APTAMER-BASED COLORIMETRIC KIT TEST FOR RAPID DETECTION OF MICROSCOPIC HEMATURIA IN URINE

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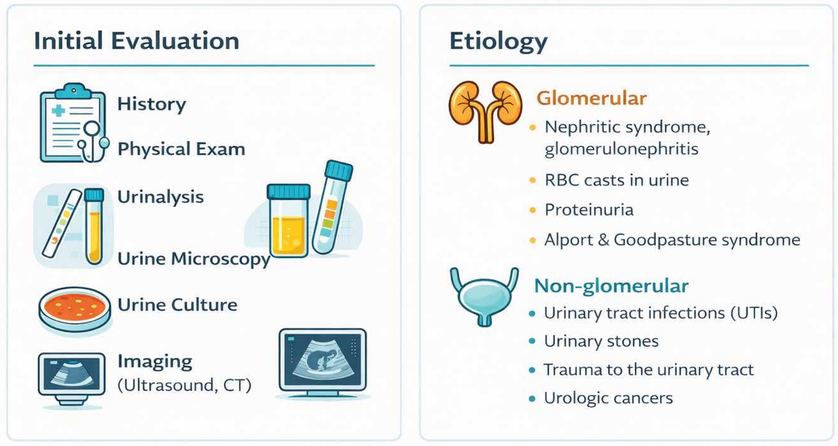
Introduction

- **Microscopic hematuria (MH)** is an early but often overlooked sign of urological disorders.



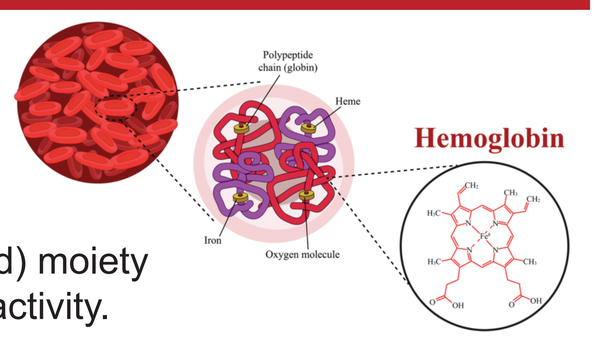
+ Reported prevalence ranges **2.4–31.1%**, depending on cohort and criteria.
+ **Microscopy** is accurate but labor-intensive.
+ **Dipsticks** are fast but prone to matrix interference (pH, vitamin C, drugs), causing false results.

There is a need for high sensitivity and accuracy, enabling practical screening and early diagnosis of urinary tract disorders

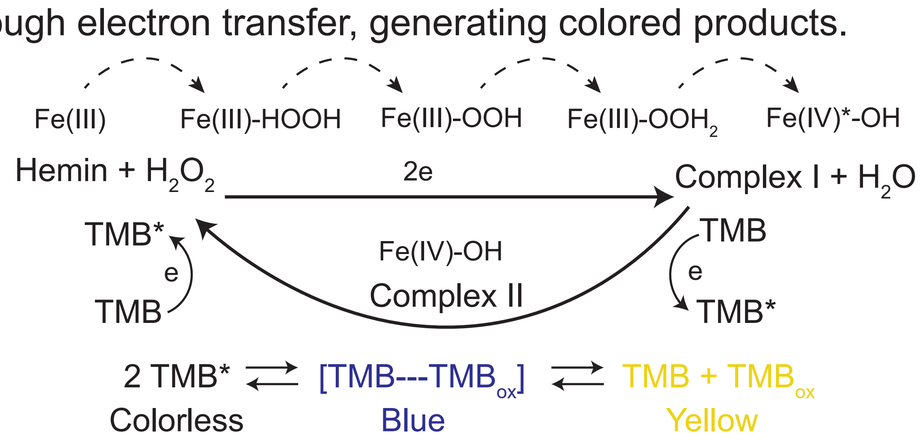
This research develop an **aptamer-based colorimetric kit test** for detecting microscopic blood in urine.

