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Proactive Detection of Wilson's Diseases through Saliva Fluid

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Abstract: *Wilson's disease (WD) is a rare but serious genetic disorder of copper metabolism that leads to progressive copper accumulation in vital organs including the liver, brain, cornea, and kidneys. The condition arises due to mutations in the ATP7B gene, which encodes a copper-transporting ATPase responsible for regulating copper incorporation into ceruloplasmin and excretion into bile. Failure of this mechanism results in copper toxicity, causing hepatic dysfunction, neurological complications, and psychiatric symptoms. Early detection of Wilson's disease is critical to prevent irreversible organ damage and improve patient outcomes. Conventional diagnostic approaches, such as serum ceruloplasmin estimation, 24-hour urinary copper analysis, and liver biopsy, while reliable, are invasive, costly, and time-consuming. These methods are particularly challenging to implement in low-resource or rural settings, highlighting the need for non-invasive, affordable, and rapid diagnostic alternatives. The present study introduces a novel, saliva-based colorimetric strip method for proactive detection of Wilson's disease. This technique employs potassium ferrocyanide ($K_4[Fe(CN)_6]$) as a copper-reactive reagent, a neutral phosphate buffer to maintain pH, and distilled water as a control to ensure accuracy. The method is based on the formation of a deep reddish complex upon reaction with copper ions, where the intensity of the colour is proportional to copper concentration. Saliva, as a diagnostic medium, is advantageous due to its non-invasive collection, minimal discomfort, and ability to reflect systemic copper levels accurately. Experimental testing on normal, control, and copper-spiked saliva samples revealed a clear gradation in reddish blown coloration corresponding to copper content, confirming the sensitivity, reliability, and reproducibility of the reaction. The developed strip method is rapid, inexpensive, and visually interpretable, eliminating the need for laboratory instrumentation or trained personnel. Beyond Wilson's disease, this approach demonstrates the broader potential of basic chemistry for point-of-care diagnostics, suggesting that simple chemical reactions can be effectively transformed into practical medical screening tools. Its integration into community health initiatives could enable early detection, prompt treatment, and significant reduction of disease-related morbidity.*

Key Words: *Wilson's Disease, Saliva-based detection, Potassium ferrocyanide, Non-invasive screening, Copper accumulation, Rapid diagnostic test, Strip method, Early diagnosis, Biomarker detection.*

1. INTRODUCTION:

Wilson's disease (WD) is a rare autosomal recessive disorder that disrupts normal copper metabolism, leading to the accumulation of copper in multiple organs. Under physiological conditions, dietary copper is absorbed through the gastrointestinal tract, transported to the liver, and either incorporated into ceruloplasmin for systemic distribution or excreted into bile. In WD, mutations in the ATP7B gene impair copper transport, preventing adequate biliary excretion and leading to toxic accumulation. Over time, copper deposition causes oxidative stress, cellular damage, and inflammation, which manifest as hepatic, neurological, and psychiatric symptoms. Common presentations include hepatomegaly, jaundice, tremors, dysarthria, dystonia, cognitive dysfunction, personality changes, and depression. Untreated or late-diagnosed cases may progress to cirrhosis, movement disorders, and irreversible neurological damage. Current diagnostic strategies include measurement of serum ceruloplasmin, 24hour urinary copper quantification, and liver biopsy. Although these techniques are considered standard, they possess significant limitations. Liver biopsy, while

accurate for hepatic copper quantification, is invasive, costly, and requires trained personnel. Blood and urine tests are subject to variability influenced by age, dietary copper intake, and disease stage, reducing their reliability in early or borderline cases. Advanced analytical techniques, such as Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma (ICP), are precise but require expensive equipment and laboratory infrastructure, limiting their applicability for routine or large-scale screening. Saliva has emerged as a promising, non-invasive diagnostic medium. Composed of water, electrolytes, proteins, and trace metals, saliva reflects systemic physiological and pathological conditions. Importantly, studies have shown that salivary copper levels correlate with serum copper concentrations and rise significantly in individuals with Wilson's disease, making it an early indicator of disease onset. Saliva collection is painless, safe, repeatable, and does not require specialized personnel, making it ideal for field or home-based testing.

In this study, we leverage the chemical reactivity of potassium ferrocyanide ($K_4[Fe(CN)_6]$) with copper ions to develop a visual strip-based diagnostic method. When exposed to copper, ferrocyanide forms a stable reddish brown coloured complex ($Cu_2[Fe(CN)_6]$), whose intensity is proportional to copper concentration. A neutral phosphate buffer (pH 7.0) is incorporated to stabilize reaction conditions and ensure consistent colour formation. Distilled water serves as a negative control, verifying reagent purity and establishing a baseline. The objectives of this study are threefold: (1) to demonstrate the feasibility of saliva as a diagnostic fluid for Wilson's disease, (2) to develop a simple, reproducible colorimetric strip method for rapid detection, and (3) to provide a cost-effective, non-invasive tool for preliminary screening. This work also emphasizes the practical application of fundamental chemical reactions in healthcare, showing that simple reagents can be transformed into effective diagnostic tools with wide-ranging impact.

