https://doi.org/10.48047/AFJBS.6.Si4.2024.1960-1971

African Journal of Biological Sciences

Development of microanalysis methodology for determination of normal and abnormal constituents in urine samples.

Meenakshi Suresh[1*], Nilam A. Patil[2], Kiran Fulzele[3],Kruttika Page[4],Shreyash Wahurwagh[5]

[1*,2,3,4,5]Department of Chemistry, Fergusson (Autonomous), Affiliated to Savitribai Phule Pune University, Pune 411004

***Corresponding author:** Meenakshi Suresh
*Meenakshi.Suresh@Fergusson.Edu

Article History
Volume 6, Issue Si4, 2024
Received: 30 May 2024
Accepted : 20 June 2024
Doi:
10.48047/AFJBS.6.Si4.2024.1960-1971

Abstract

Urine analysis is a fundamental diagnostic tool in clinical biochemistry, providing valuable insights into an individual's health status. The analysis typically includes physical, chemical, and microscopic examination of urine samples (McPherson & Pincus, 2017).

Academic institutes play a significant role in training future biochemists and clinical healthcare professionals to conduct large-scale clinical practicals. To support these efforts, our laboratory has developed a simple micro-analysis methodology using Eppendorf tubes to determine both normal and abnormal constituents in urine samples.

Developing microanalysis methods for academic institutes offers several advantages. First, they enable students to gain hands-on experience performing qualitative analysis, essential for their clinical training and development. Second, they facilitate large-scale clinical Biochemistry practices within the institute, allowing for the efficient processing of samples cost-effectively and the generation of reliable data for research and educational purposes.

Furthermore, integrating this microanalysis approach into the curriculum ensures that students are exposed to the diagnostic techniques that prepare them for their future roles in the healthcare industry. By providing access to these microanalysis methodologies, academic institutes can enhance the quality of their clinical biochemistry practical education and contribute to advancing the field. The development and utilization of micro-analysis methods for urine analysis in biochemistry laboratories of schools, colleges and research centres play a crucial role in equipping future healthcare professionals with the necessary skills and knowledge to conduct comprehensive clinical biochemistry tests. This methodology supports large-scale clinical studies and fosters a deeper understanding of the diagnostic value of urine analysis among students.

Introduction:

Formation of urine in kidneys:

The kidneys are important organs in the elimination of excess products and toxic substances that are present in the circulatory system. This process helps in maintaining the acid-base and fluid-

electrolyte balance in the body. There are three elaborate steps involved in the nephrons of kidneys for the process of urine formation: glomerular filtration, tubular reabsorption and tubular secretion. (Jensen-Jarolim et al., 2013)

Glomerular Filtration

The first step in urine formation occurs in the renal corpuscle, which comprises the glomerulus and Bowman's capsule. The urine formation is initiated by the blood filtration by the glomerulus, a tuft of capillaries carrying nutrients such as water, ions, glucose, amino acids, and other molecules. This process takes place under hydrostatic pressure and is non-selective, allowing only substances with a smaller size to pass through. Bowman's Capsule surrounds the glomerulus and collects the Glomerular Filtrate, a fluid containing filtered substances (Hall, 2010).

Tubular reabsorption

The filtrate then enters the renal tubules, where selective reabsorption occurs to retain essential substances. In Proximal Convoluted Tubule (PCT) approximately 65-70% of the filtrate, including water, glucose, amino acids, and electrolytes, is reabsorbed into the blood. This reabsorption is facilitated by active and passive transport mechanisms. When the filtrate reaches the descending limb of Loop of Henle, permeability to water increases, allowing its reabsorption into the surrounding medulla whereas, the ascending limb is impermeable to water so, it actively transports sodium, potassium, and chloride ions out of the filtrate, contributing to the concentration gradient in the medulla. In distal Convoluted Tubule (DCT), further reabsorption of sodium and water occurs, regulated by hormones such as aldosterone and antidiuretic hormone (ADH) (Shannon, 1939).

Tubular Secretion

Additional waste products and excess ions are secreted into the tubular fluid from the surrounding blood vessels in this stage. In the distal convoluted tubule and collecting duct, hydrogen ions, potassium ions, ammonia, and certain drugs are secreted into the tubular fluid. This process helps in acid-base homeostasis and removes toxins from the body (Jensen-Jarolim et al., 2013).

Collecting Duct

The final concentration of urine occurs here. ADH regulates the permeability of the collecting duct to water, increasing required water reabsorption and resulting in the formation of concentrated urine (Atherton, 2006).

