorders. *Compr Ther* 7(8), 7–15 (1971).

2. Levitt MD. Production and excretion of hydrogen gas in man. *N Engl J Med* 281, 122–127 (1969).

3. Christman NT, Hamilton LH. A new chromatographic instrument for measuring trace concentrations of breath-hydrogen. *J Chromatogr* 229, 259–265 (1982).

4. Calloway DH, Murphy EL, Bauer D. Determination of lactose intolerance by breath analysis. *Am J Dig Dis* 14, 811–815 (1969).

5. Solomons NW, Viteri FE, Hamilton LH. Application of a simple gas chromatographic technique for measuring breath hydrogen. *J Lab Clin Med* 90, 856–862 (1977).

6. Newcomer AD, McGill DB, Thomas PJ, Hofmann AF. Prospective comparison of indirect methods for detecting lactase deficiency. *N Engl J Med* 293, 1232–1236 (1975).

7. Niu HC, Schoeller DA, Klein PD. Improved gas chromatographic quantitation of breath hydrogen by normalization to respiratory carbon dioxide. *J Lab Clin Med* 94, 755–763 (1979).

8. Perman JA, Barr RG, Watkins JB. Sucrose malabsorption in children. Non-invasive diagnosis by interval breath hydrogen determination. *J Pediatr* 93, 17–22 (1978).

9. Jensen WE, O'Donnell RT, Rosenberg IH, et al. Gaseous contamination in sterilized, evacuated blood-collection tubes *Clin Chem* 28, 1406 (1982). Letter.

10. Newcomer AD, Thomas PJ, McGill DB, Hofmann AF. Lactase deficiency: A common genetic trait of the American Indian. *Gastroenterology* 72, 234–237 (1977).

11. Brown KH, Parry L, Khatun M, Ahmed MG. Lactose malabsorption in Bangladeshi village children. Relation with age, history of recent diarrhea, nutritional status and breast-feeding *Am J Clin Nutr* 32, 1962–1969 (1979).

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Effect of Atypical Creatine Kinase Isoenzymes and CK-BB on an Immunochemical CK-MB Assay

To the Editor:

Reportedly (*1*), the Roche Isomune kit (Roche Diagnostics, Nutley, NJ 07110) for measuring creatine kinase (EC 2.7.3.2) isoenzyme MB (CK-MB) is unaffected by the presence of isoenzyme BB. Recently the efficacy of this procedure in the diagnosis of acute myocardial infarct was demonstrated (*2*), except for the effect of the atypical CK isoenzymes (the cathodic and anodic CK isoenzymes). With other immunochemical procedures, these atypical isoenzymes and (or) CK-BB will be measured as CK-MB. We have determined their effect on results for CK-MB with the Roche procedure.

We measured CK isoenzymes by use of an agarose gel electrophoretic system (Corning Medical, Medfield, MA 02052), in which the CK anodic isoenzyme migrates between CK-MM and CK-MB, and the cathodic CK isoenzyme migrates cathodic to CK-MM. These isoenzymes were shown not to be adenylate kinase (EC 2.7.4.3), because they were not visible in the completed electrophoretogram when creatine phosphate was omitted from the reagent. The Roche kit was used to measure CK-MB and total CK.

CK-MB was measured in (*a*) six samples containing CK-BB; (*b*) five samples with the cathodic CK fraction, three of which contained CK-BB and one CK-MB; and (*c*) four samples with the anodic CK fraction; one contained CK-MB (Table 1). In all samples, the value for total CK was above normal; 50 U/L is the upper limit of normal for total CK.

Of the six specimens with CK-BB, five contained CK-MB. In these samples, although CK-BB was present in relatively large proportions, the results for CK-MB were not substantially different from those obtained electrophoretically. In a sample that contained only CK-BB, no CK-MB was detected. Evidently CK-BB will not contribute to the apparent CK-MB activity.

The cathodic CK activities in the five samples ranged between 8 and 30 U/L. Our results indicate that samples containing the cathodic CK band and those with both the cathodic CK and CK-BB will not be detected as CK-MB. In the sample with cathodic CK, CK-MB, and CK-BB, abnormal results for CK-MB were obtained immunochemically and electrophoretically.

Table 1. Effect of CK-BB and the (Atypical) Anodic and Cathodic CK Isoenzymes on Results (U/L) with the Roche Immunochemical CK-MB Assay

		Electrophoresis		Immunochemical:
Total CK		CK-BB	CK-MB	CK-MB
194		16	–	–
186		11	33	24
217		15	37	23
354		20	28	29
194		10	17	14
149		6	15	14
Samples with cathodic CK				
66	8[a]	–	–	–
105	30[a]	5	–	–
176	16[a]	4	21	11
247	14[a]	10	–	–
126	13[a]	–	–	–
Samples with anodic CK				
82	28[b]		–	–
179	32[b]		–	–
68	8[b]		–	–
116	9[b]		16	13

[a] Cathodic CK acty.
[b] Anodic CK acty

The anodic CK activities in the four samples ranged between 8 and 32 U/L. In the three samples with only the anodic CK fraction, no CK-MB was detected. In the sample with CK-MB in addition to the anodic CK fraction, results for CK-MB were similar by both the immunochemical and electrophoretic procedures.

The anodic and cathodic isoenzymes are not inhibited by antibodies to the M component; thus with most immunochemical procedures these isoenzymes will be measured as CK-MB. In the Roche kit a double-antibody system is used that avoids these potential interferences. The procedure involves addition of antibody to CK-M to two tubes containing serum, allowing them to stand at room temperature, then adding donkey antigoat IgG to one tube to precipitate the M-subunit–antibody complexes. CK activity in both tubes is then measured and related to CK-MB activity. The CK activity in the tube to which the second antibody was not added is due to the B activity of CK-MB, CK-BB, adenylate kinase, or to the cathodic and anodic CK isoenzymes. CK activity from the supernate in the second tube is due to CK-BB,