# The Stability of Urine for the Forensic Analysis of Samples in Alleged Driving-Under-the-Influence-of-Alcohol Cases – a Review and Case Report

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## Summary

*We report on the storage stability of urine as a body fluid for forensic investigation of driving under the influence of alcohol (DUI) prompted by a recent court case in which the urine sample was not properly preserved. Studies in this area are fewer than those for the stability of blood and were designed to address two separate problems:*

*a) suitable preservation and/or storage conditions for forensic urine samples and whether or not alcohol production in vitro post sampling could explain all or most of the analytical findings*
*or*
*b) to set clinical cut-off values for investigation and diagnosis of pathologies of carbohydrate metabolism*

*The accepted conclusions to the first set of questions are that any well-regulated drink-driving procedure must rely on adequate chemical preservation of the urine sample to preserve its integrity. A chilled (≤ 4ºC) or frozen custody chain for urine samples would also suffice but must be evidentially demonstrable by suitable temperature monitoring records.*

*For the second question regarding the glucose content of normal urine the clinical cut-off values are conservative to ensure adequate patient care and are not suitable for forensic purposes. In view of the data found in our review it would seem prudent to assume that a random urine sample from a healthy subject could have a glucose content in the upper part of the published ranges, say 60 mg/100mL, which could, if fully converted, contribute 30 mg/100mL alcohol. Thus, for a urine sample without preservative or proven chilled (≤ 4ºC) storage an alcohol result of up to 30 mg/100mL above a statutory urine limit could be unsafe to sustain a conviction.*

*The importance of the correct sampling procedure and sample temperature monitoring records should be the subject of adequate regular refresher training for any police officer authorized to take urine samples.*

# Introduction

The pathophysiology of alcohol (ethanol) and driving were briefly reviewed in our previous publication on the stability of alcohol in forensic blood samples, a study that arose from a case in which expert evidence was given by MJ Walker[1]. The subject of the storage stability of urine as a body fluid for forensic examination to test for driving under the influence of alcohol (DUI) has been less studied than that for blood.

This question arose in recent case work in the Northern Ireland courts. The circumstances were in brief thus. A driver was arrested on suspicion of DUI. An evidential breath test gave a lower of two readings of 44 µg/100mL and the statutory option available at the time under the Road Traffic (Northern Ireland) Order 1995, (the Order), Art. 19 (2) was offered and accepted by the defendant. It is for the arresting officer to decide (Art. 18 (5) of the Order), whether the option should be (subject to medical advice (Art. 18 (5A) of the Order) a blood, or a urine sample. The constable chose to require a sample of urine.

The urine sample was properly taken (two successive urinary voids of which the first was discarded, (as required by Art. 18 (6) of the Order) but was improperly stored immediately afterwards. It should have been decanted into a vial preloaded with fluoride preservative. The urine was instead decanted into the securitainer (a plastic pot with a tamper evident seal) which is not designed to store urine but rather the sample vial containing the urine. The sample was retained by the police in a secure fridge and transported 41 days later to the forensic science laboratory which reported an alcohol content of not less than 125 mg/100mL against the prescribed limit of 107 mg/100mL (Art. 13(2c) of the Order).

The authors were consulted by solicitors acting for the defendant on the forensic suitability of an unpreserved urine sample. The factors that had to be considered were:

a) the probability of the urine sample having been adequately preserved against chemical and microbiological deterioration
b) the likelihood of chemical or microbiological contamination of the urine sample
c) the impact of any such contamination on the alcohol concentration in the sample
d) the storage of the sample

In the event, the production of alcohol by microbiological fermentation of a substrate such as glucose became a prime consideration hence a further question arose:

e) what is the likely typical glucose concentration in “normal” urine?

When the case came on for hearing the experts for both prosecution and defence agreed that on the available evidence the urine sample had not been adequately preserved. The possibility of microbiological contamination was not challenged. In the event, it was accepted that the sample had been kept in a refrigerator but until receipt at the forensic science laboratory no records of temperature monitoring of the sample storage were apparent. Therefore, the literature was explored to be able to answer the questions raised by factors (c) and (e) above.

# Literature Review

The literature was reviewed between 1925 and 2020 with the aid of SciFinder, Web of Science and Google® Scholar for peer reviewed papers using appropriate search terms. Detailed evaluation of full papers followed review of relevant abstracts.