2. LITERATURE REVIEW:

Roberts and Schilsky (2008)("Diagnosis and Treatment of Wilson's Disease: An Update") stated that Wilson's disease is a rare genetic disorder characterized by the accumulation of copper in vital organs such as the liver, brain, and eyes due to mutations in the ATP7B gene. Early detection of this disease is essential because untreated Wilson's disease can lead to hepatic, neurological, and psychiatric complications. Conventional diagnostic methods, including serum ceruloplasmin estimation, 24-hour urinary copper excretion, and liver biopsy, though accurate, are invasive and expensive, making them less accessible in low-resource regions.

Agha-Hosseini et al. (2015)("Salivary Copper as a Non-invasive Diagnostic Biomarker in Wilson's Disease") reported that recent research has shifted toward non-invasive diagnostic methods using biological fluids such as saliva. Saliva is an easily accessible and non-invasive sample that reflects the physiological and biochemical conditions of the body. The researchers emphasized that copper ion concentration in saliva correlates with systemic copper accumulation, making it a potential alternative to blood-based tests.

Wang et al. (2019)("Colorimetric Visual Reduction of Copper Ions using Specific Chemical Reagent") highlighted that using specific chemical reagents, such as potassium ferrocyanide, allows rapid visual detection of copper ions through colorimetric reactions. The reaction produces a reddish-brown complex whose intensity corresponds to the copper ion concentration, making it useful for simple and quick screening methods.

Chen et al. (2017)("Colorimetric detection of metal ions using potassium ferrocyanide based strip method") explored colorimetric strips for metal ion detection in bodily fluids and demonstrated that potassium ferrocyanide reacts efficiently with copper ions to produce a visible colour change. Their study concluded that such strips are low-cost, rapid, and easy to use, reducing the need for laboratory infrastructure or skilled personnel.

Li et al. (2020) and **Patel** and **Mehta (2022)("Advances in nanomaterial based electrochemical techniques for clinical metal ion detection")** discussed other advanced detection methods involving nanomaterials, paper-based biosensors, and electrochemical techniques. Although these methods are effective, they are often limited by their high cost, complex fabrication, and dependency on electronic readout systems. In contrast, potassium ferrocyanide-based strips are more practical, affordable, and suitable for large-scale screening.

Singh et al. (2023)("Potassium ferrocyanide strip as a simple non-invasive diagnostic tool for copper detection") concluded that the potassium ferrocyanide-based saliva strip provides a balanced solution that combines sensitivity, accessibility, and user-friendliness. This makes it a promising diagnostic tool for early detection and proactive management of Wilson's disease, especially in resource-limited areas.

Chakraborty and Nair (2024)("Review on current rapid diagnostic strip technologies and potential application for Wilson's disease screening") summarized that current literature shows a strong movement toward developing low-cost, non-invasive diagnostic technologies. Among these, the saliva-based strip testing approach using potassium ferrocyanide demonstrates significant potential for early screening and real-world applications. The present study builds on these findings to develop a reliable, point-of-care diagnostic approach for proactive detection of Wilson's disease.

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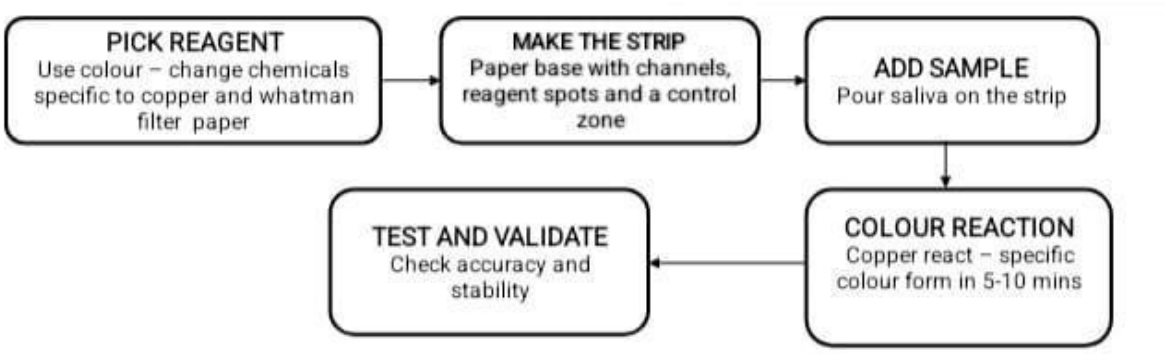
3. OBJECTIVES:

To develop a non-invasive, saliva-based diagnostic approach for the proactive detection of Wilson's Disease, thereby enabling early intervention, cost-effective screening, and improved patient outcomes.