Peroxidase-like Activity of Aptamer/Hemin

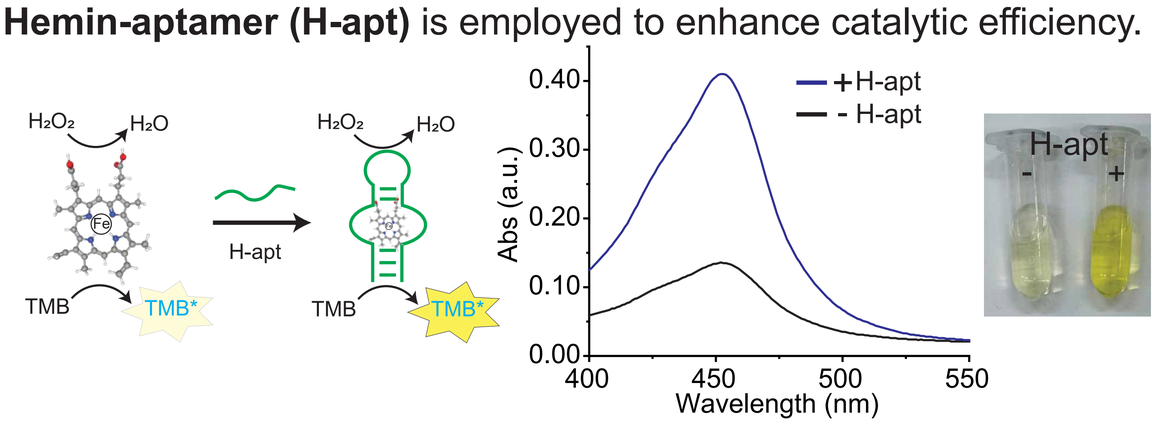
- Characterized by the presence of **red blood cells** in urine, associated with hemoglobin—the major protein component of erythrocytes.
- **Hemoglobin (Hb)** contains a hemin group—an iron–porphyrin (Fe-centered) moiety that enables peroxidase-like catalytic activity.



- The peroxidase-like hemin system catalyzes the stepwise oxidation of TMB through electron transfer, generating colored products.

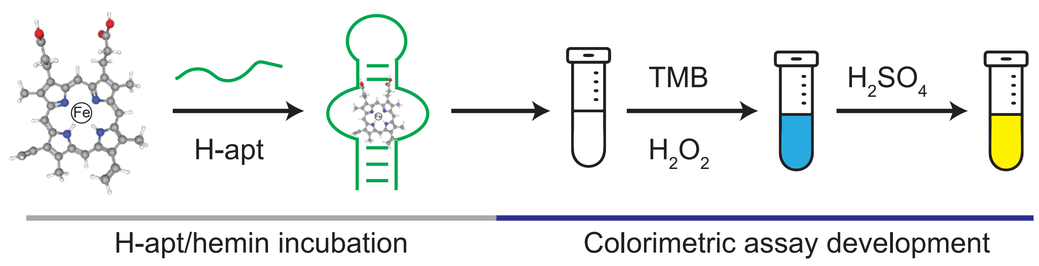


- Free heme exhibits *limited intrinsic peroxidase-like activity*; therefore, **Hemin-aptamer (H-apt)** is employed to enhance catalytic efficiency.