# Urine as a Forensic Body Fluid for Alcohol Assay

As the effects on the central nervous system depend on the concentration of alcohol delivered to the brain via the bloodstream the primary datum of interest is the blood alcohol concentration. The relationship between blood and urine ethanol concentrations were the subject of early studies by Haggard *et al* in 1940[2]. These authors noted urine collects in the bladder over time thus its alcohol concentration is not an exact proxy for that in the blood at a given time as it represents a composite average and may reflect a blood alcohol concentration at some indeterminate previous time. Their solution was to require the subject to empty the bladder but to discard this void and to base the evidential datum on a second void some 30 minutes later. This approach was confirmed by, for example, Payne *et al* in 1966[3] and entered into UK law in 1967[4]. In 1985 Biasotti and Valentine[5] reviewed historical, physiological and practical aspects of urine samples for alcohol in driving-under-the-influence investigations concluding that urine is a reliable and accurate alternative to blood if the two-void process is followed. They state with references but without discussion that urine samples can be preserved for a reasonable time without appreciable loss of alcohol. An extensive discussion of urine:blood ratios for alcohol was offered confirming the ratio of 1.3:1, originally advocated by Haggard *et al*[2], as appropriate in most instances although a potential variation up to 1.5:1 could apply[6] in some cases.

# Preservation of Forensic Urine Samples for Alcohol

That sampling and sample handling may have a substantial impact on the quality and reliability of subsequent analytical results is well known, as is the need for a proper chain of custody. The measured concentration of alcohol in urine may be suspect because of the possibility that alcohol may be produced if the urine contains sugars and is infected with microorganisms, specifically yeasts but some bacteria are also capable of fermenting sugars. The issue is familiar as a *postmortem* artefact[6] but of more relevance to the present study is alcohol produced *in vitro* after voiding urine.

Hayden *et al*[7] showed in 1977 that on re-analysing blood and urine samples for alcohol after varying periods and conditions of storage the stability of alcohol in the samples was adequate for the provisions of the Irish Road Traffic Act, 1968 which allowed for re-analysis in certain circumstances.

In 1988 Neuteboom and Zweipfenning[8] reported that at -10°C and with the addition of sodium fluoride (1%w/v), urine samples are stable for at least 12 months prior to analysis. In 1991 Kadehijian[9] reviewed urine as a forensic fluid and argued that as the physiology of alcohol elimination was by then well understood urine was a reliable sample after the urine absorption peak has been reached. It was further argued that alcohol production in urine samples is unlikely in the general population as it requires the presence of glucose and organisms capable of fermenting that substrate and more than 24 hours storage at room temperature. However, in 1993 Saady *et al*[10] found that after room temperature storage (1-21 days) of 14 initially alcohol-negative (<10 mg/100mL) urine samples five produced

alcohol (36-2327 mg/100mL) and contained glucose and yeasts. Overall ($n$ =14) the glucose contents were 0 to ≥2000 mg/100mL but six glucose-positive samples did not contain yeasts and did not evolve alcohol. In 1993 Lough and Fehn[11] reported in summary that no alcohol was produced in urine samples stored at room temperature except when supplemented with glucose, *Candida albicans*, or both. However, addition of 1% sodium fluoride, commonly used in forensic specimens such as blood at varying concentrations, completely eliminated microbial fermentation. However, in a more detailed consideration these authors found

> "…*The results of untreated urine from normal healthy subjects show a small degree of ethanol production; mean of 3 mg per 100 mL, within a range of 0 to 14 mg per 100 mL*"

They also assert

> "… *a lack of ethanol production in any of the unpreserved urine samples indicates that false DUI convictions due to endogenous ethanol production are very unlikely*"

It is, on the face of it, curious that Lough and Fehn refer to a lack of ethanol production in unpreserved urine when their own data demonstrate up to 14 mg/100mL alcohol evolved on storage. Clearly what the authors aim to refute is a defence in which substantial excess alcohol production on poor storage is claimed to explain the entirety of the alcohol found in the sample.

In 1995 Sulkowski *et al*[12] demonstrated a glucose substrate in urine could be fermented *in vitro* to alcohol by multiple *Candida* species and three bacterial species (*Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis*) although *Enterococus sp*, *Staphylococcus spp.* other than *S aureus*, and *Pseudomonas aeruginosa* were not implicated. Alcohol production could be inhibited by storage at 0°C or inclusion of about 1% sodium fluoride.

In a definitive study Jones[13] showed in 1999 that production of alcohol in urine samples by *C albicans* was stopped completely by adding 1% or 2% (w/v) sodium fluoride but not by concentrations of 0.75% (w/v) or less. Moreover, Jones also showed that storage of urine samples in a refrigerator at 4°C was equally effective in preventing alcohol production *in vitro*.

