

MAY 06, 2020  
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## Smartphone-Based Multiplex 30-minute Nucleic Acid Test of Live Virus from Nasal Swab Extract

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### Introduction

- ❑ Rapid, sensitive and specific detection and reporting of infectious pathogens is important for patient management and epidemic surveillance. [1]
- ❑ The aim of this work is to develop a point-of-care system integrated with a smartphone for detecting live virus from nasal swab media. [2]
- ❑ A panel of equine respiratory infectious diseases was used as a model system for corresponding human diseases such as COVID-19.

### Methods

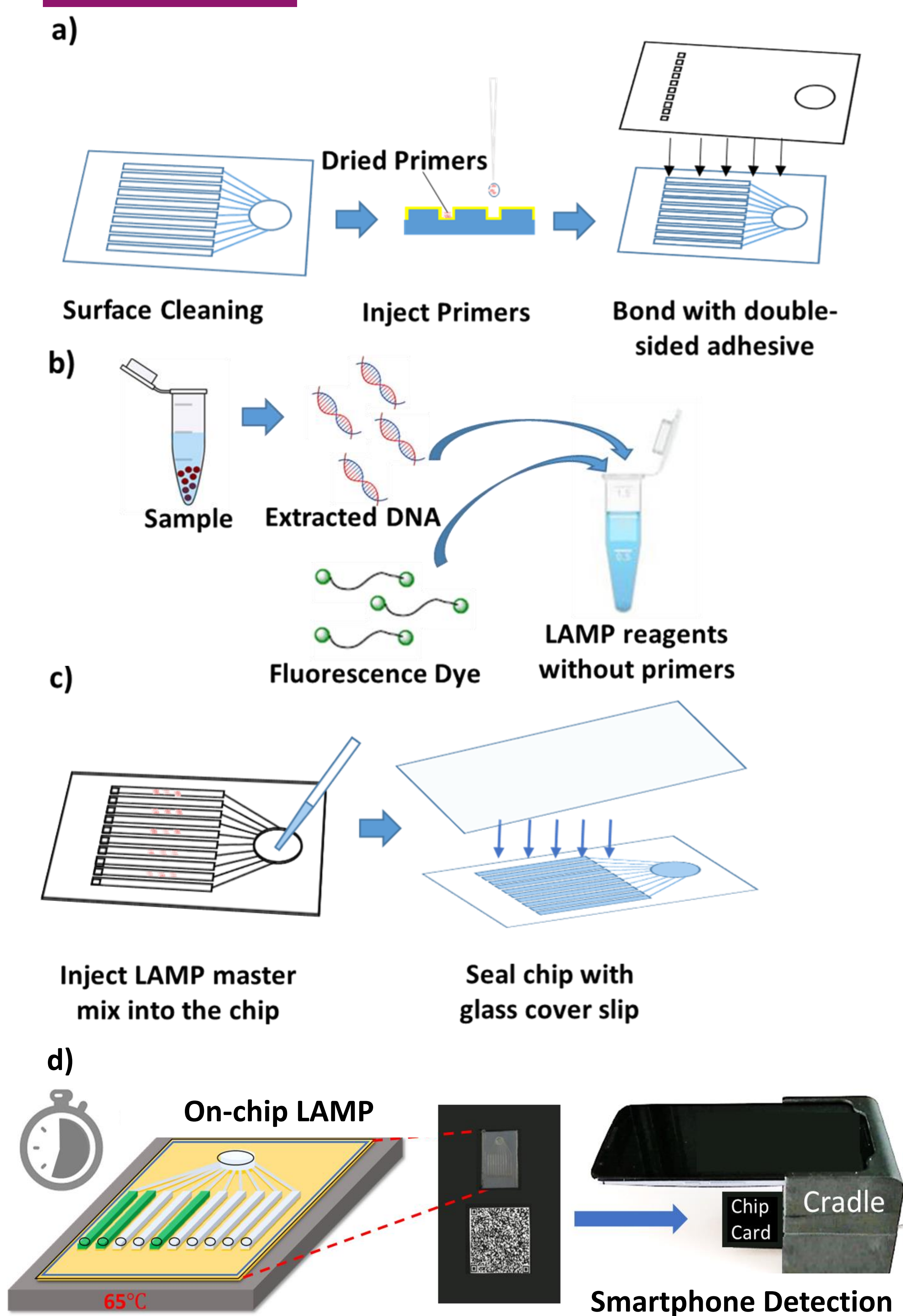


Figure 1: Detection Protocol. a) A chip will be cleaned and bound with a transparent layer of double-side adhesive after primers are deposited and dried; b) LAMP reaction master mix preparation; c) The LAMP reaction master mix will be injected through the inlet of the chip, and the chip needs to be sealed with a glass cover slip; d) after heating the chip at 65 °C for ~30 minutes, the chip will be inserted into a customized cradle for smartphone detection. Fluorescence images of the chip will be taken and used for analysis.