Normal composition of urine and excretion:

The final product of these processes is urine, composed of approximately 95% water and 5% solutes. The primary solutes include urea, creatinine, uric acid, sodium, potassium, chloride, and other dissolved ions. The urine is then collected in the renal pelvis, transported to the bladder via the ureters, and eventually excreted through the urethra during urination (Khallid & Haddad, 1999).

Regulatory Mechanisms

The formation of urine is tightly regulated by several mechanisms to maintain homeostasis. The renin-angiotensin-aldosterone system (RAAS) regulates blood pressure and fluid balance by adjusting sodium and water reabsorption, the antidiuretic hormone (ADH) controls water

reabsorption in the kidneys to maintain blood osmolarity and the natriuretic peptide hormones promote sodium and water excretion to reduce blood volume and pressure (Padilla et al., 2007).
Urinalysis is a routine clinical biochemistry practical experiment that is included in academic institutions, clinical research centres and diagnostic labs to analyse components in urine and correlate the findings to diagnose and monitor diseases such as diabetes, liver diseases, kidney disease, inborn errors of metabolism, and urinary tract infections.

Constituents of Urine and Their Significance

Normal Constituents

Water makes up about 95% of urine; essential for solubility of other constituents, Urea, a product of protein metabolism the concentration of which reflects protein intake and renal function, Creatinine, a byproduct of muscle metabolism is used as an indicator of kidney function and electrolytes that includes sodium, potassium, chloride and their levels indicate maintenance of fluid-electrolyte balance in the body (Duarte, 1980).

Abnormal Constituents

The presence of glucose indicates glycosuria when renal threshold of glucose crosses 180 mg/dl and indicates uncontrolled diabetes mellitus. The elevated levels of proteins in urine may suggest kidney disease. Detection of ketone bodies in urine may indicate uncontrolled diabetes or severe fasting or metabolic disorders. The presence of fructose and pentose in urine indicates fructosuria and pentosuria, respectively. Inborn errors of amino acid metabolism, such as cystinuria, alkaptonuria, and phenylketonuria, can also be studied by testing the presence of amino acids in urine. (Park & Shin, 2013).
Traditional methods of urinalysis involve complex and time-consuming laboratory procedures. Microanalysis kits have significantly improved the efficiency and accuracy of urine tests, providing quick results with minimal sample volumes.
Integrating microfluidic channels in analysis kits has enabled precise control and manipulation of small sample volumes, enhancing sensitivity and accuracy. Many portable micro-analysis kits have also been developed (Yager et al., 2008). The development of biosensors for detecting specific biomarkers in urine has facilitated rapid and specific analysis. These sensors often use enzymatic reactions, immunoassays, or electrochemical detection methods. Lab-on-a-chip (LOC) Technology devices incorporate multiple laboratory functions on a single chip, providing comprehensive analysis capabilities in a compact format. Dipsticks are also used to quickly detect certain components in urine (Turgeon et al., 2015).
However, these microanalysis kits and technologies cannot be used in academic institutions where clinical biochemistry tests of urine are taught to many students as a part of their practical training due to their cost factor and limited shelf life.
Our current study aims to develop microanalysis methods that can be used to analyse urine samples in academic institutions in large-scale practical sessions with cost-effectiveness and without compromising on the accuracy of the analysis.

Methodology:

Urinalysis is a key diagnostic tool for detecting various normal and abnormal constituents in urine. It provides valuable information about a person's health, particularly kidney function and metabolic processes. The general scheme for analyzing urine includes physical and microbial examination, which is classified in the table below.

Table1: General scheme for physical and microbial examination of urine sample

	Characteristic	Observation	Indication
Physical Examination	Colour	Pale yellow to amber Dark yellow or amber Red or pink Brown or black Cloudy or turbid	Normal Urine Dehydration in body Haematuria (blood in urine) Bile or myoglobin present in urine Infection, pus, bacteria or crystals in urine
	Odour	A particular but not unpleasant scent Sweet or fruity Foul or unpleasant	Normal urine Presence of ketones in urine Urinary tract infection
	Specific gravity	1.005 to 1.030 <1.005 >1.030	Normal urine Overhydration or diabetes insipidus Dehydration or congestive heart failure
Microbial Examination	Characteristic	Observation	Indication
	Cells	Red blood cells (RBCs): Increased White blood cells (WBCs): Increased Epithelial cells: Increased	Haematuria (blood in urine) Inflammation of infection Tubular damage
	Casts	Hyaline casts Granular casts RBCs cast Wbcs cast	Renal failure Chronic renal disease Glomerulonephritis Pyelonephritis
	Crystals	Calcium Oxalate, Uric Acid Cystine, Struvite	Kidney stones Metabolic disorders or infection
	Microbes	Bacteria Yeast	Infection Fungal infection
	Other	Spermatozoa Trichomonas vaginalis	Retrograde ejaculation Parasitic infection