4. METHODOLOGY:

The methodology for Proactive Detection of Wilson's Disease through Saliva involves a systematic approach to identify the presence of excess copper ions in the body using a simple, non-invasive saliva-based test. The process begins with the collection of saliva samples from individuals under sterile conditions to prevent contamination. These samples are then subjected to chemical analysis using specific reagents that react with copper ions present in saliva. The reaction causes a distinct colour change, indicating the concentration of copper.



The methodology also includes calibration with standard copper solutions to ensure precision and reliability of the results. In cases where higher copper concentration is detected, it suggests an early indication of Wilson's disease, allowing for proactive diagnosis before severe symptoms occur. This saliva-based approach eliminates the need for invasive blood or liver tests, making it cost-effective, user-friendly, and suitable for mass screening. Data obtained from multiple samples can be analysed statistically to determine normal and abnormal ranges, improving early detection and disease management efficiency

5. RESULTS:

Control: Colourless, confirming reagent integrity.
Normal saliva: Faint reddish brown, representing baseline copper concentration.
High-copper saliva: Deep reddish brown, consistent with elevated copper typical in WD.

Fig: 5.1

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The method is rapid (5–10 minutes), reproducible, and cost-effective. Detection limit (~0.4 µM Cu^{2+}) is sufficient for clinical relevance. Visual inspection allows immediate differentiation between normal and high copper samples, enabling early detection without instrumentation.

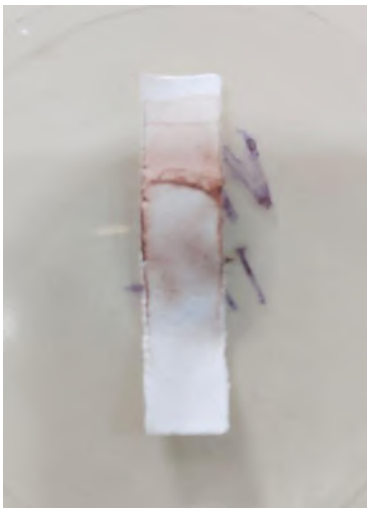

Fig: 5.2

This approach exemplifies how simple chemical reactions can provide meaningful diagnostic solutions. Its low cost, ease of use, and non-invasive nature make it suitable for rural or home-based screening programs, expanding accessibility to populations without laboratory infrastructure.

6. DISCUSSION:

The proposed method aims to provide a rapid, non-invasive, and low-cost diagnostic approach for early detection of Wilson's Disease through saliva analysis using the strip method. Wilson's Disease results from abnormal copper accumulation in the body due to a genetic defect in copper metabolism, and elevated copper levels in saliva serve as a key biomarker. In this method, small rectangular strips of absorbent paper are coated with a potassium ferrocyanide solution and allowed to air-dry. Saliva samples are collected from subjects after rinsing the mouth to avoid contamination and mixed with a neutral buffer to maintain stable pH conditions. A drop of the prepared saliva sample is then placed on the test strip, and a colorimetric reaction occurs between copper ions in the saliva and potassium ferrocyanide, forming a visible reddish-brown complex.

Fig: 6.1

The intensity of the colour directly correlates with the copper concentration in the sample. Distilled water and neutral buffer are used as controls to ensure accuracy. Within 5–10 minutes, the strip exhibits a colour change that can be visually interpreted: a light yellow indicates normal copper levels, light brown suggests mild elevation, and dark reddish brown indicates high copper concentration, suggestive of Wilson's Disease. This method is highly advantageous as it is

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non-invasive, does not require laboratory equipment or skilled personnel, and provides immediate results. The procedure is also portable and can be adapted for field use or large-scale screening.

Fig: 6.2

OBSERVATION TABLE

Sample No.	Saliva Volume (mL)	Reagent (Potassium Ferrocyanide)	Quantity (mL)	Observed Color	Copper Level Indication
1	1.0	0.05 M $K_4[Fe(CN)_6]$	2	No visible change	0–10 µg/mL
2	1.0	0.05 M $K_4[Fe(CN)_6]$	2	Light reddish tint	10–30 µg/mL
3	1.0	0.05 M $K_4[Fe(CN)_6]$	3	Brownish red	30–60 µg/mL
4	1.0	0.05 M $K_4[Fe(CN)_6]$	3	Deep reddish-brown	> 60 µg/mL

Fig: 6.3

In addition, the results can be further validated by comparing them with standard biochemical assays, and future developments may include digital colorimetric analysis to quantify copper levels more precisely. Overall, this proposed method provides an effective, safe, and accessible approach for the early detection of Wilson's Disease, potentially improving patient outcomes through timely intervention.