### Results

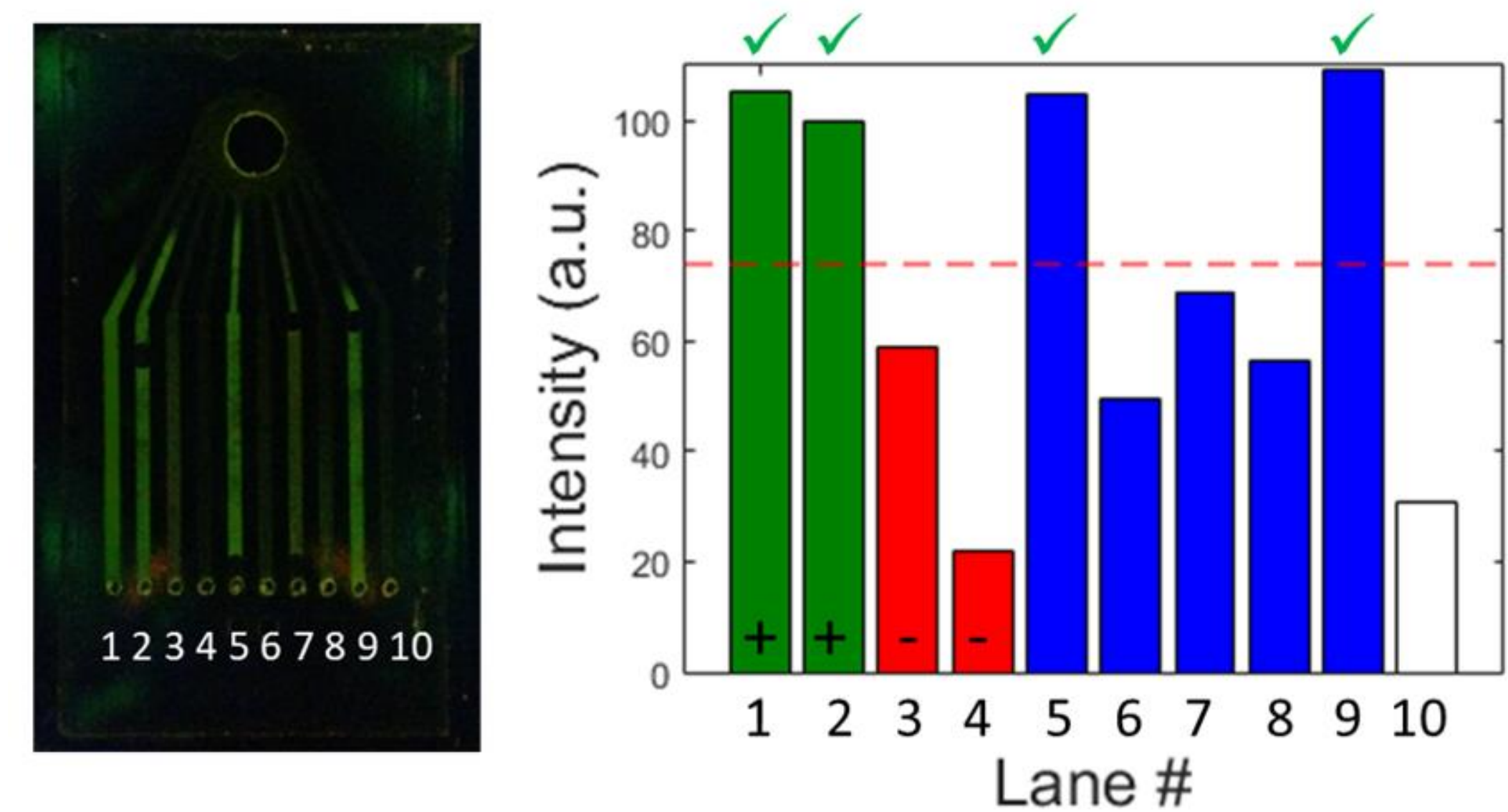


Figure 2: On-chip multiplex detection of equine respiratory infection pathogen DNA. The smartphone images of amplified chips and the corresponding average channel intensities are shown for detecting *Streptococcus equi subspecies equi* (*S. equi*) accompanied with equine influenza virus (EIV) at 1000 copies/ $\mu$ L. (Channel 1 and 2: positive controls, Channel 3: a negative control with primers, Channel 4: no-primer negative control, Channel 5-9: tests for *S. equi*, *Streptococcus equi subspecies zooepidemicus* (*S. zoo*), equine herpesvirus 1 (EHV1), equine herpesvirus 4 (EHV4) and EIV, respectively. Channel 10 was an unused test channel.

