

# Isothermal amplification and lateral flow detection of HPV 16 and 18 E7 DNA

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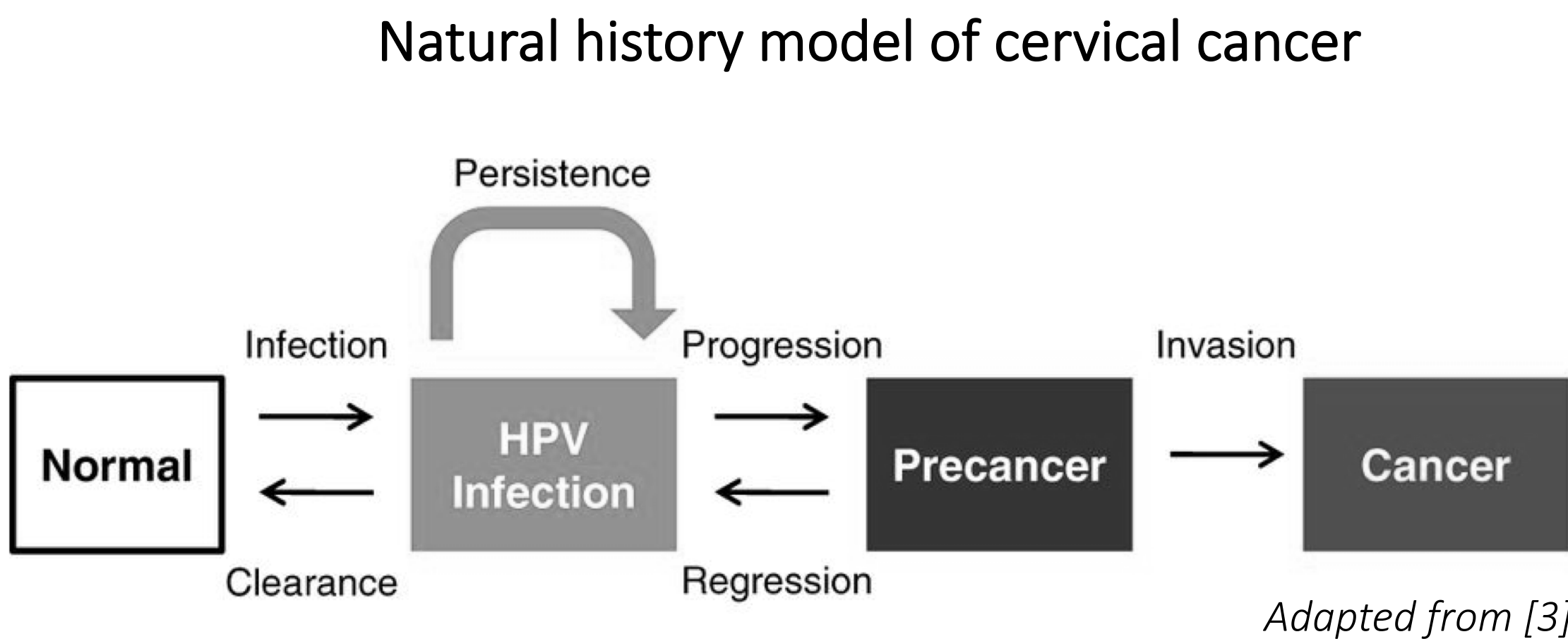
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## INTRODUCTION

- Cervical cancer is preventable with vaccination against human papillomavirus (HPV) and screening and treatment of precancerous lesions
- Approximately 570,000 women are diagnosed with and 311,000 die of cervical cancer annually [1]; 90% of cervical cancer deaths occur in low-resource settings [2]; HPV 16 & 18 account for 70% of cancers
- Currently available HPV tests are not accessible in resource-limited settings due to cost and complexity
- There remains a need for an easy-to-use, low-cost, point-of-care HPV screening test.