In our study, the normal constituents tested were urea and ions like chloride, ammonium, potassium and phosphate, while the abnormal constituents in the test sample included glucose, fructose, pentose ketone bodies, protein, cysteine, and bilirubin. The above-mentioned tests were carried out in various Eppendorf tubes.

Tests for normal constituents:

Urea: 0.5 ml of the test sample with urea was taken, and diacetyl monoxime was added and checked for colour change.

Chloride ion: 0.5 ml of the test sample with chloride was mixed with silver nitrate and checked for precipitate formation.

Ammonium ion: 0.5 ml of the test sample containing ammonium ions was mixed with Nessler's Reagent and checked for precipitate formation.

Potassium ion: 0.5 ml of the test sample containing potassium was mixed with picric acid to obtain a precipitate with distinct colourisation.

Phosphate ion: Concentrated nitric acid and ammonium molybdate was used to a 0.5 ml test sample containing phosphate ion and was checked for a change of colour.

Sulphate ion: 0.5 ml of the sample containing sulphate was taken in two tubes and mixed with barium nitrate and lead acetate to check for precipitate.

Test for abnormal constituents:

Glucose: 0.5 ml of the test sample with glucose was mixed with Benedict's solution and heated to observe for a colour change.

Fructose: 0.5 ml of the test sample containing fructose was mixed with resorcinol and concentrated hydrochloric acid to check for a colour change.

Pentose: A 0.5 ml of test sample containing pentose sugar was mixed with orcinol in Conc. HCl to observe for a colour change.

Ketone bodies: 0.5 ml of the test sample having ketone bodies (acetone, acetoacetate) was treated with sodium nitroprusside and sodium hydroxide and checked for a colour change.

Protein: 0.5 ml of the test sample that contained albumin was mixed with copper sulphate and sodium hydroxide to observe a change of colour.

Cysteine: 0.5 ml of the test sample containing cysteine was mixed with lead acetate and hydrogen sulphide was added to observe for a precipitate.

Bilirubin: 0.5 ml of the test sample containing bile pigment bilirubin was sprinkled with sulphur powder to check if it floats at the surface of the test sample or sinks to the bottom of the tube.

Results and Discussions:

In the current research study, microscale analysis was performed using Eppendorf tubes to examine the presence of selected normal and abnormal constituents in the test sample and the results were interpreted as shown in Table 2 and Table 3.

Table 2: Tests for normal constituents

Test	Observation	Inference
0.5 ml of test sample with urea + diacetyl monoxime	Colourless solution to pink	Presence of urea confirmed
0.5 ml of test sample with chloride ion + silver nitrate	Milky white precipitate obtained	Presence of chloride ion confirmed
0.5 ml of test sample containing ammonia + Nessler's reagent	Reddish-brown precipitate formed	Presence of ammonium ion confirmed
0.5 ml of test sample containing potassium + picric acid	Deep yellow precipitate formed	Presence of potassium ion confirmed
0.5 ml of test sample containing phosphate + conc. nitric acid + ammonium molybdate	Canary yellow precipitate formed	Presence of phosphate ion confirmed
0.5 ml of sample containing sulphate + barium nitrate 0.5 ml of sample containing sulphate + lead acetate	Milky white precipitate formed Curdywhite precipitate formed	Presence of sulphate ion confirmed

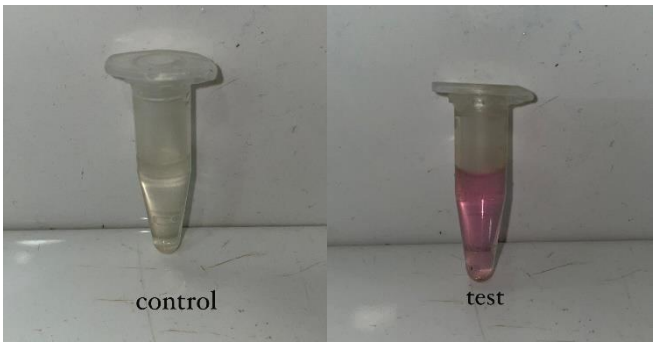

Figure1: Test for presence of urea

Figure 1 shows that pink colour was obtained by mixing the test sample containing urea with Diacetyl monoxime solution that confirmed the presence of urea after comparing with control tube