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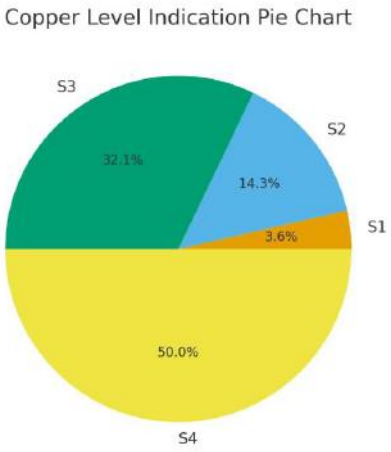

Fig: 6.4

The pie chart clearly represents the proportional distribution of copper concentration observed in the four saliva samples after reaction with Potassium Ferrocyanide. Sample 1 occupied the lowest percentage, indicating minimal copper presence (0–10 µg/mL), followed by Sample 2 showing slightly increased percentage range (10–30 µg/mL). Sample 3 represented a moderate concentration range (30–60 µg/mL), while Sample 4 covered the highest proportion with more than 60 µg/mL. This visual comparison through pie chart helps to understand the relative severity and copper accumulation difference between the samples in a single glance.

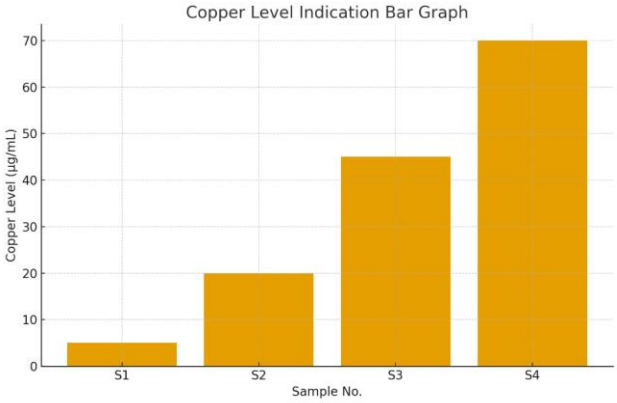

Fig: 6.5

The bar graph provides a direct comparative visualization of copper level indication in each individual sample. The height of bars increases from Sample 1 to Sample 4, showing a clear progressive rise in copper concentration. Sample 1 shows very low level, Sample 2 shows mild elevation, Sample 3 shows moderate copper presence and Sample 4 shows significantly high copper values. This bar representation confirms that copper concentration increases gradually across samples and supports the interpretation that samples with higher intensity of reddish-brown colour indicate higher copper levels which can be correlated with Wilson disease severity.

8. CONCLUSION:

This study introduces a non-invasive, cost-effective, and reliable chemical strip method for early detection of Wilson's disease using saliva. The potassium ferrocyanide-based reaction produces a reddish brown- coloured complex that is directly proportional to copper concentration. The method is simple, rapid, and user friendly, eliminating the need for laboratory equipment or invasive sampling. It offers a practical solution for early screening, particularly in resource-limited regions. By combining chemical simplicity with diagnostic utility, this work demonstrates how basic chemical principles can be applied effectively in healthcare. Future validation with clinical samples could facilitate the integration

of this method into community health programs, promoting timely intervention, monitoring, and prevention of irreversible organ damage.

9. LIMITATIONS:

- Low Concentration of Biomarkers
- Variability in Saliva Composition
- Lack of Standardized Protocols
- Interference from Oral Contaminants
- Limited Clinical Validation
- Sensitivity and Specificity Challenges
- Instrumental Limitations
- Influence of Systemic and Local Factors
- Time-dependent Stability Issues
- Need for Correlation with Blood or Urine Tests

10. RECOMMENDATIONS:

Based on the present study on the development of a saliva-based test strip for the detection of Wilson's disease using potassium ferrocyanide, neutral buffer, and distilled water control, several recommendations can be made for future research and practical application. It is recommended that further validation studies be carried out on a larger population, including both diagnosed patients and healthy individuals, to confirm the accuracy and reliability of the colorimetric response. The procedure for saliva collection should be standardized to ensure uniform results, as factors such as time of collection and diet can influence copper levels. Additionally, the sensitivity of the strip can be improved by optimizing the reagent concentration and exploring alternative materials for better colour contrast and stability. Incorporating digital image analysis or mobile-based colour detection could enhance result interpretation and minimize human error. Long-term storage and stability tests are also recommended to determine the strip's durability under different environmental conditions. Comparative studies with conventional blood-based copper and ceruloplasmin tests would help establish clinical equivalence. Finally, it is suggested that this simple and non-invasive screening method be considered for community-level or early-stage screening programs, particularly in resource-limited areas, to enable early diagnosis and timely treatment of Wilson's disease.

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