A literature review regarding the verification of creatinine and urea produced by local point-of-care devices compared to an in-house analytical platform.

Lauryn Bailey

TABLE OF CONTENTS

1. ABSTRACT

Point-of-care testing (POCT) is an integral part of urgent care as it allows for quick and informed decisions to be made regarding the care of a patient. This can greatly impact patient care by ensuring patients receive vital treatment as quickly as possible. It allows for a better flow of patients through departments by assessing their situations rapidly resulting in a better use of NHS resources. Although there are many benefits to POCT, there are only a small number of tests that are designed to be run on these analysers, and not all these tests are verified on every instrument. The POCT analysers at Queen Alexandra Hospital, of Portsmouth Hospitals University Trust, are an example of this. Despite evidence supporting the importance of creatinine and urea tests in urgent care, the three POCT analysers in the emergency department are not verified to complete these tests which means a patient sample must be sent to the in-house laboratory for testing. This significantly increases the wait time for results and requires more patient sample. It could be argued that the verification of creatinine and urea on the POCT instruments could benefit patient care. To do this, results from POCT analysers can be compared to the in-house chemistry analysers to determine if there is a significant difference and if the POCT analyzers are fit to run these tests. In this example, Radiometer ABL90 Flex Plus POCT analyzers are to be compared with Roche's COBAS c702.

Keywords: creatinine, urea, point-of-care, verification, comparison, urgent care.

Word Count: 250

A literature review regarding the verification of creatinine and urea produced by local point-of-care devices compared to an in-house analytical platform.

2. INTRODUCTION

Kidney disease is a growing concern due to rising cases and incidents in urgent care (Bagshaw *et al.*, 2007). Serum creatinine and urea concentrations can be an indicator of renal function and therefore can be used to determine the severity of a patient's illness as well as how they should be treated (Gounden *et al.*, 2022). The introduction of creatinine and urea to point-of-care testing (POCT) could be highly beneficial to patient care by allowing for quicker results, however, this method is not currently verified in many hospitals meaning that testing is required to be undertaken in a biochemistry laboratory. This literature review aims to assess the importance of creatinine and urea testing and the benefits of POCT as well as explore current verification comparison studies in this area and how POCT can be used in the future.

3. CREATININE AND UREA

3.1. SYNTHESIS

Creatinine and urea can both be described as non-protein nitrogenous waste products, with urea accounting for approximately 80-90% of these (Salazar, 2014). The excretion of these waste products is mainly controlled by the kidneys (Gounden *et al*., 2022).

Creatinine is the product of the spontaneous conversion of creatine and phosphocreatine (Da Silver *et al*., 2019). Creatine is synthesised in the liver, kidney, and pancreas and is due to the transamination of amino acids (Salazar, 2014). The amino acids involved in this are arginine,

methionine, and glycine. The enzyme L-arginine-glycine amidinotransferase (AGAT) produces guanidinoacetate acid (GAA). The enzyme guanidinoacetate methyltransferase (GAMT) produces creatine by the methylation of GAA (Silver *et al*., 2019). Phosphorylation then converts the creatine into phosphocreatine. This mainly occurs in the skeletal muscle and the brain (Salazar, 2014). This is where creatinine is spontaneously synthesised.

The urea cycle is described by Barmore *et al.* (2022). It takes place in the hepatocyte cells of the liver and begins with the synthesis of N-acetyl-glutamate (NAG) in the mitochondria. NAG is formed from glutamate and acetyl-CoA via the NAG synthase enzyme. NAG is an activator that is required for the use of the enzyme carbamoyl phosphate synthetase (CPS I). CPS I is used to form carbamoyl phosphate from CO and ammonia. Carbamoyl phosphate is then used alongside ornithine to create citrulline using the enzyme ornithine transcarbamoylase (OTC). Ornithine translocase then transports citrulline to the cytoplasm where an active reaction with aspartate takes place to form argininosuccinate. This uses the enzyme argininosuccinate synthetase. Argininosuccinate lyase converts this into arginine which then undergoes hydrolysis using arginase. This then forms urea and ornithine. Most of the urea is excreted through the kidneys however some is excreted through the gastrointestinal tract (Gounden *et al.*, 2022).

