171: From Tissue to Test: An RNA-First Workflow for *In Situ* Target Validation and Point-of-Care Diagnostics

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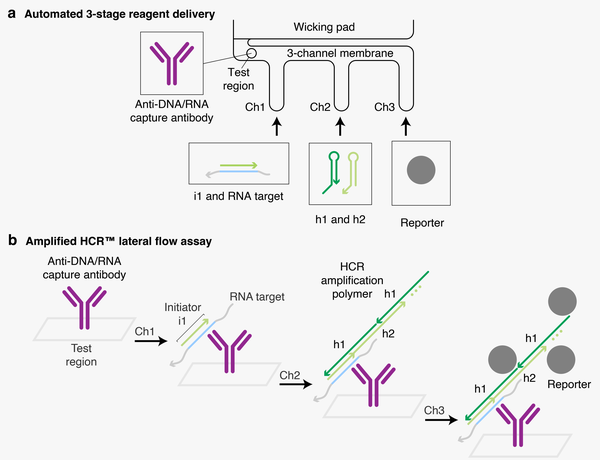
Introduction

Bringing molecular discoveries to point-of-care (POC) diagnostics requires robust target validation that spans species and tissue contexts. For lateral flow assay (LFA) development, commercial translation is often hindered by challenging sensitivity and specificity requirements, cross-reactivity, and poor target accessibility — these are compounded by unreliable or unavailable antibodies. Traditional pipelines depend on *in vitro* systems and/or antibody-based assays that may fail to perform optimally. The HCR™ technology is a foundational platform capable of enabling the close study of a molecular target from early discovery in diverse species, through *in situ* validation in established animal models and human tissues, and ultimately to POC diagnostics. Here, we present a translational workflow leveraging clinical-grade HCR™ HiFi Probes, HCR™ Pro, and a novel HCR™ diagnostic platform to validate RNA targets *in situ* and support the development of antibody-independent POC assays.

HCR™ Lateral Flow Assay Platform



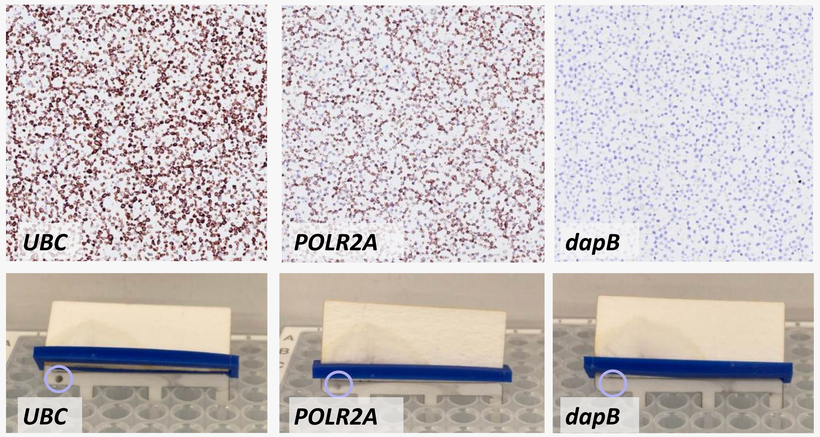
Methods

Candidate RNA targets were screened using HCR™ HiFi Probes applied to formalin-fixed paraffin-embedded (FFPE) tissue sections from preclinical models and clinical samples. *In situ* validation was performed using HCR™ Pro, providing quantitative and spatially resolved expression data. Based on these validated RNA target results, nucleic acid-based LFAs are in development using a novel HCR™ diagnostic platform designed for deployment in clinical settings in combination with a novel, non-invasive method for analyte collection in human skin cells.

Approach

We validated the methodology by first testing HCR™ HiFi Probes *in situ* on FFPE cell pellet sections. After acquiring this data, we collected the total RNA content from adjacent sections, and the resultant RNA extract was screened for the same housekeeping targets using our HCR™ LFA. From this initial validation of the workflow, we continued onto more clinically relevant targets to serve as a proof-of-concept for a diagnostic test at the POC. We completed this demonstration with a workflow that detects mRNA, from a novel skin sample collection method, in less than three hours of total runtime.

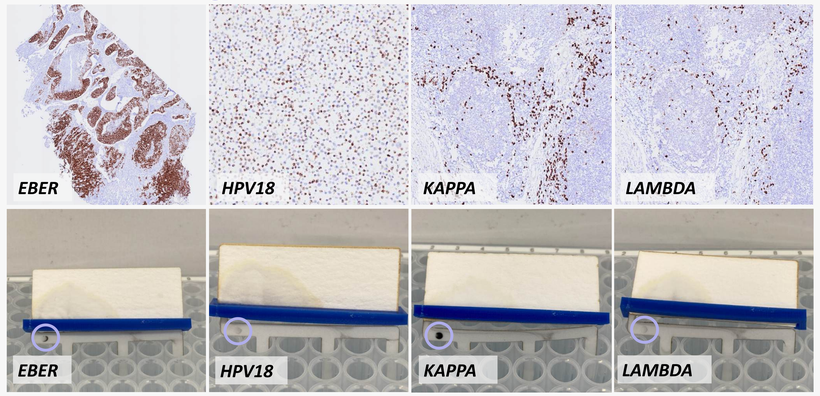
Results: Housekeeping Target Detection



HeLa cell pellet *in situ*. Top Row. We performed HCR™ Pro on sections of FFPE cell pellet to demonstrate the presence of housekeeping targets UBC (left column) and POLR2A (middle column). A negative control probe, DapB (right column), was used as a metric for background signal. We see that the abundance of the target and the intensity of the staining correlate with each other as expected. **Lateral flow assay detection.** Bottom row. Total RNA was collected from adjacent sections of HeLa cell pellets using a third-party RNA extraction kit. The RNA extract was renormalized to a concentration of 1 µg/mL and was used as the target RNA for three lateral flow assays that detect the presence of UBC (left column), POLR2A (middle column), and dapB (right column). Much like the *in situ* staining, we see that the lateral flow assay staining intensity correlates with the expected abundance of the desired target.

