Optimizing Urine HPV Detection via Ultra-Short Fragment Targeting: A Comparative Analysis of DNA Fragmentation in Urine versus Cervical Samples

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INTRODUCTION

- **The Global Gap:**

  While the WHO aims for a 70% screening rate by 2030 to eliminate cervical cancer, China's current rate for women aged 35-44 is only 36.9%.

- **The Urine Challenge:**

  Urine offers an ultimate non-invasive alternative to clinical swabs. However, legacy testing methods (e.g., HC II, FTH) require long DNA sequences (>200bp).

- **The Barrier:**

  HPV DNA in urine exists as highly fragmented cell-free DNA (cfDNA). Traditional assays targeting longer amplicons fail to capture these degraded fragments, leading to high discordance between urine and cervical samples.

OBJECTIVES

- To quantitatively map the fragmentation profile of HPV DNA in paired urine versus cervical samples.
- To validate if targeting "ultra-short" fragments (e.g., 50bp) significantly improves the detection yield in urine.

METHODS

- **Sample Selection:**

  Paired clinical samples positive for high-risk HPV 16, 18, and 56.

- **Amplicon Gradient Strategy:**

  Custom primers targeting *E6*/*E7* genes were designed to generate a gradient of amplicon lengths: 50 bp, 100 bp, 150 bp, and 200 bp.

- **Technological Innovation:**

  Applied Fluorescence PCR Melting Curve Analysis (MCA) utilizing a modified reverse primer with a *C3*/*C6* spacer (Polymerase Extension Arrest) to accurately target ultra-short amplicons (<80bp).

- **Quantification:**

  Absolute copy numbers (*copies*/μL) were evaluated via Droplet Digital PCR (ddPCR).

RESULTS

- **Cervical Samples Exhibit High DNA Integrity:**
  - No significant differences in quantification were observed across varying amplicon lengths.
  - Linear regression showed almost no correlation between target length and detection concentration: HPV 16 (R^2=0.1986), HPV 18 (R^2=0.0473), and HPV 56 (R^2=0.0431).
- **Urine Samples Exhibit Severe Degradation:**
  - Detected concentration showed a strong negative linear correlation with amplicon length.
  - The shorter the amplicon, the higher the recovered concentration: HPV 16 (R^2=0.8973), HPV 18 (R^2=0.9459), and HPV 56 (R^2=0.9303).
- **Yield Breakthrough:**

  Primer pairs targeting ultra-short fragments of 50 bp detected HPV DNA concentrations that were at least **10 times higher** than those targeting long fragments of 200 bp in urine samples.

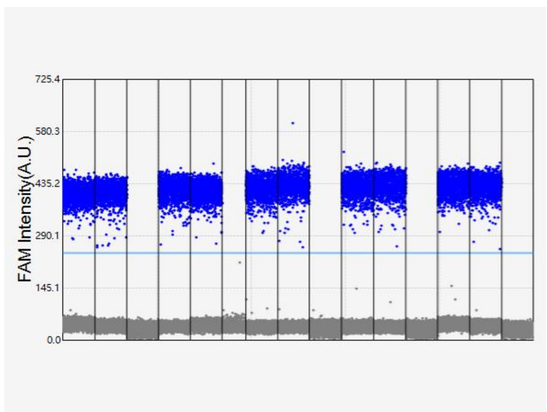

Fig. 1 Results - HPV16, CERVIC

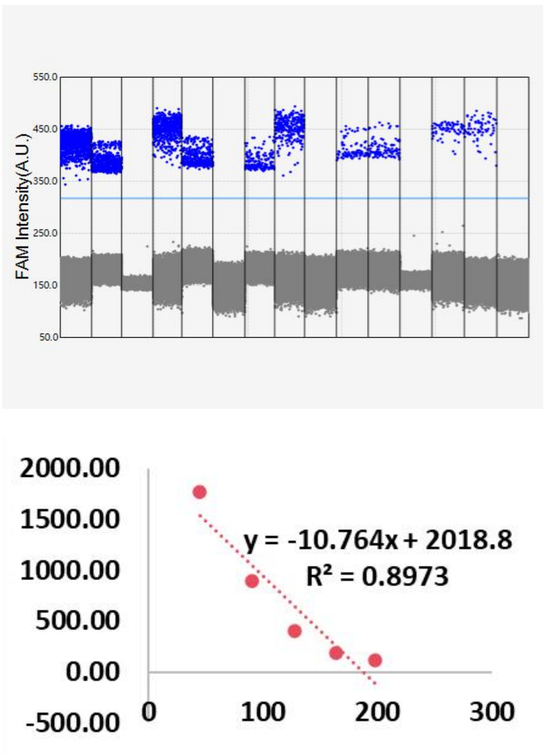

Fig. 2 Results - HPV16, URINE

CONCLUSIONS

- **Distinct Strategies Required:**

  Urine and cervical samples require fundamentally distinct primer design strategies due to significant fragmentation differences.

- **The Ultra-Short Imperative:**

  Targeting ultra-short fragments (50-80 bp) is essential for urine-based assays to recover "lost" signals and enhance detection sensitivity.

- **Clinical Feasibility:**

  This strategy successfully overcomes the cfDNA fragmentation barrier, making reliable, non-invasive urine HPV screening a feasible reality for clinical practice.

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