3.2. ACUTE RENAL FAILURE

Acute renal failure is described by Van Biesen *et al.* (2006) as a rapid and often reversible decreased glomerular filtration rate (GFR). It is stated that this can occur alongside renal disease but can also arise in people with a previously normal GFR.

There are three described categories of acute renal failure causes. These are prerenal, postrenal, and renal. The prerenal cause is when there is a reduction in GFR caused by decreased renal perfusion. This leads to a rise in serum creatine and urea levels; however, it is reversible. An obstruction of the urinary collect system causes postrenal acute renal failure. The renal form occurs when the internal structures of the nephron are impacted. These include the tubules, vessels, interstitium, and glomeruli (Kamal, 2014).

Renal function tests can be utilised to aid the diagnosis of acute renal failure and assess how well the kidneys are performing (Gounden *et al.*, 2022). As well as this, renal function tests can be used to monitor the progression of kidney disease and how well the kidneys respond to certain treatments. Kidney function can be indicated by testing levels of urea and creatinine in serum. Studies have supported this by showing the relationship of these analytes with the occurrence of decreased renal function. An increase in urea and creatinine serum levels can indicate reduced renal function (Kamal, 2014).

Creatinine is mainly synthesised in skeletal muscle. Therefore, a patient's muscle mass can impact their serum creatinine results. This means that creatinine tests alone may not be a suitable indicator for people with reduced muscle mass such as in cases of amputees, those suffering from muscle diseases, and those who have experienced lengthy starvation (Kamal, 2014). Creatinine levels can also be impacted by other factors that affect muscle mass such as age, gender, and diet. It has been shown that consuming red meat can cause creatinine level changes of up to 30% (Gounden *et al.*, 2022).

Some factors impact urea levels in serum. The main factor is diet. Due to urea being a by-product of protein metabolism, a high-protein diet could cause an increased level of urea. A patient's hydration status and any previous issues they may have with protein synthesis may also impact urea results (Salazar, 2014).

3.3. GFR

In acute and chronic renal failure there is a decrease in GFR which causes a build-up of urea and creatinine in the blood (Amin *et al.*, 2014). Due to the affecting factors of urea, it is not recommended to use this analyte alone to assess renal function. Creatinine is a stronger indicator since it is not as easily impacted (Salazar, 2014).

Assessing GFR is an integral part of patient care because it allows for an accurate determination of kidney function and therefore assesses the likelihood of kidney disease. It can also be used to aid drug dosing and monitoring kidney disease that has already been diagnosed (Kamal, 2014).

The level of GFR can indicate how far renal disease has progressed. It is often split into 5 stages with stage 5 being end stage renal disease. Stage one is where the GFR result is normal (>90mL/min/1.73m^2). Stage 2 is when the GFR has slightly decreased (60-89mL/min/1.73m^2). In stage 3, the GFR has moderately decreased to 39-59mL/min/1.73m^2. Stage 4 is where the GFR is between 15-29mL/min/1.73m^2, indicating a severe reduction in GFR. Stage 5 is renal failure which is shown by a GFR of <15mL/min/1.73m^2 (Mula-Abed *et al.*, 2012).

4. POINT-OF-CARE TESTING

4.1. OVERVIEW

Point-of-care testing is when a test is undertaken at the same location as the patient rather than sending the sample somewhere else for analysis. One benefit of this is shorter turn-around times for results. This is because POCT instruments often have a reduced measurement time and testing at the same location also irradicates any delays due to sample transport (Luppa *et al.*, 2011). Because of this, clinical decisions can be made sooner including treatment options for the patient or further testing.

One study has shown how using POCT can be cost-efficient. The study used POCT instruments to test ventilated paediatric patients prior to transporting them by air to a specialised hospital to evaluate whether it could be used to save time and money for the referring hospital. It was determined that over $4000 was saved by shortening wait times due to not having to spend as much on factors such as paramedic overtime, and aircraft waiting charges (Macnab *et al.*, 2003).