In 2007 Mandić-Radić *et al*[14] emphasised proper collection, handling and storage of the blood or urine specimens as being essential to the evidential probity of the resulting data and reported loss of alcohol from 15 samples of urine stored at 4°C for >1 month.

# The Glucose Content of Urine from Healthy Subjects

Since microbial contamination in taking a urine sample is unavoidable a critical consideration is the extent to which urine from healthy subjects contains glucose. The early studies of glucose in urine were unreliable due to the non-specific methods in use[15], however its determination by glucose oxidase has, since the 1950's, provided a specific and reliable method[16]

For 164 samples of urine from normal patients (100 children, 27 adults) Apthorp[17] reported in 1957 that all contained glucose and other sugars, except sucrose and galactose. The glucose ranged from 1 to 12

mg/100mL. In patients with renal or hepatic disease, although study numbers were small, some instances of glucose over 12 mg/100mL were found. Patients with a gross glycosuria of known cause were excluded.

In an extensive study, Fine[18] in 1965 applied a glucose oxidase method with 80% average recovery of added glucose. Of the 700 volunteers studied (163 F, 537 M) the data exhibited two groups. The major group of glucose data, 91% of the population studied, appeared approximately normally distributed with a mean of 6 mg/100mL and standard deviation of 3.2 mg/100mL. Urine samples containing glucose over 16 mg/100mL, 9% of the data, exhibit no characteristic pattern and Fine observed no very sharp demarcation between the two groups. The overall glucose range was between 0.2 mg/100mL and 9328 mg/100mL. Oral administration of 50g of glucose in a small number of subjects ($n$=8) showed a slight increase in glucose excretion. Male subjects had a higher percentage of urine samples with raised glucose (12.1% against 3.7% females) and showed an age-related increase in the number with urine glucose contents above 16 mg/100mL, rising to 23% in the age range 60-70. Thus, a random sample for a healthy male aged 60-70 could contain over 16mg/100mL glucose in urine. Fine investigated any subjects with urine glucose levels over 15 mg/100mL wherever possible by blood-sugar assay, which suggested in most cases undiagnosed diabetics or other glycosuria pathologies. In 1965 Bernard and Ginsburg[19] concurred with Fine and reported briefly that over 90% of apparently normal adults have a mean urinary glucose content of 7 mg/100mL.

In 1982 Gupta *et al*[20] investigated by proficiency testing the reliability of routine clinical urine glucose analyses and went on[21] to suggest differences in literature values for the upper limits of normal urine glucose are probably due to different analytical methods. Applying an *o*-toluidine procedure to urines from 261 healthy subjects (180 M, 81 F) Gupta *et al*[21] found in 1982 an apparently nearly normal distribution with mean of 36.5 mg/100mL and SD of 12.1 mg/100mL. The normal range (mean ± 2SD) rounded to the nearest unit was 12-61 mg/100mL covering 94% of the data. The subjects were aged 1-70 years, none were galactosemic and the females were not pregnant or lactating. For older men Gupta *et* al found a urine-glucose range of 5-35 mg/100mL. In 1986 Bitzén and Scherstén[22] recommended further diagnostic tests for possible diabetes are required above a urine glucose concentration of 1.1 mmol/L (20 mg/100mL)[23].

In 1999 Jones *et al*[24] rehearsed Fine's data (1-15 mg/100mL glucose, 90% of samples) but went on to state that a random sample of urine might contain up to 30 mg/100mL glucose citing a standard text of the day[25]. The 2015 edition of the same textbook[26], collating reference intervals, which

> "*… attempt to describe the typical results found in a defined population of apparently healthy people*"

gives 1-15 mg/100mL for urine glucose.

In 2006 Jones[27] published a general review of urine as a biological specimen for forensic analysis of alcohol and restated Fine's data of 1-15 mg/100mL for urine glucose in healthy individuals concluding that only trace amounts are to be expected as glucose is almost totally reabsorbed into the blood in the renal tubules.

## Diabetes

Many studies focus on patients with diabetes[28-31] for whom glucose in the urine is very probable and can be as high as >2000 mg/100mL.

## Laboratory Considerations

Analysis for fluoride in a urine sample is readily carried out by means of a fluoride ion selective electrode after dilution of an aliquot of the sample[32]. If the *in vitro* synthesis of alcohol is considered to be a post-sampling artefact two sets of laboratory analyses can be carried out with the second performed after the sample or a portion of it has been left at room temperature for several days[33]. Absence of an increase in the alcohol concentration would rule out alcohol synthesis in the laboratory.