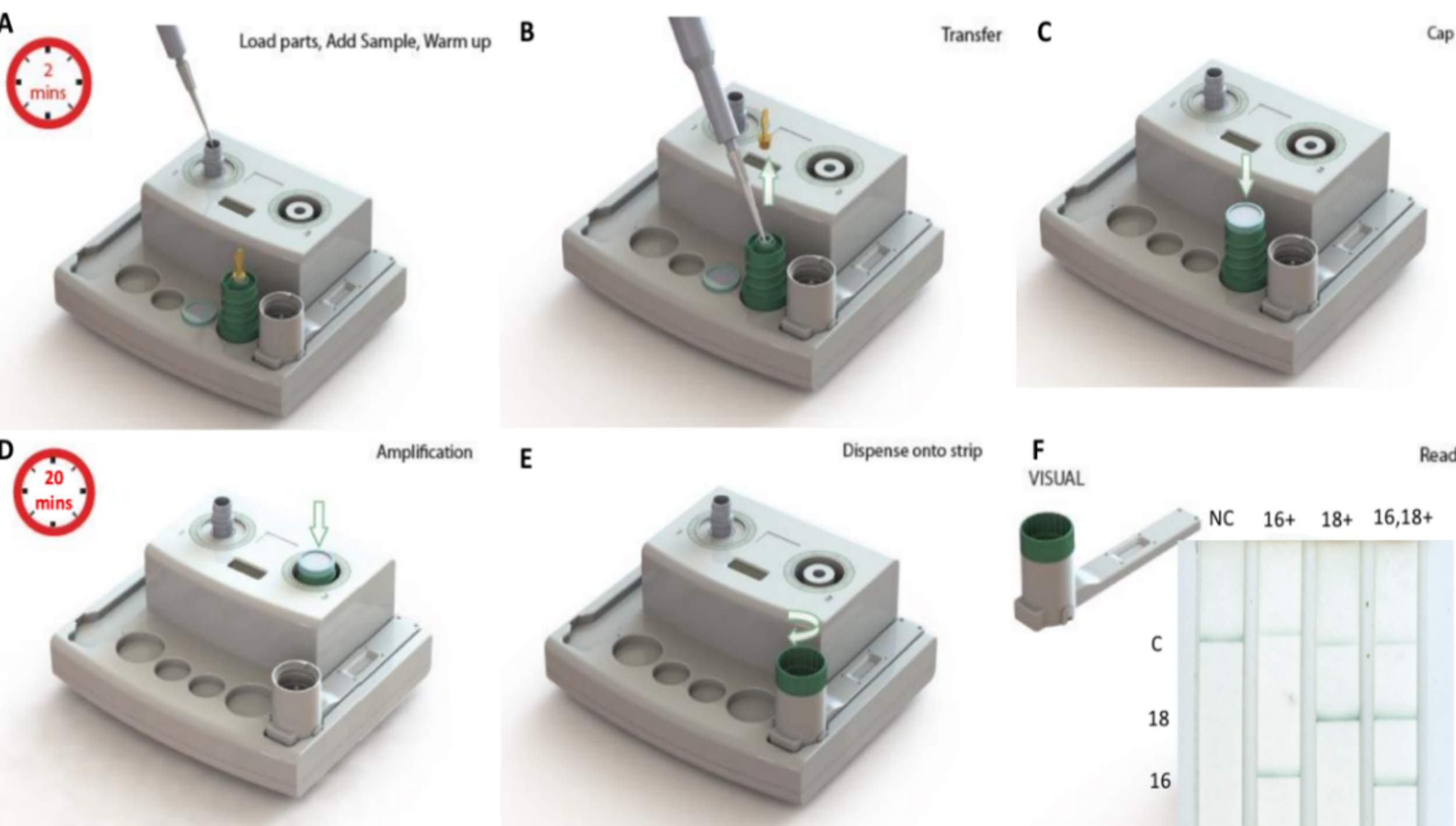


Adapted from [3]

Goal: Amplify HPV 16 and 18 E7 DNA isothermally & detect amplicons by lateral flow

