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Naomi Q. Hansen
Charissa L. Oliphant
Walid G. Yasmineh

Dept. of Lab. Med. and Pathol.
Univ. of Minnesota Med. School
Minneapolis, MN 55455

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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Kay P. Maguire
Graham D. Burrows
Trevor R. Norman
Bruce A. Scoggins[1]

Dept of Psychiatry and Physiol. and Med.
[1]*Howard Florey Instit. of Exp. Physiol. and Med.*
University of Melbourne
Parkville, Australia

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