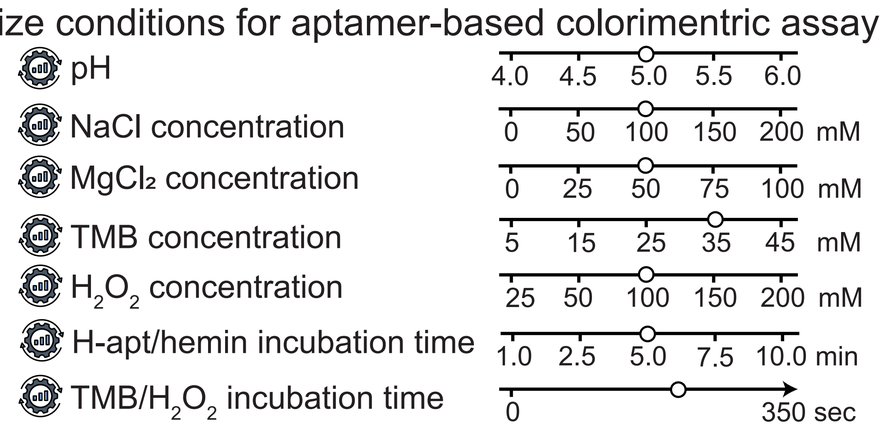


Experimental procedure

- Schematic of aptamer-based colorimentric assay for blood detection

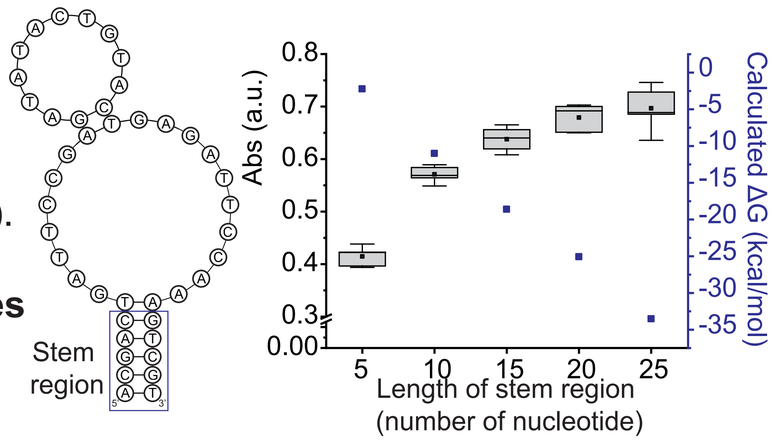


- Optimize conditions for aptamer-based colorimentric assay



Optimized sequence of H-apt

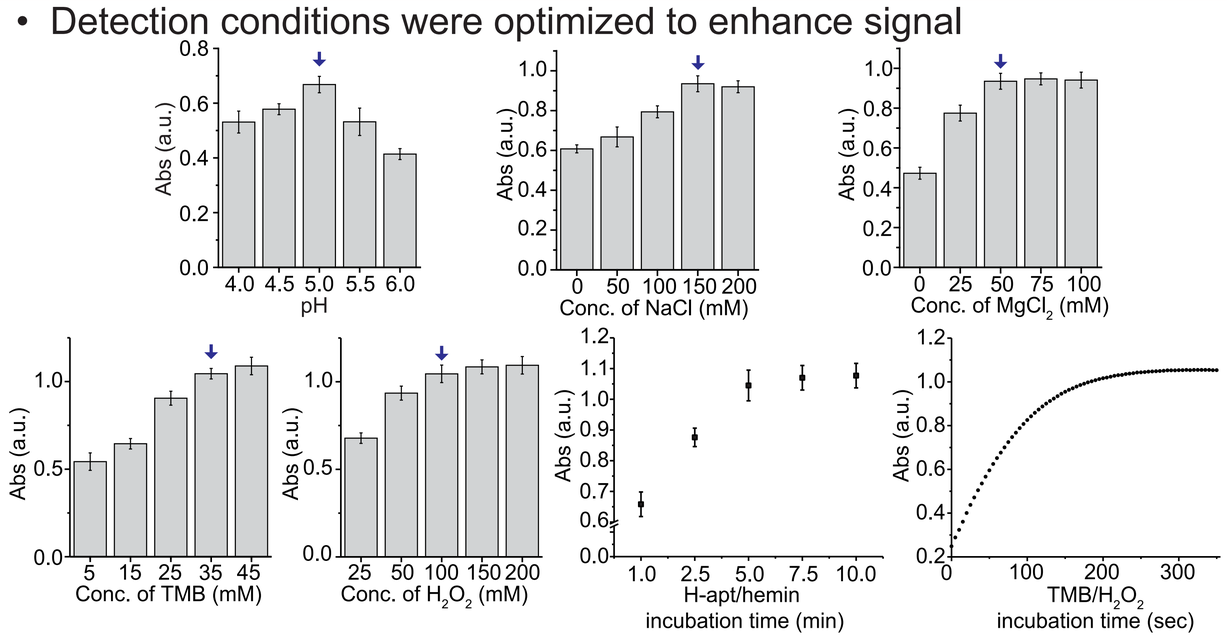
- **The stem length of H-apt** was systematically varied to modulate structural stability.
- **Increasing** stem length **enhances** thermodynamic stability (more negative ΔG).
- Catalytic activity **increases** with stem elongation.



