reduction of pyruvate by lactate dehydrogenase (EC 1.1.1.27). Thus two simple and practicable reagent modifications, either of which is likely to essentially or completely eliminate pyruvate interference, appear to be feasible.

Firstly, the 4000 U of exogenous lactate dehydrogenase per liter [reported to be present in the reagent mixture by Yasmineh and Hanson (*1*) but not by the package insert] could be replaced by a specific inhibitor of lactate dehydrogenase. In fact by using oxamate, an inhibitor of lactate dehydrogenase (*2*), another manufacturer has completely eliminated interference by pyruvate up to 1.1 mmol/L in another enzymic kinetic amylase method (*3*). Alternatively, addition of an adequate amount of NADH would allow the 4000 U of exogenous lactate dehydrogenase per liter to reduce the pyruvate during the pre-incubation period and thus eliminate its effect during measurement of amylase activity. Pyruvate interference is routinely eliminated or minimized by this approach in the determination of aspartate aminotransferase (EC 2.6.1.1) activity (*4*). I anticipate that either of my two suggested modifications would result in reliable amylase measurements with the Enzymatic Amylase—DS Reagent in the presence of abnormally high pyruvate concentrations.

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This Letter, sent to the authors of the paper in question, elicited the following response:

To the Editor:

Addition of an adequate amount of NADH to allow the reduction of pyruvate during the pre-incubation is a worthy suggestion. This is essentially what we do in our clinical laboratory, by selecting a reagent lot containing a minimum of 22 μmol/L NADH. This amount of NADH will eliminate interference in a specimen with a normal α-amylase activity [20–90 U/L (*1*)] and a pyruvate concentration of 0.8 mmol/L, as shown in Table 1.

Table 1. Effect of Increasing Concentrations of Pyruvate on α-Amylase Activity as Measured with Amylase-DS Reagent Containing NADH, 22 μmol/L

Pyruvate, mmol/L serum	Amylase, U/L
0	33
0.2	33
0.4	34
0.6	31
0.8	33
1.0	20
1.5	2

We believe it unnecessary for us to add additional NADH to the reagent, because during 18 months of routine use of this reagent we have done 7921 serum and 2711 urine α-amylase determinations and have encountered only three specimens with pyruvate concentrations exceeding 0.8 mmol/L. These specimens, with pyruvate concentrations of 0.85, 1.0, and 1.4 mmol/L, were from a kidney-transplant patient with steroid-induced diabetes mellitus, an acute lymphocytic leukemia patient on steroid treatment, and a patient in septic shock, respectively. Pyruvate interference in these specimens was detected, not by routine measurement of pyruvate in all α-amylase samples, as stated by Lustig, but by examination of the reaction rates for a prolonged lag phase (*1*, *2*).

Lustig also suggested the use of oxamate to eliminate pyruvate interference.[1] We have added oxamate to the Amylase—DS reaction mixture to give final concentrations ranging from 0.5 to 60 mmol/L and found that pyruvate interference, at a concentration of 1.0 mmol/L serum, was not eliminated. This is probably because of the extremely high amounts of lactate dehydrogenase (LD, EC 1.1.1.27) present in the reaction mixture. For example, we found that LD activity in five lots of Amylase—DS reagent ranged from 400 to 12 000 U/L. Since the reagent:sample ratio for the α-amylase assay is 500 μL:20 μL, this activity is comparable to 10 000 to 300 000 U/L of serum, respectively.

The "Statzyme" amylase kit (Worthington Diagnostics, Division of Millipore Corp., Freehold, NJ 07728) shows no interference with α-amylase activity at pyruvate concentrations of 1.1 mmol/L (*3*), which Lustig suggests is due to the addition of 10 mmol of sodium oxamate per liter to inhibit LD activity. Using this kit, we have similarly found that pyruvate, at a concentration of 2.0 mmol/L of serum, did not interfere with the α-amylase reaction (Table 2, no LD added). However, after dialysis of the reagent to remove oxamate, we found very little (<20 U/L) LD activity in the reagent. Pyruvate did interfere, however, when we added rabbit muscle LD (Sigma Chemical Co., St. Louis, MO 63178) to the Statzyme reagent, as shown in Table 2. For example, at LD activities of 40 and 400 U/L of reagent and a pyruvate concentration of 1.0 mmol/L of serum, the α-amylase activity dropped from 63 U/L to 57 and 35 U/L, respectively. This is equivalent to 10 and 44% inhibition, respectively. We conclude, therefore, that a specimen with above-normal values for both pyruvate and LD could give falsely low results for α-amylase activity with the Statzyme reagent, despite the presence of oxamate.

Table 2. Effect of Increasing Concentrations of Pyruvate on α-Amylase Activity with the Statzyme Amylase Reagent in the Absence and Presence of Added LD

Pyruvate, mmol/L serum	Amylase, U/L		
	No LD	40 U/L LD [a]	400 U/L LD [b]
0	62	62	64
0.2	63	60	60
0.4	61	60	52
0.6	63	58	46
0.8	62	58	41
1.0	63	57	35
1.4	62	55	24
2.0	63	54	5

[a] This activity is the equivalent of 1000 U/L of serum.

[b] This activity is the equivalent of 10 000 U/L of serum.

[1] Seeing this response, Dr. Lustig commented, in part: "I suggested that the oxamate should *replace* the exogeneous lactate dehydrogenase in the reagent. I did not imply or expect that the oxamate will be used in *conjunction* with the lactate dehydrogenase in the Enzymatic Amylase—DS Reagent.