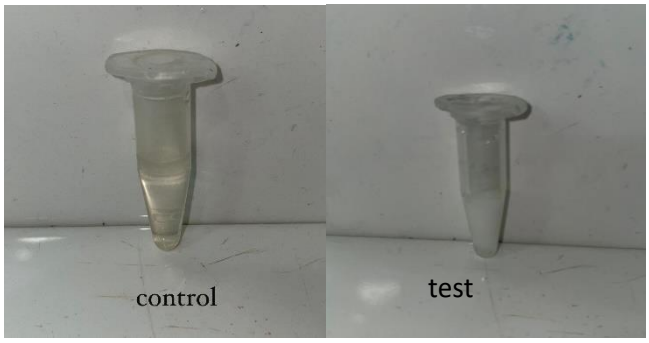

Figure 2: Test for presence of chloride

As per Figure 2, a milky white precipitate of silver chloride was formed on mixing the sample of chloride with silver nitrate, which confirmed the presence of chloride ions in comparison with the colourless control.

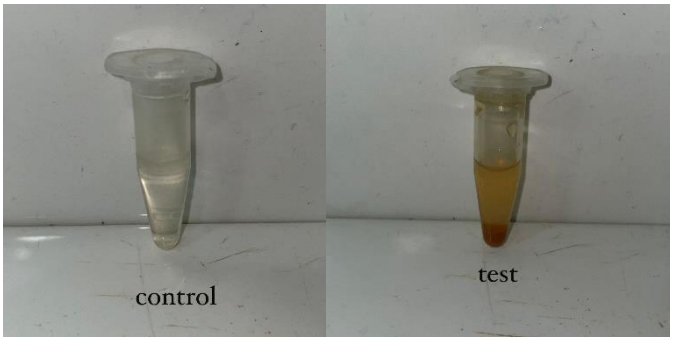

Figure 3: Test for the presence of ammonia

The Figure 3 suggests that a reddish-brown precipitate was formed by adding Nessler's reagent to the sample that contained ammonium ion that confirmed the presence of ammonia contrasting with the colourless control.

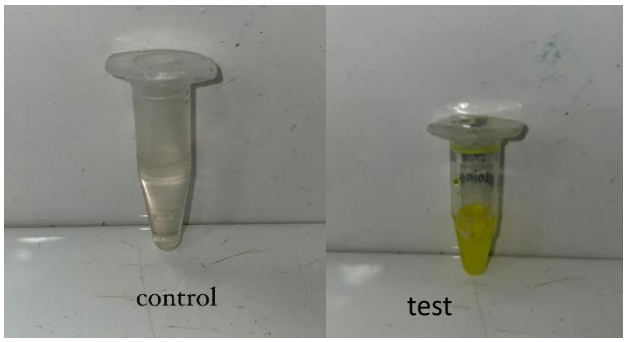

Figure 4: Test for potassium ion

As seen in Figure 4, the presence of potassium ions was confirmed due to the formation of a deep yellow precipitate on mixing the sample containing potassium with picric acid. The colour developed was compared with the control tube.

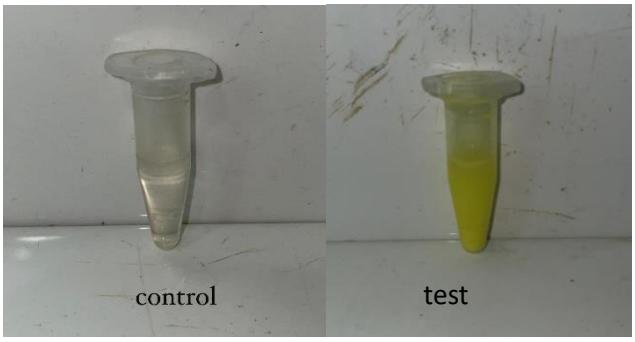

Figure 5: Test for phosphate ion

As Figure 5 shows, when the sample containing phosphate was mixed with concentrated nitric acid and Ammonium molybdate, a canary yellow precipitate was formed that confirmed the presence of the phosphate ion in contrast to the colourless control.