Verifying tests for creatinine and urea could be beneficial to POCT because they can be used to assess renal function. Bagshaw & Gibney (2008) state that testing for acute kidney injury (AKI) should be a priority for patients in urgent care due to its severity and rising cases. It is also described that investigations have shown that mortality following a diagnosis of AKI has only slightly increased in recent years although the prevalence is rapidly growing (Bagshaw & Gibney, 2007).

Renal function tests such as creatinine and urea are essential to evaluating the sequential organ failure assessment (SOFA) score of a patient. This is explained by Klick & Guins (2011). A SOFA score is often used to diagnose and assess the severity of sepsis by looking at the function of coagulation and the respiratory, liver, cardiovascular, renal, and central nervous systems. Renal function tests are required for this to review the condition of the kidneys. It is important to recognise severe sepsis as soon as possible and begin treatment quickly as it has a high mortality rate. Testing creatinine and urea on POCT analysers can aid the quick turnaround of results and therefore impact rapid clinical decisions.

One example of a POCT instrument is the ABL90 Flex Plus which is a blood gas analyser manufactured by Radiometer. It has many benefits including a short 35 second measurement time. It also only requires a small 65µL sample which can be beneficial to patients such as those who must take frequent samples and paediatric patients (Seeger *et al.*, 2011). This analyser also has built-in calibrator and quality control samples and can be programmed to test them at set intervals to assess the accuracy and reliability of the results (Lim *et al.*, 2023). It also features an automatic sampler which may reduce sampling errors and be beneficial to staff training (Salvagno *et al.*, 2019).

4.2. MEASUREMENT PRINCIPLE

The measurement principles for urea and creatinine on the ABL90 Flex Plus are detailed in the instructions for use by Radiometer (2021).

Urea is measured by potentiometry. This is where a voltmeter measures the potential of an electrode chain and applies it to the Nerst equation to determine the concentration.

Creatinine is measured using a 2-sensor amperometry system. One sensor detects only creatine whereas the other can detect creatine and creatinine. This information can be used to deduce the creatinine only result. In this system, an ammeter measures the current of a polarised electrode chain. A solution of creatine and creatinine is passed over the outermost layer of a multilayer membrane which holds the enzymes creatinase, creatininase, and sacrosine oxidase. These enzymes convert the creatine and creatinine molecules into hydrogen peroxide. It is the hydrogen peroxide which converted into a current that can be measured by the analyser.

5. IN-HOUSE TESTING

5.1. OVERVIEW

At Portsmouth Hospitals University Trust, urea and creatinine tests are routinely run on in-house biochemistry analysers. The analyser installed at Queen Alexandra Hospital is manufactured by Roche. Roche is a major producer of clinical biochemistry analysers with over 2000 instruments installed in hospitals across the world. There are a variety of analysers designed to test creatinine and urea such as the cobas c501/c502, however, the Portsmouth site uses the cobas c702 (Jørn Erlandsen & Randers, 2018).

5.2. MEASUREMENT PRINCIPLE

An enzymatic method is used to determine the creatinine content of a serum sample using the cobas c702. It is based on the conversion of creatinine with the use of aiding enzymes to ultimately form quinone imine chromogen. The creatinine concentration is directly proportional to the colour intensity of the formed quinone imine chromogen. The reactions

involved in this conversion are explained by Roche Diagnostics (2019) and are shown in Figure 1.

