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Short Communication

Is there a role for ethanol assay using alcohol dehydrogenase in a basic drugs and alcohol screen of blood following routine autopsies?

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The external examination system of determining the cause of death as operated in Dundee, Scotland is controversial. The addition of a blood or urine specimen for drugs and alcohol processed by hospital autoanalysers will reduce error in death certification. This is even more convenient to organise with a shorter turn-around time than imaging by computed tomography (CT) or magnetic resonance imaging (MRI). These measures may reduce the autopsy rate and ameliorate the distress of families of the deceased.

Key words: Autopsy, alcohol, death.

INTRODUCTION

For over a decade, there has been controversy over the appropriateness of the high rate of coroner's autopsies (Pounder, 2000; Rutty et al., 2001). Advocacy of the "view and grant" system of issuing the cause of death as operated in Dundee, Scotland has been challenged and imaging is suggested as a partial answer to lessen the likely inaccuracy in death certification (Pounder et al., 2011; Palmer, 2011). We suggest the use of widely available autoanalysers in hospital laboratories to estimate drugs, and alcohol could prove a useful adjunct to the external examination that might also include imaging to reduce error. Gas chromatography (GC) is more time consuming and expensive than the automated enzymatic assay for ethanol (Nine et al., 1996). With the short turn-around times of less than one day, families would be less distressed by the death certification system.

BRIEF REPORT

Microgenics DRI Ethyl Alcohol Assay uses alcohol dehydrogenase and the back reaction nicotinamide adenine dinucleotide (NAD) to NADH measured spectrophotometrically at 340 nm on the Olympus 5400 autoanalyser for the analysis of alcohol. The data sheet claims that the assay can accurately quantitate alcohol concentrations within the range of 10 to 600 mg/dl. The stated sensitivity is 10 mg/dl and is defined as the lowest concentration that can be differentiated from a negative sample. The method package insert quotes the percentage cross reactivity with other common alcohols and at a level of 2,000 mg/dl, but only n-butanol at 1.7% and n-propanol at 10.7% register. Within run precisions are 2.7% at 50 mg/dl; 1.2% at 100 mg/dl and 0.6% at 300 mg/dl, and between run precision is 4.5% at 50 mg/dl. But the data sheet warns that increased levels of lactic acid and lactate dehydrogenase in post-mortem samples may cause elevated ethyl alcohol results.

Table 1 includes the data when an enzymatic ethanol assay was added to the routine lactate dehydrogenase (LDH) assays in the laboratory. Values with asterisk* were post-mortem samples. The enzymatic values below 10 mg/dl are technically indistinguishable from zero. GC is the gold standard and this small study confirms that in autopsy specimens with LDH values greater than 2,000 IU/L, ethanol values are unsafe to interpret, because of the likelihood of false positives. The question of whether

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Table 1. False positive blood ethanol values associated with high LDH levels using GC alcohol analysis as the reference standard.

LDH	Ethanol-alcohol dehydrogenase (mg/dl)	Ethanol-GC (mg/dl)
296	0.6	-
325	0	-
332	0	-
371	0.8	-
379	0.2	-
459	0.4	-
474	0.5	-
590	0.8	-
599	0.7	-
913	0.4	-
1,145	1.2	-
2,100*	14.9	ND
2,514*	6.4	ND
2,520*	6.3	ND
5,123	1.8	-
6,008	1.9	-
6,178*	22.1	ND
6,497	3.1	-
7,174	4.3	-
7,400	3.3	-
7,867	3.6	-
8,181	2.6	-
9,852*	30.1	ND
16,677*	99.6	ND
44,898*	248	ND
77,054*	450	ND
77,337*	450	ND
119,513*	>600	23

Upper reference limit for LDH is 225 IU/L. ND = none detected. *Indicates post-mortem samples.

enzyme assay can legally validly rule out the presence of ethanol without reverting to GC as the gold standard remains a matter of opinion. This should not be a problem because using GC for ethanol analysis, the limit of detection is 8 mg/dl, and the limit of quantitation is 10 mg/dl as reported in the literature. The analytical range is 10 to 400 mg/dl. False positives using GC have been reported with acetonitrile poisoning (Jones et al., 1992).

DISCUSSION

Drugs which may increase the plasma LDH, include anaesthetics, aspirin, clofibrate, fluorides, mithramycin, narcotics and procainamide. Ten years ago, a case report published autopsy LDH values in one case of 6,419 IU/L (ref 191-428) without stating the site of sampling (Kubo et al., (2001). Really high antemortem levels of LDH have been reported in pulmonary and disseminated toxoplasmosis, 8 cases of 9 had AIDS with levels ranging from 2,868 to 16,000 IU/L, and a median value of 10,222 (Pugin et al., 1992). False positive alcohols appear not to occur when the method eliminated LDH activity by protein precipitation as occurs with the EMIT-11 Plus® ethyl alcohol assay (Levy et al., 2000). Elevated LDH values were reported in post-mortem samples and in end stage liver and kidney disease (Nine et al., 1995). False positive ethanol results were found in post-mortem samples from infants with LDH values of 2,800 IU/L or greater with blood alcohol results less than 10 mg/dl (Badcock and O'Reilly, 1993), and his statement was published "many forensic and clinical laboratories use this blood alcohol concentration value as a cut-off concentration" (Winek et al., 2004). Given the limits of analyte detection in clinical laboratories, this is simply conventional. The intracellular concentration of LDH is about 160 fold greater than in plasma. With haemolysis, for each 1 g/L of haemoglobin, the concentration of LDH was increased by

670 IU/L (Kroll and Elim, 1994). Haemolyse all blood cellular components in normal people and the ranges of post-mortem values of LDH in blood are partly predictable. At 140 g/L of haemoglobin in normal male, total haemoglobin derived LDH at complete haemolysis would be about 93,800 IU/L. Add a moiety for normal or elevated pre-haemolysis serum values, white cell lysis and necrosis, and the levels reported here are predictable. High levels in these data show progressively higher false positive alcohol values by enzyme assay. The lactate values at post-mortem were un-assayed, but will be higher than the normal living values. Post-mortem LDH values are likely to be associated with higher lactate values and tend to give greater false positive alcohol values.

Blood alcohol produced by post-mortem decomposition rarely exceeds 50 mg/dl (Winek, 1975). Femoral vein blood is the best sampling point and all blood alcohols less than 10 mg/dl should be reported as negative. This is close to the limit of detection of head-space GC methods. False negatives may occur in putrefying corpses due to the presence of the putrefactive bases cadaverine, putrecine and phenethylamine (Richardson, 2000).

In any contentious case, comparing ethanol concentrations in cardiac and femoral vein blood, urine and vitreous humour is virtually essential to ensure reaching a correct diagnosis on the alcohol status of the person at death (Kugelberg and Jones, 2007), which is not the issue here.

Conclusions

Blood ethanol measured by alcohol dehydrogenase may have a role as a rule-out for blood alcohols at non-contentious post-mortem examinations in association with the autoanalyser analysis of drugs of abuse. Same day turnaround at the local hospital laboratory would speed the pathologists report and facilitate an early decision from the coroner. The grieving process by relatives would be facilitated.

LDH driven NAD+ oxidation will cause false positive alcohol values with the coupled reaction, and the elevated LDH levels in post-mortem specimens are likely to result in false positive ethanol values. All positive alcohols greater than 10 mg/dl should be analysed by GC.

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