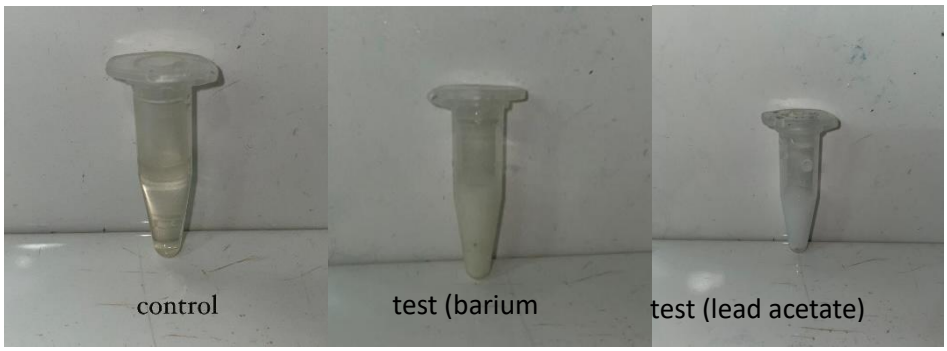

Figure 6: Test for sulphate ion

In Figure 6, a white precipitate is formed when barium nitrate and lead acetatewere added to the test samples of sulphate taken in two different Eppendorf tubes, indicating the presence of sulphate ions.

As seen in Table 2 and Figures 1 to 6, the presence of ions and molecules like urea, chloride, ammonium, potassium, phosphate and sulphate suggests that these test samples indicate the constituents normally present in urine. The toxic ammonia converts to urea in the liver via a urea cycle of reactions, which gets excreted in the urine, whereas the ions (Cl^-, NH_4^+, K^+, PO_4^{3-},

SO42-) are excreted in urine to maintain the fluid-electrolyte balance (*Fluids and Electrolytes*, 1996).

Table 3: Tests for abnormal constituents

Test	Observation	Inference
0.5 ml of test sample with glucose + Benedict's solution	Brick red colorization observed	Glucose present
0.5 ml of test sample containing fructose + resorcinol + conc. hydrochloric acid	Red pink precipitate formed	Fructose present
0.5 ml test sample containing pentose sugar + orcinol + conc. HCl	Dark green colour observed	Pentose present
0.5 ml of test sample containing ketone bodies + sodium nitroprusside + sodium hydroxide	Colourless solution turned red	Ketone bodies present
0.5 ml of test sample containing protein + Biuret solution	Blue solution turned violet	Protein present
0.5 ml of test sample containing cystine + lead acetate + hydrogen sulphide	Black precipitate formed	Cysteine present
0.5 ml test sample containing bile + sulphur powder	Sulphur powder sank to the bottom of the test tube	Bilirubin present

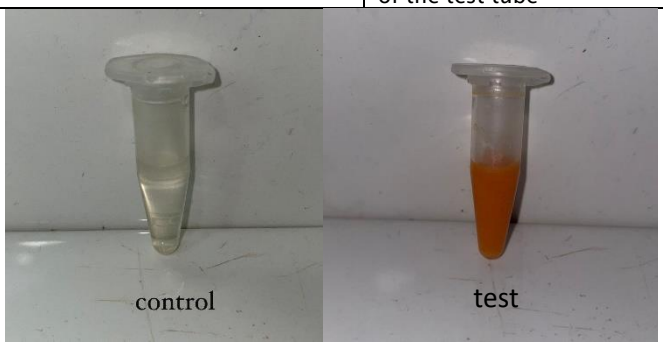

Figure 7: Test for glucose

As observed in Figure 7, a brick-red colouration indicated the presence of glucose in the test sample when it was mixed with Benedict's solution.

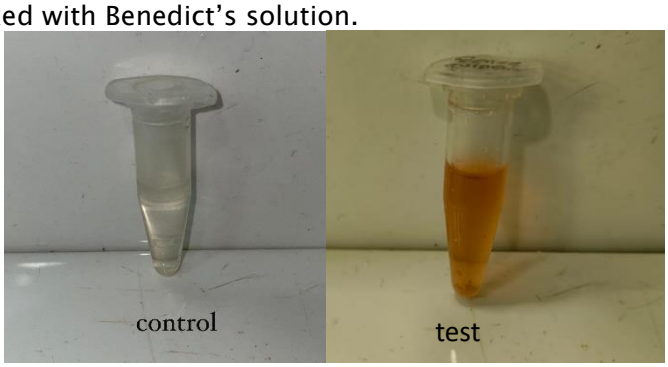

Figure 8: Test for fructose

Figure 8 shows a light red-pink colour that was formed when resorcinol and conc. HCl were added to the test sample containing fructose, confirming its presence in comparison to the colourless control.