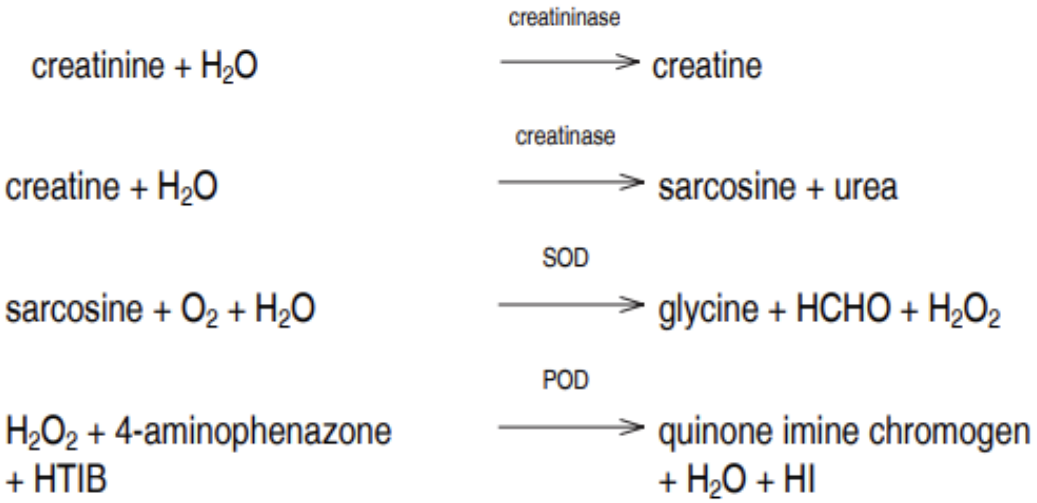

Figure 1: The reactions involved in the conversion of creatinine to quinone imine chromogen as described by Roche Diagnostics (2019).

The method for determining the concentration of urea in serum samples using the cobas c702 begins by hydrolysing urea to create ammonium and carbonate using the enzyme urease. The ammonium then reacts with 2-oxoglutarate with the aid of enzyme glutamate dehydrogenase and coenzyme NADH. This produces L-glutamate. This reaction is shown below in Figure 2.

Figure 2: Reaction of ammonium and 2-oxoglutarate (Roche Diagnostics, 2020).

For every mole of urea hydrolysed, two moles of NADH are oxidised to NAD^+. Urea concentration is directly proportional to the decrease in NADH concentration. This is measured photometrically (Roche Diagnostics, 2020).

6. COMPARISONS

There have already been several documented studies comparing urea and creatinine results between POCT instruments and in-house biochemistry analysers. A recent study by Lim *et al.* (2023) compared the Radiometer ABL90 Flex Plus to four different biochemistry analysers and found that there was no significant difference between test results, therefore deeming results from the ABL90 Flex Plus clinically reliable. Furthermore, it was stated that the Roche c702 showed little to no bias in between results whereas the Siemens ADVIA 1800 showed a slight negative bias, and the Hitachi Sekisui showed a slightly positive bias, however, there was no clinically significant difference.

A similar study by Pizarro Sánchez *et al.* (2020) determined that urea and creatinine results from the ABL90 Flex Plus are interchangeable with results from the biochemistry analysers Dimension Vista 1500, Cobas c702, and Architect c16000. It was concluded that the results had no impact on patient care.

Bargnoux *et al.* (2021) also conducted a study that proved that the ABL90 is suitable for testing creatinine and urea. Additionally, it was demonstrated that this test method was not impacted by haemolysis or icterus.

7. FUTURE

There are many studies that have shown the variety of options for the future of POCT. One example of this is the measurement of Nt-proBNP on the AQT90 FLEX POCT analyser. In this study, results from the POCT instrument were compared to another assay to assess the

correlation between the results. It was determined that there was no significant difference between the results and the results showed a significant correlation to the severity of heart failure (Lepoutre *et al.*, 2013). Goble & Rocafort (2016) explain how the addition of Nt-proBNP to a POCT testing panel can be beneficial to patient care and to the healthcare trust. It is stated that in case of congestive heart failure, it is expected that 50% of patients will be readmitted within 1 year. An early diagnosis of this could lead to earlier treatment and a better outcome for the patient, therefore improving patient care whilst also reducing the costs of patient admission.

Vashist *et al.* (2015) list a variety of analytes that are showing potential to be added to POCT instruments such as markers for strokes and sepsis, immunosuppressant levels for transplant patients, and hormones such as parathyroid. They also address the potential for disposable POCT kits to be used in less developed countries for the diagnosis of sexually transmitted infections and malaria. Furthermore, it is explained how POCT can be used for at-home testing of international normalised ratio for patients on anticoagulants or that have clotting disorders.