HCR™ Pro enabled high-specificity and high-dynamic-range *in situ* detection across a range of abundance of mRNA targets. Validated probe sets demonstrated robust expression in LFA analysis as well, noting the qualitative intensity change between the UBC and POLR2A results, forming the foundation for ongoing LFA assay development.

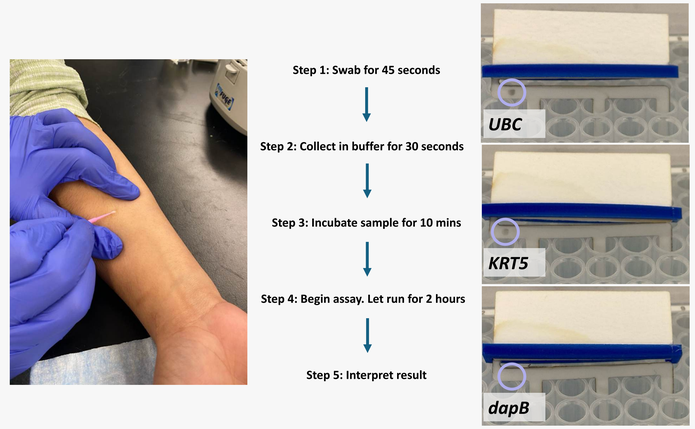
Results: Clinical Target Detection



Clinical *in situ* data. Top row. Again, we performed HCR™ Pro on FFPR section to validate the presence of biomarkers *in situ.* From left to right we illustrate the presence of: EBERs (a small, non-protein coding RNA that indicates the presence of the Epstein-Barr virus) in EBER+ gastric cancer tissue, HPV18 in the HPV18+ HeLa cell line, kappa light chain RNA in tonsil tissue, and lambda light chain RNA in tonsil tissue. **Lateral flow assay positive detection.** Bottom row. Total RNA was collected from adjacent sections for each respective column using the same third-party RNA extraction kit as before. The resultant extract was then renormalized to a concentration of 1 µg/mL and used as the RNA target for the detection of EBERs, HPV18, kappa, and lambda via LFA and we see a positive result in the test region of the LFA, indicating the presence of the desired analyte.

HCR™ Pro enabled high-specificity, high-dynamic range *in situ* detection of RNA targets across tissue types. *In situ* imaging allowed for easier validation of targets, reducing downstream development risk. This validation of mRNA detection from FFPE tissue informed our decision to pursue the development of HCR™ LFAs without specialized instrumentation, enabling future deployment in healthcare settings at the POC.

Results: Skin Sample Collection



Skin sample collection demonstration. Left and middle column. The novel, non-invasive sample collection allows for the downstream analysis of epidermal cells via HCR™ LFA. The entire process, shown in the middle column, can take researchers from sample collection to analysis in less than three hours total. **mRNA detection from skin sample.** Right column. Three independent skin samples were collected and served as our target RNA for the HCR™ LFA. From top to bottom, we detect for UBC, KRT5 and dapB.

HCR™ LFA enables the downstream detection of mRNA targets from a novel sample collection method. The general housekeeping target UBC indicates that mRNA is present in the sample. Furthermore, we can demonstrate that targets like KRT5, more relevant to the sample type, can also be detected. The detection of dapB serves as a negative control and as a metric for background staining relative to the other targets. Adjacent studies using HeLa cell lysate have indicated a sensitivity limit of nearly 2,000 cells. This translates into an ability to detect mRNAs at an estimated 1,000 transcripts per µL of the working sample solution.

Conclusion

This study establishes an RNA-first translational workflow for diagnostic development that bypasses antibody limitations and enables early *in situ* target validation. Current HCR™-powered LDTs supports diagnostic utility today, while ongoing LFA development aims to expand accessibility at the POC. The HCR™ platform enables seamless molecular tracking from discovery through *in situ* validation to clinical diagnostics. Next steps include a continued collaboration with the Department of Dermatology at the Yale School of Medicine to use HCR™ LFAs as a diagnostic tool. We also aim to achieve an assay that allows for analyte collection and detection with minimal equipment necessary, such that a diagnosis can be provided at the POC.

References

[1] Harry M. T. Choi, Maayan Schwarzkopf, Mark E. Fornace, Aneesh Acharya, Georgios Artavanis, Johannes Stegmaier, Alexandre Cunha, Niles A. Pierce; Third-generation in situ hybridization chain reaction: multiplexed, quantitative, sensitive, versatile, robust. Development 15 June 2018; 145 (12): dev165753. doi: https://doi.org/10.1242/dev.165753

[2] Samuel J Schulte, Jining Huang, Niles A. Pierce; Hybridization chain reaction lateral flow assays for amplified instrument-free at-home SARS-CoV-2 testing. ACS Infect. Dis. 3 February 2023; 9, 3, 450-458. doi: https://doi.org/10.1021/acsinfecdis.2c00472

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