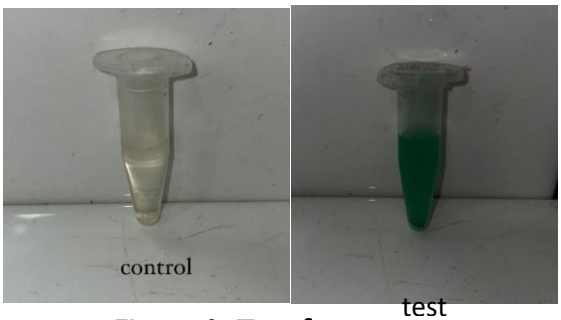

Figure 9: Test for pentose

As per Figure 9, a green colour change was seen when the test sample containing pentose sugar was mixed with orcinol in conc. hydrochloric acid in comparison to the colourless control.

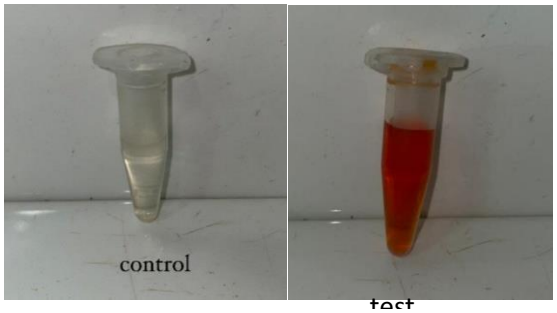

Figure 10: Test for ketone bodies

As seen in Figure 10, when the test sample containing acetone was mixed with sodium nitroprusside and sodium hydroxide, a deep red solution was formed, which confirmed the presence of ketone bodies that were absent in thecontrol solution.

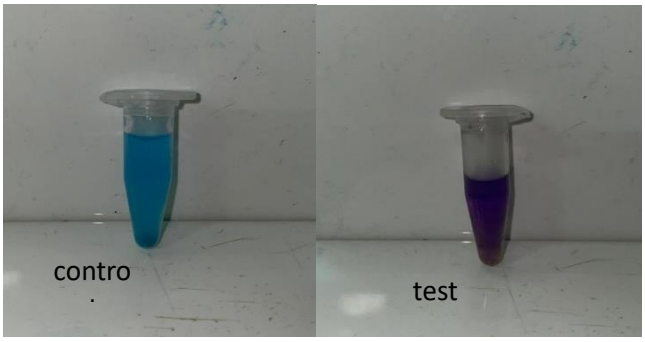

Figure 11: Test for protein

According to Figure 11, when the test sample comprising protein was mixed with copper sulphate and sodium hydroxide (NaOH) solution, a violet colour was observed, confirming the presence of protein in the test sample compared to the blue-coloured control.

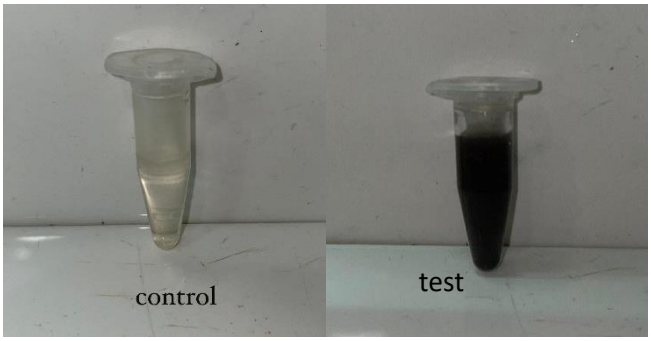

Figure 12: Test for cysteine

As shown in Figure 12, the test sample containing cysteine showed a brownish-black precipitate when mixed with lead acetate, proving the presence of cysteine that was not seen in the control.