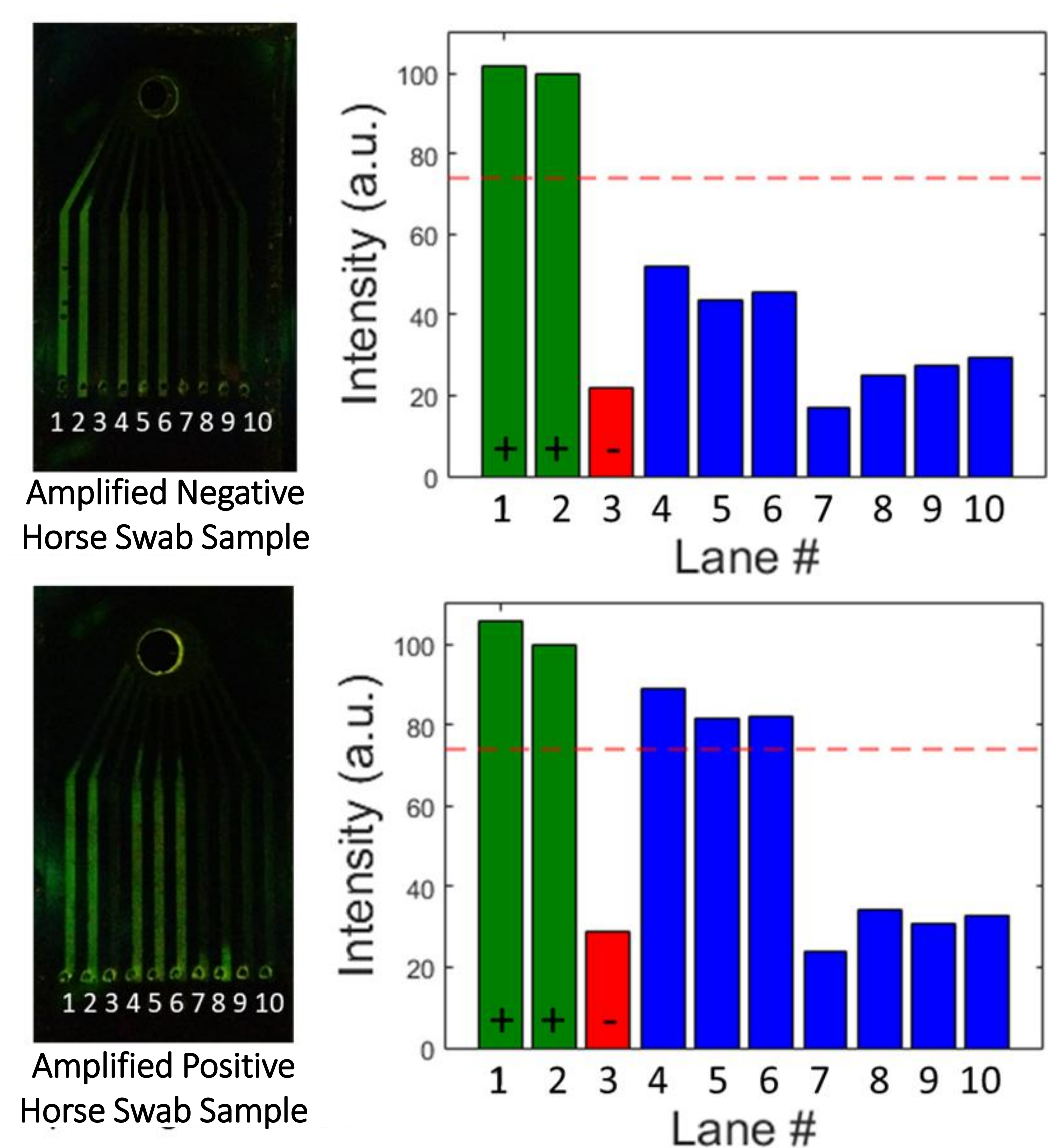


Figure 3: On-chip test results of a negative and a positive horse swab sample ( $\sim 5.48 \times 10^4$  EHV1 genome copies/mL). (Channel 1 and 2: positive controls, Channel 3: a negative control with primers, Channel 4-6: tests for EHV1, Channel 7-10: tests for *S. equi*, *S. zoo*, EHV4 and EIV, respectively.

### Conclusions

This study has demonstrated a smartphone-based system for rapid and multiplex detection of specific nucleic acids of pathogens. The sensitivity of the system is adequate for early-stage detection of viruses in nasal swab samples, down to  $5.5 \times 10^4$  copies/mL, which corresponds to about 18 copies per reaction and is comparable to the limit of detection of a PCR assay run on a commercial thermocycler.

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### REFERENCES

- [1] P. Yager, G. J. Domingo and J. Gerdes, *Annu. Rev. of Biomed. Eng.*, 10 (2008): 107-144.
- [2] F. Sun, A. Ganguli, J. Nguyen, R. Brisbin, K. Shanmugam, D. L. Hirschberg, M. B. Wheeler, R. Bashir, D. M. Nash and B. T. Cunningham, *Lab on a Chip*, 2020.

