Rising CO_2 – identifying the cause of a recuring Jaffé creatinine assay failure

Craig McKibbin, Lowri Rooke, Dylan Jones
Glangwili General Hospital, Hywel Dda University Health Board
Dolgwili Road, Carmarthen SA31 2AF

Abstract

The Jaffé creatinine method is susceptible to various exogenous and endogenous interferents but remains the method of choice for nearly half of clinical laboratories in the UK. Here we describe a largely forgotten cause of analytical interference with clinical significance for patient results – increased atmospheric carbon dioxide levels.

Trouble-shooting investigations into a repeated intermittent internal quality control (IQC) failure of the Roche Jaffé creatinine assay at Glangwili General Hospital over several months led to the discovery that events coincided with frozen IQC material deliveries to the laboratory. Staff were in the practice of disposing of solid CO_2 pellets in the metal sink in a room housing the clinical chemistry automation. It was hypothesised that the increased concentration of atmospheric carbon dioxide, dissolved in the analyser water supply, lowered the Jaffé reaction pH leading to a decreased reaction rate and/or absorbance intensity of the alkaline creatinine picrate complex.

Repeat analysis of patient specimens following return of the assay to acceptable IQC performance revealed a maximum creatinine deviation of -64 %, developing within 10 minutes on one occasion, at the assumed peak of atmospheric CO_2 accumulation.

Although the laboratory did not have CO_2 monitors installed during this period, the probable cause was effectively confirmed several months later when a member of staff again sublimated dry ice in this sink, resulting in a recurrence of the analytical failure.

The Jaffé creatinine method

The Jaffé method for the estimation of creatinine level was originally described by Folin in 1914 [1] and, with various modifications, is still used by over 40 % of clinical laboratories in the UK participating in the WeQas EQA scheme. In the Jaffé reaction creatinine in a plasma or serum specimen forms a yellow-orange complex with alkaline picrate, the rate of its development being proportional to the concentration of creatinine in solution.

Creatinine + picric acid —*Alkaline pH*→ yellow-orange complex

The Jaffé method is considerably cheaper than enzymatic alternatives for this high volume test. However the reaction is susceptible to analytical interference from a range of endogenous and exogenous compounds (see box below [2]).

Positive interferences:

- Acetoacetate, acetone, ascorbic acid, fructose, glucose, pyruvate, uric acid, drugs such as cephalosporins, flucytosine, levodopa, methyldopa, nitrofurantoin, piperacillin

Negative interferences:

- bilirubin, haemoglobin, lipaemia, dipyrone, N-acetylcysteine

The Roche Cobas c501 Jaffé creatinine generation 2 assay employs a kinetic colorimetric method with rate-blanking for serum bilirubin and a -26 µmol/L absolute correction for serum/plasma pseudo-creatinine chromogens.

Background

During late 2018 to 2019 the Biochemistry department at Glangwili General Hospital experienced a recurring intermittent failure of internal quality control (IQC) ranges for the Roche c501 Jaffé generation 2 serum creatinine and serum bicarbonate assays. Patient results released between scheduled IQC events were also affected by the problem, resulting in significantly lower serum creatinine results being generated.

Analytical issues were typically only identified when in-patient results failed the LIMS delta-check rule during authorisation, with the majority of erroneous results reporting to the user undetected.

Trouble-shooting investigations and preventative measures for this recurring analytical issue involved assessment of the water supply (as both analysers were affected and culminating in early replacement of the old purification system), increased IQC frequency and a trial of running patient specimens in duplicate for short periods in an attempt to identify discrepancies, and an investigation by Roche. Eventually it was identified that increased levels of atmospheric CO_2 may interfere with the Jaffé creatinine assay.

At this point a connection was made between creatinine assay failures on at least 5 separate occasions and scheduled reagent deliveries packaged on dry ice received on the same day.

Hypothesis

The Blood Sciences laboratory at Glangwili Hospital is awaiting modernisation, having not benefited from material refurbishment since its opening in 1949. The laboratory comprises several small rooms with one of the largest and better ventilated containing the biochemistry automation.

Staff had been in the practice of using a metal sink installed in this room to sublimate dry ice pellets received in frozen reagent and consumables deliveries. It was suspected that this practice was responsible for raising the atmospheric CO_2 level sufficiently to influence the measurement of creatinine.

It is known that variation of the pH of the reaction mixture by altering the concentration of sodium hydroxide or by the use of buffer solutions has a profound effect on the rate of formation and intensity of the colour due to alkaline creatinine picrate [3]. The below figure is reproduced from Cook, 1975.