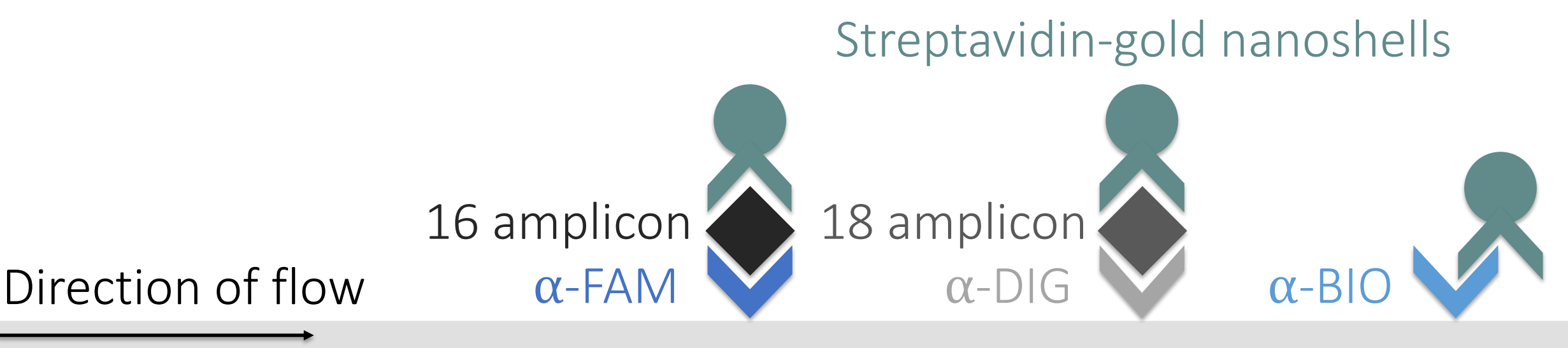
## APPROACH

NATflow platform developed by Axxin Diagnostics, Ltd.

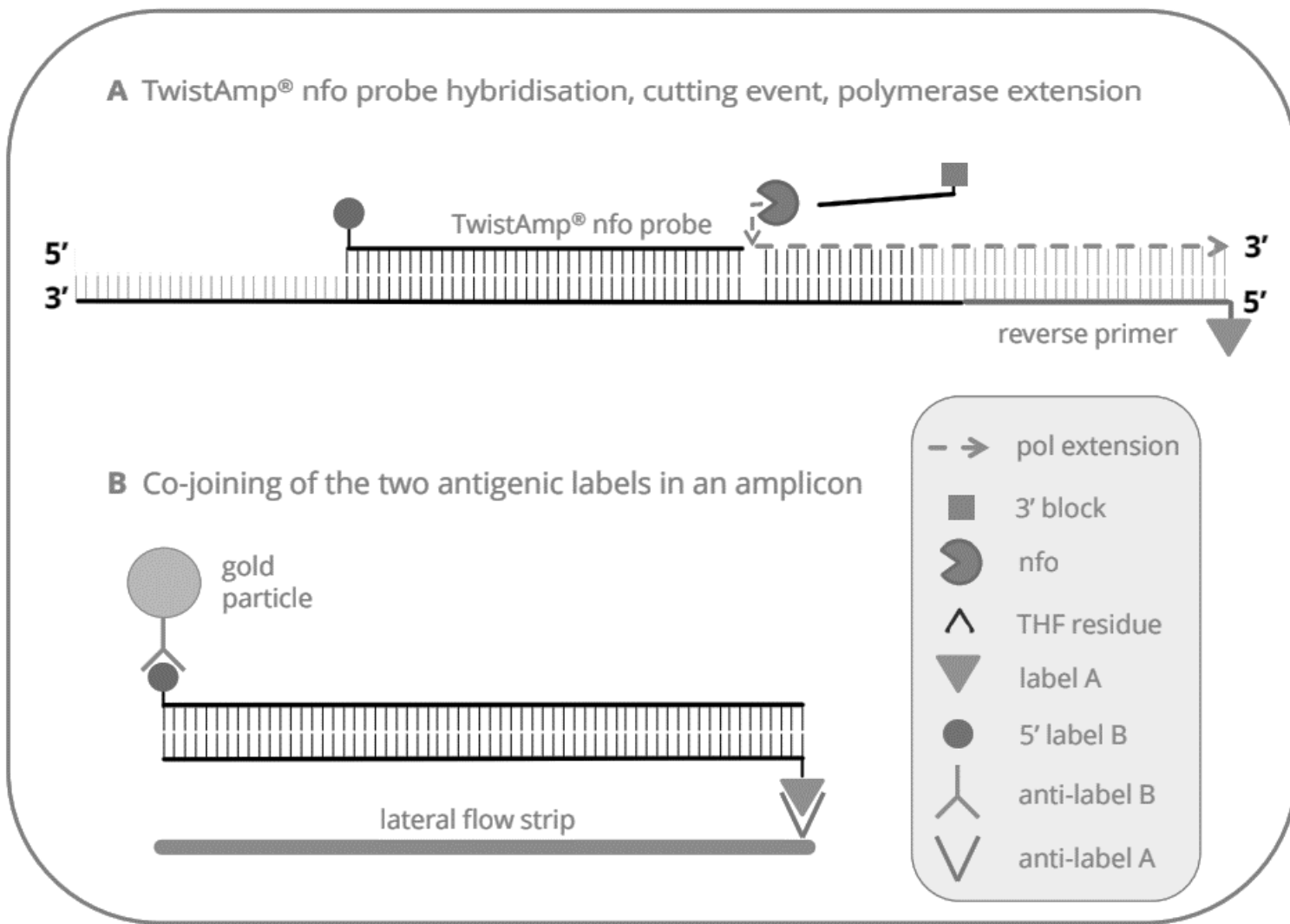


Adapted from Axxin Diagnostics, Ltd.