8. CONCLUSION

Urea and creatinine serum tests have proven to be integral for assessing a patient's renal function (Gounden *et al.*, 2022). Renal function is integral in the diagnosis of kidney disease and other illnesses such as sepsis (Klick & Guins, 2021). Recent studies have shown that, in many cases, POCT analysers such as the ABL90 Flex Plus have shown no significant difference in creatinine and urea results when compared to verified biochemistry analysers such as the Cobas c702 (Lim *et al.*, 2023). Introducing creatinine and urea to POCT panels could greatly impact the time it takes to recognise and treat kidney disease, therefore improving

patient care (Seeger *et al.*, 2011). This also opens up the idea that POCT can be used for other analytes in the future to improve patient care such as Nt-pro-BNP for the early detection of congestive heart failure (Goble & Rocafort, 2016).

Word count: 2489

9. REFERENCES

- Amin, N.U. *et al.* (2014) "Evaluating Urea and Creatinine Levels in Chronic Renal Failure Pre and Post Dialysis: A Prospective Study," *Journal of Cardiovascular Disease* [Preprint]. Available at: https://www.researchgate.net/profile/Muhammad-Noorulamin/publication/309319865_Evaluating_Urea_and_Creatinine_Levels_in_Chronic_Renal_Failure_Pre_and_Post_Dialysis_A_Prospective_Study/links/581495be08aeb720f68477e0/Evaluating-Urea-and-Creatinine-Levels-in-Chronic-Renal-Failure-Pre-and-Post-Dialysis-A-Prospective-Study.pdf (Accessed: April 19, 2023).
- Bagshaw, S.M., George, C. and Bellomo, R. (2007) "Changes in the incidence and outcome for early acute kidney injury in a cohort of Australian Intensive Care Units," *Critical Care*, 11(3). Available at: https://doi.org/10.1186/cc5949.
- Bagshaw, S.M. and Gibney, R.T. (2008) "Conventional markers of kidney function," *Critical Care Medicine*, 36(Suppl). Available at: https://doi.org/10.1097/ccm.0b013e318168c613.
- Bargnoux, A.-S. *et al.* (2021) "Evaluation of a new point-of-care testing for creatinine and urea measurement," *Scandinavian Journal of Clinical and Laboratory Investigation*, 81(4), pp. 290–297. Available at: https://doi.org/10.1080/00365513.2021.1914344.
- Barmore, W., Azad, F. and Stone, W.L. (2022) *Physiology, Urea Cycle*. Treasure Island, Florida: StatPearls. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513323/#_NBK513323_pubdet_ (Accessed: April 23, 2023).
- Da Silva, R.P. *et al.* (2009) "Creatine synthesis: Hepatic metabolism of guanidinoacetate and creatine in the rat in vitro and in vivo," *American Journal of Physiology-Endocrinology and Metabolism*, 296(2). Available at: https://doi.org/10.1152/ajpendo.90547.2008.