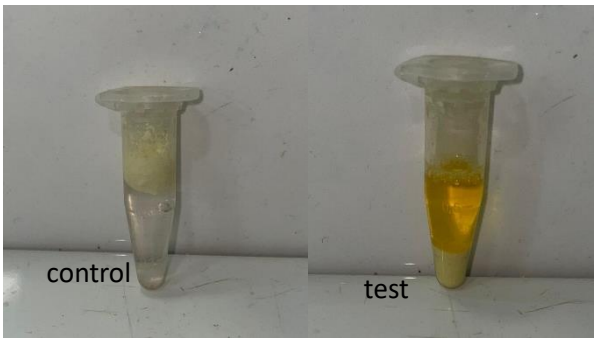

Figure 13: Test for bilirubin

Figure 13 shows the sulphur powder sinking when sprinkled on the test sample containing bilirubin, confirming its presence, whereas in the control, the powder floats on the surface.
Glucose is present in urine during Glycosuria (Liman &Jialal, 2020). It happens due to the elevation of blood sugar levels. The normal range of blood sugar is between 80 mg/dl and 120 mg/dl, and when the level crosses the renal threshold of glucose (180mg/dl), it is indicative of diabetes mellitus, where the excess of glucose in the body is excreted out through the urinary system.
The condition where ketone bodies like acetoacetic acid and acetone are excreted along with urine is known as ketonuria. The same occurrenceindicates fasting, untreated diabetes mellitus, and consumption ofa high-fat diet(Ruzsányi& Kalapos, 2017).

The presence of obvious amounts of protein in urine is known as proteinuria which is observed in variety of kidney disorders. The presence of protein in urine, even in lesser amounts, is also indicative of pregnancy and a high-protein diet(Lamb et al., 2009).
Cystinuria is an inherited autosomal recessive disease characterized by high concentrations of the amino acid cystine in the urine, leading to the formation of cystine stones in the kidneys, ureters, and bladder. It is a type of aminoaciduria(Biyani & Cartledge, 2006).
Fructosuria is a condition in which the metabolism of fructose is affected. It happens due to a defect in the gene that codes for a protein called fructokinase, which is responsible for the first step in the breakdown of fructose. Excess fructose is excreted in urine(Kishnani& Chen, 2021).
Bilirubin, if found in a urine sample, indicatesliver disorders (Tubule, P. C. et al., 2015).During jaundice, bilirubin can be present in the urine, but the amount and type of bilirubin vary depending on the type.In Haemolytic or Prehepatic Jaundice, bilirubin is not typically present in the urine, as it is not produced by the liver. Instead, it is produced by the breakdown of haemoglobin in the blood.Bilirubin is produced by the liver in hepatic or hepatocellular jaundice and can be present in the urine. The amount of bilirubin in the urine can be increased and may be conjugated or unconjugated.Bilirubin is produced by the liver in post-hepatic or obstructive jaundice and can be present in the urine. The amount of bilirubin in the urine can be increased and may be conjugated or unconjugated.
Pentosuria is a condition where the sugar xylitol, a pentose, presents in the urine in unusually high concentrations. It was characterized as an inborn error of carbohydrate metabolism in 1908. It is

associated with a deficiency of L-xylulose reductase, necessary for xylitol metabolism. (Khachadurian, A. K. 1962).

Conclusion:

Through a combination of physical, chemical, and microscopic examinations, Urinalysis provides a comprehensive assessment of urine constituents. Identifying normal and abnormal components is crucial for diagnosing and monitoring various health conditions, particularly those related to the urinary and metabolic systems. The tests mentioned are essential tools in clinical practice, enabling early detection and management of diseases.

The development of microanalysis kits represents a significant advancement in the urinalysis field, providing rapid, accurate, and accessible diagnostic tools. Continued innovation in this area promises to further improve healthcare outcomes through enhanced detection capabilities and broader application of these technologies.

Although many microanalysis kits and advanced technologies are available on the market, they are not feasible due to their cost factor and limited shelf life in academic institutions where clinical biochemistry tests of urine samples are taught to a large number of students as part of their practical curriculum.

Therefore, our current study aims to develop microanalysis methodologies that can be used to analyse urine samples in clinical laboratories of academic and research institutes, with cost-effectiveness and without compromising on the accuracy and reliability of the analysis.