### Lateral flow detection



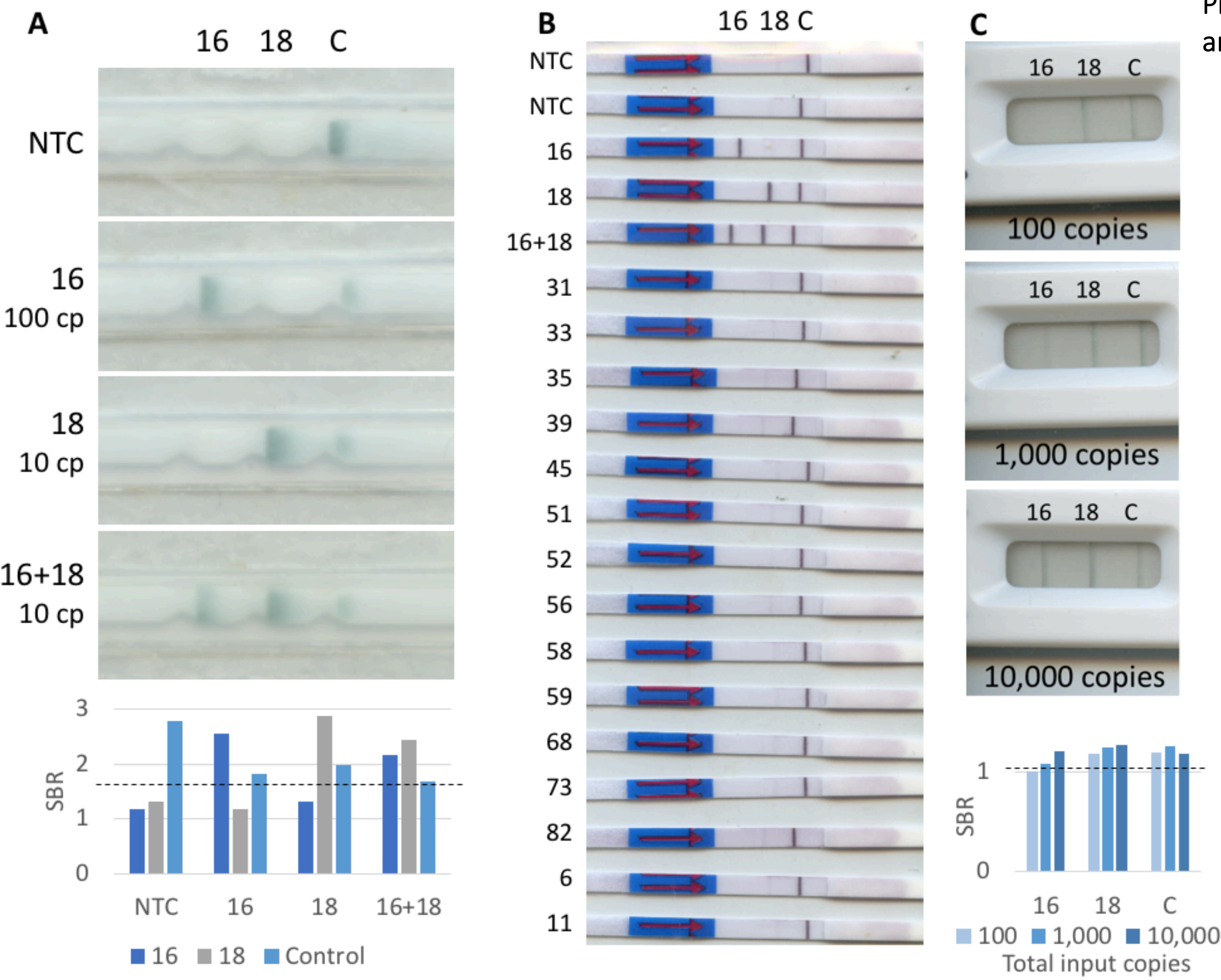
Isothermal amplification assay:  
RPA nfo (39°C)



Adapted from TwistDx, Ltd.

