False-Positive emt*-st™ Ethanol Screen with Post-Mortem Infant Plasma, N.R. Badcock and D.A. O'Reilly (Dept. of Chem. Pathol., Adelaide Children's Hoep., 72 King William Road, North Adelaide, South Australia, 5006 Australia)

When post-mortem plasma and vitreous humor from infants, negative for ethanol by gas chromatography, were analyzed for ethanol with a commercially available homogeneous enzyme immunoassay (EMIT*-st™; Syva Co., Palo Alto, CA), anomalous results were obtained in the majority of cases with sudden infant death syndrome (SIDS) and, to a lesser extent, in non-SIDS cases (age-matched control infants who died of known causes). Results for all other EMIT-st drug tests were negative. The incidence of positive findings in plasmas and vitreous humors was respectively 84/125 and 9/70 in SIDS cases, and 16/49 and 0/18 in non-SIDS cases. We suspected an interfering endogenous substance, such as is found in the digoxin radioimmunoassay of post-mortem serum from infants (1). We also hoped that, in identifying the cross-reactant, we might uncover a biochemical marker for SIDS.

To determine the cross-reactant, we prepared special EMIT-st alcohol vials containing all the usual assay reagents except alcohol dehydrogenase (EC 1.1.1.1) and stabilizers, as well as bottles of each of the special diluent stabilizers. Reactions involving the vials minus alcohol dehydrogenase produced the same false-positive results. The data suggested that the positive samples contained an NAD*-based enzyme, probably a dehydrogenase, and its substrate in sufficient quantities to convert NADH in the absence of added enzyme.

At this stage, lactate and lactate dehydrogenase (LDH; EC 1.1.1.27) were suspected. We tested this hypothesis by adding lactate and oxamide to positive samples. By showing an enzyme rate increase with lactate and a rate decrease with oxamide, an LDH inhibitor, we demonstrated the contribution of LDH and its substrate to a false-positive result. Further analysis showed that for a lactate concentration of 20 mmol/L, an LDH concentration of ~2800 U/L or greater was required to produce a positive absorbance difference. The mean LDH value for the 125 SIDS plasma and vitreous humor samples tested was 6430 (SD 6165, range 590–38 400) and 2965 U/L (SD 2840, range 11–14 892), respectively; for the 51 non-SIDS plasma and vitreous humor samples assayed, mean LDH was 4218 (SD 3733, range 950–31 723) and 1077 U/L (SD 802, range 21–4642), respectively. Mean plasma and vitreous humor lactate concentrations were also higher in the SIDS group (91 and 23 mmol/L, respectively, vs 63 and 15 mmol/L).

The average LDH of 20 adult post-mortem plasma samples was 2194 U/L and all of these plasma specimens gave a negative EMIT-st result; plasma samples from 15 patients surviving myocardial infarction with an average LDH of 1024 U/L were similarly negative. The much lower LDH values in these two groups explains why previously reported post-mortem specimens, shown by gas chromatography to be alcohol-free, were similarly negative by EMIT-st assay (2).

The significantly higher LDH concentration in the SIDS infants than in the pediatric control group (P < 0.01 for each comparison) has been reported previously (3). The increase seems to be largely but not entirely consistent with the longer post-mortem interval in the SIDS group (27 vs 20 h). The higher value could also suggest agonal time differences and reflect various mechanisms of death.

The correlation for the SIDS and non-SIDS cases between the change in absorbance (AA) for plasma EMIT-st and LDH was significant: r = 0.770 (P < 0.01) and r = 0.857 (P < 0.001), respectively. A similar good correlation existed for SIDS vitreous humor (r = 0.821; P < 0.001) but not for non-SIDS vitreous humor samples (r = –0.268, P > 0.5); we cannot explain this anomaly.

We conclude that the EMIT-st ethanol test is unreliable for use with post-mortem specimens, especially those from infants with increased concentrations of lactate and LDH. The increased lactate and LDH in the SIDS group may also be relevant.

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References

Protein 1 is a Secretory Protein of the Respiratory and Urogenital Tracts Identical to the Clara Cell Protein, A. Bernard,1 H. Roels,2 R. Lauwereys,4 R. Witters,6 C. Gielens,6 A. Soumillion,2 J. Van Damme,2 and M. De Ley5 (1 Unit of Industrial Toxicol. and Occupational Med., Catholic Univ. of Louvain, Clos Chapelle-aux-Champs, 30.54, B-1200 Brussels, Belgium; 2 Lab. voor Biochem., Dekenstraat 6, and 3 Rega Inst. for Med. Res., Catholic Univ. of Louvain, Minderbroedersstraat 10 B-3000 Leuven)

Protein 1 (P1) is an α-microprotein discovered in the urine of patients with tubular dysfunction (1). Little is known about this protein except that it is a small protein