The ratio of 5-hydroxytryptophol (5HTOL) to 5-hydroxyindole-3-acetic acid (5HIAA) in urine provides a useful method to distinguish between ethanol that might have been synthesized *postmortem*, or generated *in vitro*, from ethanol excreted in urine as a result of drinking. The 5HTOL: 5HIAA ratio is altered during the metabolism of alcohol and remains elevated in urine for several hours after systemic alcohol is no longer detectable[34]. Urine ethyl-glucuronide (ETG) and ethyl sulphate (ETS) may also be useful biomarkers. ETG arises from the metabolism of alcohol by conjugation with glucuronic acid and remains detectable in urine for several days after alcohol consumption has ceased and when systemic alcohol is no longer present. ETG may, however, also arise from microbiological fermentation. ETS also arises from the human metabolism of alcohol and is similarly detectable in urine when systemic alcohol is no longer detectable[35]. In 2018 Foley[36] describes a case in which, by ETG and ETS data, it was accepted that alcohol in the urine sample arose by post-sampling microbiological fermentation.

## The Case Concludes

The instant case was listed before a District Judge in the Magistrate's court. Expert reports and witness statements were exchanged. The defendant is not diabetic however based on the above literature it was argued that normal urine generally contains some glucose, and that a random specimen of urine might contain sufficient glucose, if fully fermented, to jeopardize the safety of a conviction. The judge adjourned the case indicating that he required oral evidence from the police property officer on the storage conditions of the sample. In the meantime, the COVID-19 pandemic supervened, and it appears the defendant accepted responsibility for a lesser charge which did not result in the loss of his driving license and the matter did not come to trial.

## Conclusions

It is important to recall that none of the studies cited herein were designed to address the situation of a case of improper storage or preservation leading to a small increase in alcohol in the urine. Instead, they were for two separate reasons:

*a) to assess suitable preservation and/or storage conditions for forensic urine samples and whether or not alcohol production in vitro post sampling could explain all or most of the analytical findings*
*or*
*b) to set clinical cut-off values for investigation and diagnosis of pathologies of carbohydrate metabolism*

The accepted conclusions to the first set of questions are that any well-regulated drink driving procedure must rely on adequate chemical preservation of the urine sample to preserve its integrity. A chilled (≤ 4ºC) or frozen custody chain for urine samples would also suffice but must be evidentially demonstrable by suitable temperature monitoring records.

Blood samples must be similarly preserved and in addition chilled[1]. It is interesting to note a recent recall that occurred in the US; in 2019 a voluntary nationwide recall of a specific lot of blood collection tubes was instigated when a small portion were found to lack the required chemical preservatives (sodium fluoride and anticoagulant potassium oxalate). Rodda *et al*[37] have discussed the scientific and legal implications.

For the second question, the glucose content of normal urine, clinical cut-off values are conservative to ensure adequate patient care and are not suitable for forensic purposes. The data due to Fine[18] and Gupta[22] remain persuasive. Correcting Fine's data for the 80% recovery noted in his paper suggests that the data are approximately normally distributed in the lower range of the dataset imply 1-19 mg/100mL urine glucose for most urine samples with a long upper tail of higher values for 9% of the data. Gupta's data confirm a higher range of values is possible with 94% of the data covered by 12-61 mg/100mL urine glucose. It would seem prudent therefore to assume that a random urine sample could have a glucose content in the upper part of the published ranges, say 60mg/100mL, which could, if fully converted, contribute 30 mg/100mL alcohol (One mole of glucose (MW 180), fully fermented, produces two moles of ethanol (MW 46). Thus 60 mg/100mL could produce up to 60x2x(46/180) mg/100mL of ethanol = 30.7 mg/100ml). Thus, urine results in a sample without preservative or proven chilled (≤ 4ºC) storage of up to 30 mg/100mL above the statutory limit would be unsafe to convict on.

Fundamentally, any well-regulated drink driving procedure must rely on adequate chemical preservation of the sample (urine or blood) to preserve sample integrity rather than a chilled or frozen custody chain. To do otherwise runs counter to well-accepted scientific studies and long-established practice. Taking a urine specimen in the investigation of DUI has become an increasingly rare occurrence. Thus, the importance of the correct sampling procedure and suitable temperature monitoring of the stored sample should be the subject of adequate regular refresher training for any officer authorized for urine sampling or storage in the investigation of DUI.

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