- Goble, J.A. and Rocafort, P.T. (2016) "Point-of-care testing," *Journal of Pharmacy Practice*, 30(2), pp. 229–237. Available at: https://doi.org/10.1177/0897190015587696.
- Gounden, V., Bhatt, H. and Jialal, I. (2022) *Renal Function Tests*, *National Library of Medicine*. Treasure Island, Florida: StatPearls. Available at: https://www.ncbi.nlm.nih.gov/books/NBK507821/#_NBK507821_pubdet_ (Accessed: April 20, 2023).
- Jørn Erlandsen, E. and Randers, E. (2018) "Challenges in the measurement of plasma creatinine on the Roche cobas C702," *Scandinavian Journal of Clinical and Laboratory Investigation*, 78(6), pp. 490–495. Available at: https://doi.org/10.1080/00365513.2018.1501090.
- Kamal, A. (2014) "ESTIMATION OF BLOOD UREA (BUN) AND SERUM CREATININE LEVEL IN PATIENTS OF RENAL DISORDER," *Indian Journal of Fundamental and Applied Life Sciences* [Preprint]. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=6ffdd0fb1a2162328233ca22b492be5261d2c66e (Accessed: April 19, 2023).
- Klick, B. and Guins, T. (2021) "Sepsis in the Urgent Care Setting," *Current Problems in Pediatric and Adolescent Health Care*, 51(2), p. 100968. Available at: https://doi.org/10.1016/j.cppeds.2021.100968.
- Lepoutre, T. *et al.* (2013) "Measurement NT-proBNP circulating concentrations in heart failure patients with a new point-of-care assay," *Clinical Laboratory*, 59(07+08/2013). Available at: https://doi.org/10.7754/clin.lab.2012.120418.
- Lim, H.-J., Lee, S.-Y. and Choi, H.-J. (2023) "Evaluation of the accuracy of CR and Bun using the abl90 flex plus blood gas analyzer and the equivalence of candidate specimens for assessment of renal function," *Journal of Clinical Medicine*, 12(5), p. 1940. Available at: https://doi.org/10.3390/jcm12051940.

- Luppa, P.B. *et al.* (2011) "Point-of-care testing (POCT): Current techniques and future perspectives," *TrAC Trends in Analytical Chemistry*, 30(6), pp. 887–898. Available at: https://doi.org/10.1016/j.trac.2011.01.019.
- Macnab, A.J. *et al.* (2003) "Cost: Benefit of point-of-care blood gas analysis vs. laboratory measurement during stabilization prior to transport," *Prehospital and Disaster Medicine*, 18(1), pp. 24–28. Available at: https://doi.org/10.1017/s1049023x00000649.
- Mula-Abed, W.-A.S., Al Rasadi, K. and Al Riyami, D. (2012) "Estimated glomerular filtration rate (egfr): A serum creatinine-based test for the detection of chronic kidney disease and its impact on clinical practice," *Oman Medical Journal*, 27(2), pp. 108–113. Available at: https://doi.org/10.5001/omj.2012.23.
- Pizarro Sánchez, C. *et al.* (2020) "Analytical evaluation of ABL90 FLEX PLUS BLOOD GAS analyzer for urea and creatinine," *Point of Care: The Journal of Near-Patient Testing & Technology*, 19(2), pp. 37–42. Available at: https://doi.org/10.1097/poc.0000000000000200.
- Radiometer (2021) *ABL90 FLEX PLUS Instructions for use*. Radiometer.
- Roche Diagnostics (2019) "CREP2 Creatinine Plus Method Sheet." Mannheim, Germany.
- Roche Diagnostics (2020) "UREAL Urea/BUN Method Sheet." Mannheim, Germany.
- Salazar, J.H. (2014) "Overview of urea and creatinine," *Laboratory Medicine*, 45(1). Available at: https://doi.org/10.1309/lm920sbnzpjrjgut.
- Salvagno, G.L. *et al.* (2019) "Analytical evaluation of radiometer ABL90 FLEX plus enzymatic creatinine assay," *Journal of Laboratory and Precision Medicine*, 4, pp. 26–26. Available at: https://doi.org/10.21037/jlpm.2019.07.01.
- Seeger, C., Kawiecki, R.W. and Kristensen, H.B. (2011) "Analytical performance of the abl90 flex blood gas analyzer," *Point of Care: The Journal of Near-Patient Testing &*

Technology, 10(3), pp. 108–115. Available at: https://doi.org/10.1097/poc.0b013e31822813c5.

- Van Biesen, W., Vanholder, R. and Lameire, N. (2006) "Defining acute renal failure," *Clinical Journal of the American Society of Nephrology*, 1(6), pp. 1314–1319. Available at: https://doi.org/10.2215/cjn.02070606.
- Vashist, S.K. *et al.* (2015) "Emerging technologies for next-generation point-of-care testing," *Trends in Biotechnology*, 33(11), pp. 692–705. Available at: https://doi.org/10.1016/j.tibtech.2015.09.001.