"I agree with Hanson et al. that the *simultaneous* presence in serum of improbably high lactate dehydrogenase [activities] and greatly elevated pyruvate concentrations would result in falsely low amylase activities as measured by the Statzyme amylase kit, but I [would] expect the above two conditions to occur less frequently than elevations of serum pyruvate alone, which, as shown by Hanson et al., leads to a spurious decrease of amylase activity as measured with the Enzymatic Amylase—DS Reagent. Consequently, pyruvate interference should occur less often with the Statzyme Amylase kit than with the Enzymatic Amylase—DS Reagent."

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Evaluation of a Kit for Measuring Tricyclic Antidepressants

To the Editor:

A commercial kit for measuring the concentration of tricyclic antidepressants in plasma has recently become available (Tri-Cy; Wien Laboratories, Succasunna, NJ 07876). The antiserum was raised in rabbits against succinyl-nortriptyline coupled to bovine serum albumin. It cross reacts with tertiary amine tricyclics (e.g., amitriptyline) as well as secondary amine tricyclics (e.g., nortriptyline). The manufacturers suggest that the kit can be used to measure amitriptyline, nortriptyline, imipramine, desipramine, protriptyline, and doxepin in plasma. For all these drugs, a linearized standard curve prepared by using pure imipramine is used, the resulting concentration being corrected by a cross-reactivity factor supplied with the kit for each particular drug.

We have carefully evaluated the kit, using the above protocol, for measurement of nortriptyline in patients' samples. We compared results obtained with the kit with those obtained by another radioimmunoassay procedure (*1*), in which a nortriptyline standard curve is used; the procedure has been validated by comparison with a specific double-isotope-derivative dilution assay (*1, 2*). We found poor agreement between the two radioimmunoassays, as shown by the calculated line of best fit: $y = 1.8x - 8.4$ where x = our procedure and y = kit. Although a reasonable correlation coefficient was obtained ($r = 0.96$, n = 18), the slope of the line was almost twice that expected.

Looking for the cause of this discrepancy, we obtained samples of nortriptyline standards from the manufacturers, and samples were again measured by both procedures, with use of nortriptyline standards instead of imipramine in the kit protocol. Agreement was improved ($y = 0.80x - 0.90$, $r = 0.95$, n = 27) and would have been even better if a standard of <50 μg/L had been included, because several of the samples were below this concentration.

This second comparative study led us to suspect that the cross-reactivity factors supplied with the kit, to be used with the imipramine standard, were resulting in over-correction and hence erroneously high values. The cross-reactivity factor supplied for nortriptyline, to be used with an imipramine standard curve, was 1.6. In our hands, this factor varied from 1.0 at 50 μg/L, to 1.2 at 100 μg/L, to 1.4 at 200 and 400 μg/L. Only at the higher concentrations was this factor constant, but it still was less than 1.6. We recommend that, to obtain accurate results with this kit, standard curves should be for the drug to be measured and avoid any correction factor.

Because both tertiary and secondary amine tricyclics cross react with the antiserum, but not to the same extent, only total immunoreactive material is quantitated for samples from patients being treated with amitriptyline or imipramine. The manufacturers claim that this "total" correlates well with the "total" tricyclic concentration (tertiary and secondary) as determined by gas–liquid chromatography/mass fragmentography (*3*). Their comparison again showed acceptable correlation coefficients, but slopes and intercepts were not satisfactory (personal communication from G. H. Wien, Director, Wien Laboratories). Comparing 29 samples containing imipramine and desipramine, we calculated a slope of 0.77, and a y-intercept of 30.6 ($r = 0.93$). Amitriptyline and nortriptyline samples (n = 19) compared gave a slope of 1.2 and a y-intercept of −8.4 ($r = 0.92$), whereas nortriptyline samples alone gave a slope of 1.06 and a y-intercept of 43.6 ($r = 0.90$, n = 10). The reason for the discrepancies may be the fact that the tertiary and secondary tricyclics do not cross react equally with the antiserum at all concentrations. However, they highlight the need for caution in using most of the radioimmunoassay methods published (including our own) for measurement of "total" tricyclics.

A further problem in measuring "total" tricyclic concentrations is deciding whether the secondary or the tertiary amine should be used as the standard. For example, in a sample containing both amitriptyline and nortriptyline, using either amine for the standard curves resulted in a "total" concentration of 200 μg/L when amitriptyline was used and 400 μg/L when nortriptyline was used.

In conclusion, we wish to stress three points:

Firstly, before commercially available kits are used, each laboratory should rigorously check out the methodological protocols. Results from the kit should be compared with those obtained by an established method for patients' plasma and standards. For the "Tri-Cy" kit, we recommend the use of appropriate standards for the drug to be measured.

Secondly, we caution against the use of radioimmunoassay for "total" tricyclic concentrations and recommend the use of specific techniques capable of determining both the secondary and the tertiary components.

Thirdly, in reporting comparative methodological studies, the slope and intercept of the calculated line of best fit obtained from the two methods should be quoted, because correlation coefficients alone can be misleading.

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The president of Wien laboratories replies:

To the Editor:

We are indebted to Dr. Maguire's team for their letter in which they verify good correlation between our Tri-Cy test set and the other methods with which it was compared. The following comments are in reply to the questions raised in Dr. Maguire's letter; however, it is difficult to comment without having access to the actual data generated by their research.

The Tri-Cy test set has shown excellent correlation with another radioimmunoassay (RIA) method and produced a linear regression equation of $y = 0.99 x + 8.1$ with a correlation coefficient of 0.97, n = 25, range 0–1000 μg/L. The comparison RIA method involved a [^{3}H]-nortriptyline tracer and ammonium nium sulfate separation; our Tri-Cy test