|              | Sequence (5'→3')  |
|--------------|---|
| HPV 16 FWD   | CATGGAGATACACCTACATTGCATGAATAT                                      |
| HPV 16 PROBE | [FAM]-CAATTAAATGACAGCTCAGAGGAGGAGGATGAAA[dSP]AGATGGTCCAGCTGG-[C3SP] |
| HPV 16 REV   | [BIO]-CACAAACGAAGCGTAGAGTACACATTGCAA                                |
| HPV 18 FWD   | ATTGCATTAGAGCCCCAAATGAAATTC   |
| HPV 18 PROBE | [DIG]-CAGAGGAAGAAAACGATGAAATAGATGGAGTTAA[dSP]CATCAACATTACCA-[C3SP]  |
| HPV 18 REV   | [BIO]-TACTAGCTCAATTCTGGCTTACACTTACA                                 |

## RESULTS



SBR: Signal-to-background ratio; 16: HPV 16 test line; 18: HPV 18 test line;

C: control line; dashed line indicates positivity threshold

Preliminary data for HPV 16 and 18 amplification test:

A. Limits of detection for the multiplexed amplification assay for HPV 16 and 18. Blue colorimetric signal under the 16, 18, or C (control) headings indicate the presence of an amplicon, showing successful amplification. The limits of detection were found to be between 10-100 copies of type 16 DNA, 10 copies of type 18 DNA, and 10 copies of 16 and 18 DNA.

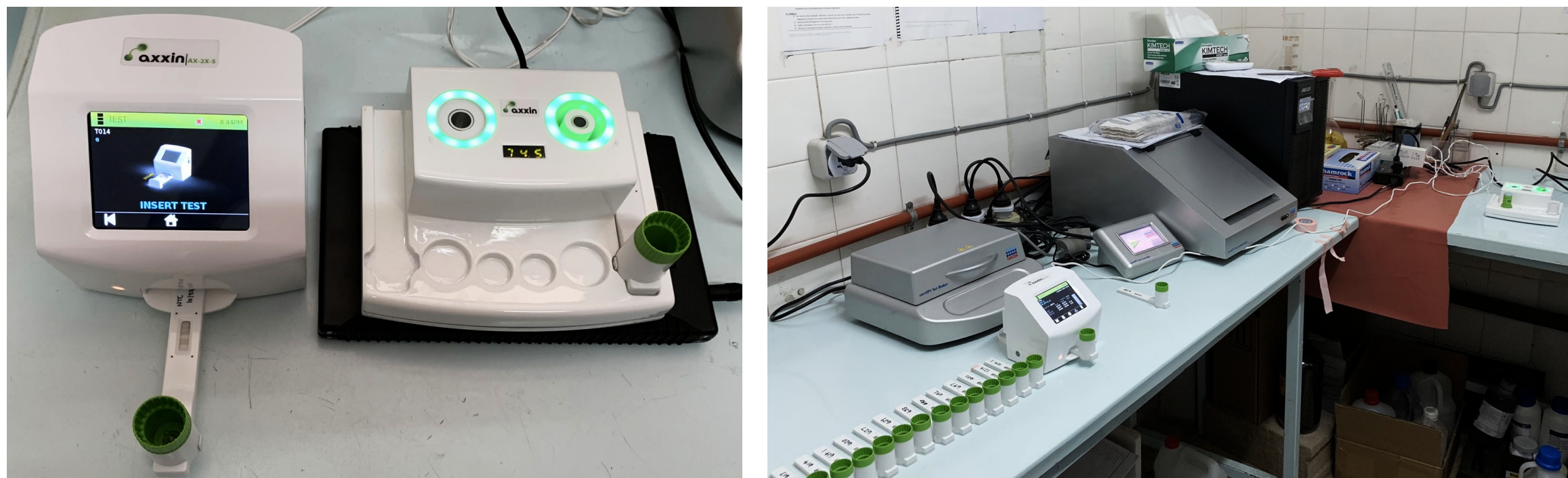
B. Specificity of HPV 16 and 18 assays. Positive signals are only visible at the 16 test line with the 16 and 16+18 samples and at the 18 test line with the 18 and 16+18 samples, showing specificity of the amplification reaction against other high- and low-risk genotypes.

C. Integrated amplification and detection within the Axxin NATflow cartridge. Limits of detection in this integrated format are 1,000 input copies of type 16 and 100 copies of type 18 DNA. Both limits of detection are acceptable clinically.

## NEXT STEPS

- Develop and incorporate an assay for pan high-risk HPV detection, e.g. with L1 consensus primers
- Refine point-of-care sample preparation methods; characterize DNA extraction from clinical samples
- Validate integrated sample preparation, amplification, and detection with clinical samples

Clinical testing in Maputo, Mozambique



## ACKNOWLEDGEMENTS

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