The optimized **10-nucleotide stem** configuration was selected to maximizes peroxidase-like activity of the aptamer/hemin complex.

Optimization conditions

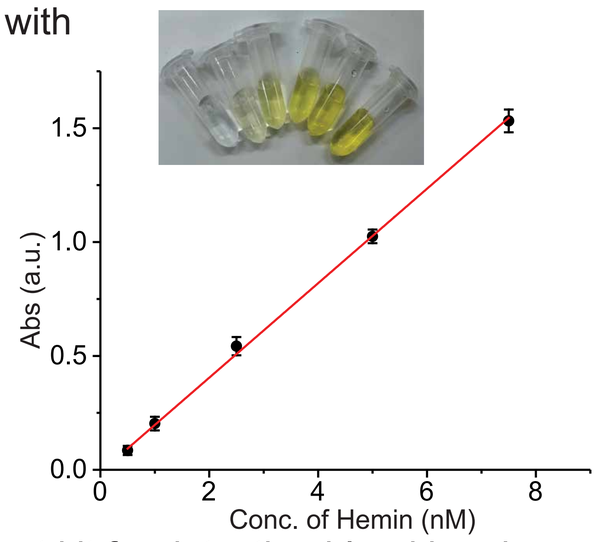
- Detection conditions were optimized to enhance signal



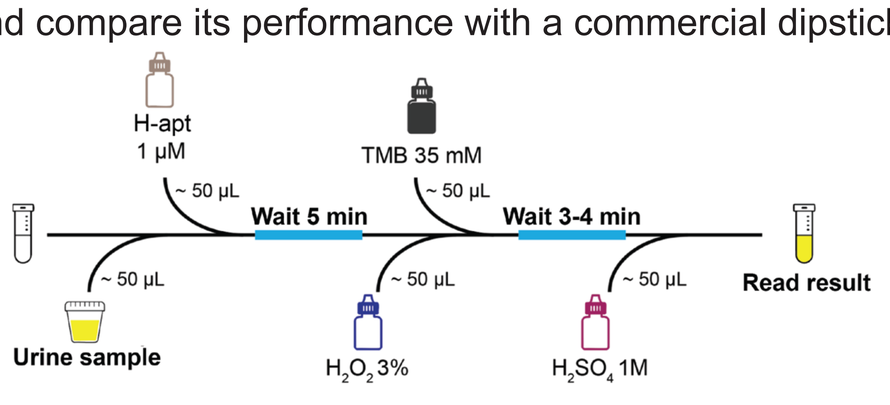
Optimum conditions: **pH 5.0; NaCl 150 mM; $MgCl_2$ 50 mM; TMB 35 mM; $[H_2O_2]$ = 100 mM.**
Selected incubation times: **5.0 min for H-apt/hemin and 200 s for TMB/H_2O_2 reactions.**

Detection blood in urine sample

- Absorbance increases proportionally with hemin concentration (≈0.5–8 nM).
- Small error bars indicate **good repeatability**, and the **visible color change** supports simple colorimetric detection.
- The LOQ is **4–10× lower** than the heme level associated with a typical microscopic hematuria screening threshold (~2 nM).



- Design a prototype aptamer-based test kit for detecting blood in urine and compare its performance with a commercial dipstick.



The prototype aptamer-based kit reliably detects **low-level hematuria (5–10 RBC/μL)** in urine and **outperforms** a commercial dipstick.

Conclusions

A high simple and sensitivity aptamer-based kit test for microscopic hematuria detection was developed.

Further develop and validate a fully integrated test kit.

References

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