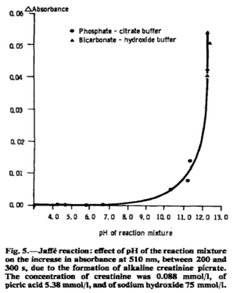
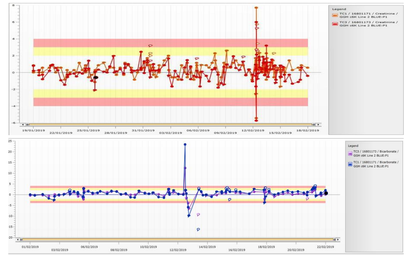


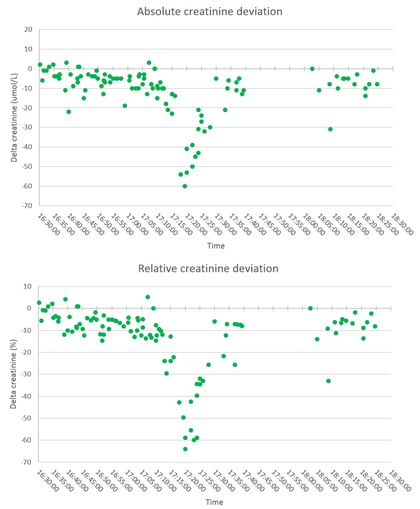
Fig. 5.—Jaffé reaction: effect of pH of the reaction mixture on the increase in absorbance at 510 nm, between 200 and 300 s, due to the formation of alkaline creatinine picrate. The concentration of creatinine was 0.088 mmol/l, of picric acid 5.38 mmol/l, and of sodium hydroxide 75 mmol/l.

It was hypothesised that the reduction in Jaffé reaction rate brought about by partial acidification of the water supply or uncapped reagents by dissolution of CO_2 at higher atmospheric concentrations resulted in a lower level of absorbance reached in the assay's kinetic time window.

Quantitation of the effect

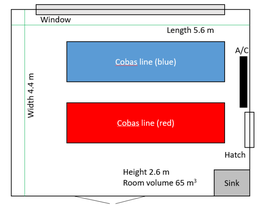


Levy-Jennings IQC plots for serum creatinine and serum bicarbonate on one occasion

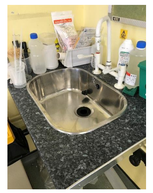


Interference time course on one occasion to illustrate the rate of interference onset and magnitude of the deviation under the particular laboratory conditions

Laboratory environment



Room plan



Laboratory sink

Outcome

To avoid this environmental effect a separate area of the laboratory building was found to safely dispose of dry ice. Unfortunately an opportunity presented itself to test the hypothesis several months later when a member of staff once again sublimated dry ice in the automation room, leading to the expected failure of the Jaffé creatinine assay.

Discussion points

- A literature search yields few results for atmospheric CO_2 as an interferent and it is not listed in the Roche creatinine Jaffé kit insert as a source of interference.
- The magnitude of the effect will be dependent on many factors including amount of dry ice sublimated, ventilation and room volume. However in this real world example a maximum interference of -64 % was detected in the Jaffé creatinine assay developing within 10 minutes and resolving within 20 minutes.
- The falsely low creatinine results generated during these periods posed a clinical risk due to the potential for missed acute kidney injury and incorrect dosage of renally cleared medications in the acute setting. Implementation of delta-checks in result validation middleware may have helped identify the assay problem. Specimens run between acceptable IQC events were reanalysed and amended reports issued for result deviations outside expected combined biological and analytical variation.
- CO_2 is a colourless odourless gas that can lead to asphyxiation at high concentrations and is denser than air, causing the gas to accumulate in confined spaces, particularly at ground level.
- A CO_2 monitor would have been useful in identifying exposure and also in confirming atmospheric CO_2 levels had returned to normal prior to assay recalibration to avoid over-correction in patient results when CO_2 eventually dissipated.
- Not having the ability to quantify the atmospheric CO_2 concentration is a limitation of this study.

Take-home message

- Be aware that atmospheric CO_2 produced from sublimation of dry ice in the laboratory environment is capable of clinically significant negative interference in the Jaffé creatinine method
- Consider acquiring a CO_2 monitor if it is not possible to eliminate exposure.

References

1. Folin O. On the determination of creatinine and creatine in urine. *J Biol Chem* 1914; 17:469
2. Tietz Clinical Guide to Laboratory Tests, 4th edition (2006)
3. Cook JGH. Factors influencing the assay of creatinine. *Ann Clin Biochem* 1975; 12:219-232.