Acknowledgements:

The authors acknowledge the support of infrastructure and chemicals from the Department of Chemistry, Fergusson College (Autonomous), Pune-4

References

1. Atherton, J. C. (2006). Regulation of fluid and electrolyte balance by the kidney. Anaesthesia & Intensive Care Medicine/Anaesthesia and Intensive Care Medicine, 7(7), 227-233. https://doi.org/10.1053/j.mpaic.2006.04.002
2. Biyani, C. S., & Cartledge, J. J. (2006). Cystinuria—Diagnosis and Management. EAU-EBU Update Series, 4(5), 175-183. https://doi.org/10.1016/j.eeus.2006.06.001
3. Buttaro TM, Trybulski JA, Polgar-Bailey P, Sandberg-Cook J (2012). Primary Care: A Collaborative Practice (4th ed.). Elsevier Health Sciences. p. 690. ISBN 978-0-323-07585-5.
4. Duarte,C. G. (1980). Renal Function Tests. Little Brown. http://books.google.ie/books?id=hiJsAAAAMAAJ&q=Laboratory+tests+of+renal+function&dq=Laboratory+tests+of+renal+function&hl=&cd=1&source=gbs_api
5. Fluids and Electrolytes. (1996).http://books.google.ie/books?id=7kT7jwEACAAJ&dq=Human+Water+and+Electrolyte+Balance&hl=&cd=4&source=gbs_api
6. Hall, J. E. (2010). Guyton and Hall Textbook of Medical Physiology E-Book. Elsevier Health Sciences. http://books.google.ie/books?id=Po0zyO0BFzwC&printsec=frontcover&dq=Medical+Biochemistry+Guyton%5D&hl=&cd=1&source=gbs_api
7. Jensen-Jarolim, E., Schöpper, H., &Gabner, S. (2013). Volume and Clearance: Kidneys and Excretory Systems (pp. 161-177). https://doi.org/10.1007/978-3-7091-1559-6_10

8. Khallid, N. S., & Haddad, F. H. (1999). Routine urine analysis in university candidates: is it worthwhile? Eastern Mediterranean Health Journal/Eastern Mediterranean Health Journal, 5(1), 118–122. https://doi.org/10.26719/1999.5.1.118
9. Khachadurian, A. K. (1962). Essential pentosuria. American Journal of Human Genetics, 14(3), 249. Tubule, P. C., Tubule, D. C., Tubules, C., Tubes, U., Containers, U. C., Tubes, U. T., ... & Protection, L. (2015). CHAPTER CONTENTS. Linne &Ringsrud's Clinical Laboratory Science-E-Book: The Basics and Routine Techniques, 383.
10. Kishnani, P. S., & Chen, Y. T. (2021). Disorders of Carbohydrate Metabolism. In Elsevier eBooks (pp. 105–156). https://doi.org/10.1016/b978-0-12-812535-9.00004-2
11. Lamb, E. J., MacKenzie, F., & Stevens, P. E. (2009). How should proteinuria be detected and measured? Annals of Clinical Biochemistry, 46(3), 205–217. https://doi.org/10.1258/acb.2009.009007
12. Liman, M. N. P., &Jialal, I. (2020). Physiology, glycosuria.
13. McPherson, R. A., & Pincus, M. R. (2017). Henry's Clinical Diagnosis and Management by Laboratory Methods E-Book. Elsevier Health Sciences. http://books.google.ie/books?id=xAzhCwAAQBAJ&printsec=frontcover&dq=Henry%27s+Clinical+Diagnosis+and+Management+by+Laboratory+Methos+(23+ed.).+Elsevier+Health+Sciences.&hl=&cd=1&source=gbs_api
14. Padilla, M. J., Miaoulis, I., Cyr, M., & Coolidge-Stolz, E. (2007). Human Biology and Health. http://books.google.ie/books?id=_Vw8SwAACAAJ&dq=Human+Biology+and+Health&hl=&cd=1&source=gbs_api
15. Park, S. J., & Shin, J. I. (2013). Overview of the Formation, Components, Color, and Abnormal Findings of Urine. Daehan Soa SinjangHakoeji/Daehan so'aSinjangHaghoeji, 17(2), 29. https://doi.org/10.3339/jkspn.2013.17.2.29
16. Ruzsányi, V., & Kalapos, M. P. (2017). Breath acetone as a potential marker in clinical practice. Journal of Breath Research, 11(2), 024002. https://doi.org/10.1088/1752-7163/aa66d3
17. Shannon, J. A. (1939). RENAL TUBULAR EXCRETION. Physiological Reviews, 19(1), 63–93. https://doi.org/10.1152/physrev.1939.19.1.63
18. Turgeon, M. L. (2015). Linne &Ringsrud's Clinical Laboratory Science-E-Book: The Basics and Routine Techniques. Elsevier Health Sciences.
19. Yager, P., Domingo, G. J., & Gerdes, J. (2008). Point-of-Care Diagnostics for Global Health. Annual Review of Biomedical Engineering, 10(1), 107–144. https://doi.org/10.1146/annurev.bioeng.10.061807.160524.