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False Results with the N-Acetyl-
cysteine-Activated, 
Immunoinhibited CK-MB 
Determination

To the Editor:

Several societies of clinical chemistry recommend use of N-acetyl cysteine (NAC) to reactivate creatine kinase (CK, EC 2.7.3.2) and its MB isoenzyme (CK-MB) before their determination. The reactivating properties of NAC have been shown to be more potent and reliable than those of previously used activators such as glutathione. However, we found a remarkably high number of false, especially of false-positive, results with this method.

We determined CK-MB activity in 56 sera with above-normal total CK from patients with excluded myocardial infarction (MI), according to the recommendations of the German Society for Clinical Chemistry (1) at 37 °C and using Merck (Darmstadt, F.R.G.) reagents no. 14336 and 14327. We found 32% false-positive results when using a limit for the MB portion of total CK of 5%, and 12.5% false-positives with a 10% limit. The absolute normal-value limit was 23 U/L. Of the 18 false-positive results with the 5% limit, 13 were for cases with a skeletal-muscle lesion.

The percentage of false-negative results with a correctly timed determination of CK-MB in 94 sera with above-normal total CK from patients with proven (by symptoms, electrocardiogram, and enzymes) MI was 15% (10% limit) and 4% (5% limit).

To check the specificity of the NAC-activated test in the absence of increased total CK, we determined CK-MB according to the above-mentioned technique in a further series of 210 sera from outpatients without MI. There were seven cases (3.3%) with false-positive results (mean CK-MB value 29.83 ± 8.15 U/L).

We conclude, therefore, that in cases with increased total CK the NAC-activated CK-MB assay does not permit one to differentiate with sufficient reliability between skeletal and myocardial muscle lesions, especially when a low relative normal value for the CK-MB portion of total CK is applied.

Reference

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Modified Direct 
Enzymoimnoassay for Salivary 
Progestrone

To the Editor:

In the assessment of ovarian function, the value of measuring progesterone in serial saliva samples is becoming increasingly clear (1, 2). We recently reported an enzymoimmunoassay for directly measuring progesterone in saliva with no extraction step (3). A solid-phase antiserum was used, and the method had the required specificity and sensitivity. Concentrations measured in saliva were in general agreement with those of other workers using a radioimmunoassay with an extraction step (4). More recently, we have found that this direct assay overestimated the progesterone concentrations in some saliva samples (as ascertained by an assay with an extraction step), by up to 10-fold in most samples from some individuals.

We investigated this phenomenon by assaying 134 saliva samples from 103 healthy non-pregnant women, ages 21 to 40 years, who were not taking oral contraceptives. Each sample was assayed both by our direct method and by a similar method incorporating an extraction step (3). In addition, portions of the saliva samples were heat-treated before direct assay, by transferring 300 µL of saliva to a small glass bottle, securing the cap, incubating in an oven at 56 °C for 2 h, and centrifuging at 8000 × g for 1 min. Aliquots of the supernatant fluid were then assayed without extraction.

We compared the values from the direct assay of unheated samples with those from the extraction assay and found a poor but significant correlation (r = 0.587, p < 0.001), showing overestimation of the concentration in many samples (Figure 1A). However, when the same samples were heat-treated before direct assay, a much better correlation with the extraction assay was obtained (r = 0.921, p < 0.001), with a concomitant decrease in the slope of the regression line from 1.7 to 1.1 (Figure 1B). With some saliva samples, heat treatment had a remarkable effect on the values for apparent progesterone. For example, one sample that was found to have 526 pmol/L with extraction gave values of 2426 and 776 pmol/L without and with heat treatment, respectively. However, with saliva samples where there was no significant difference between the values obtained from the extraction and "unheated" direct assays (p > 0.05, n = 33, Student's two-tailed paired t-test), subsequent heating of the samples did not significantly alter the values after direct assay (p > 0.05). Therefore, heat treatment may not be necessary for all saliva samples, although we now routinely heat all samples at 56 °C for 2 h before assay.

At present, we cannot fully explain how the heat treatment reduces the apparent progesterone concentrations in the direct assay. However, there are at least three mechanisms by which a