# Point-of-care Workflow for Screening cCMV (Congenital Cytomegalovirus)

**Anastasia Giyoun Kim[1], Lesley Chan[2], Xuanchang Hu[3], Nuttada Panpradist[4]**

1 Department of Molecular Cellular Biology, 2 Bioengineering, 3 Electrical and Computer Engineering, and 4 Center for Integrated Global Health of Women, Adolescents, and Children, School of Public Health, University of Washington, Seattle, WA, USA

**Corresponding author:**
NP (nuttadap@uw.edu)

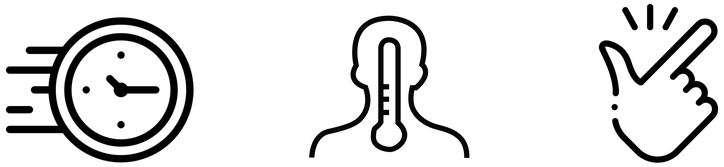
## Background

### Why cCMV?

- 6-14% in low- and middle-income countries **[1]**
- 20% of infants born to seropositive mothers affected **[2]**

### Why need our onsite, equipment-free testing?

- Delayed diagnosis → higher incidence of disease and long-term complications (50% of infants develop complications including hearing loss, vision loss, and neurodevelopment delay.) **[3, 4, 5]**
- Traditional PCR method has long turnaround.



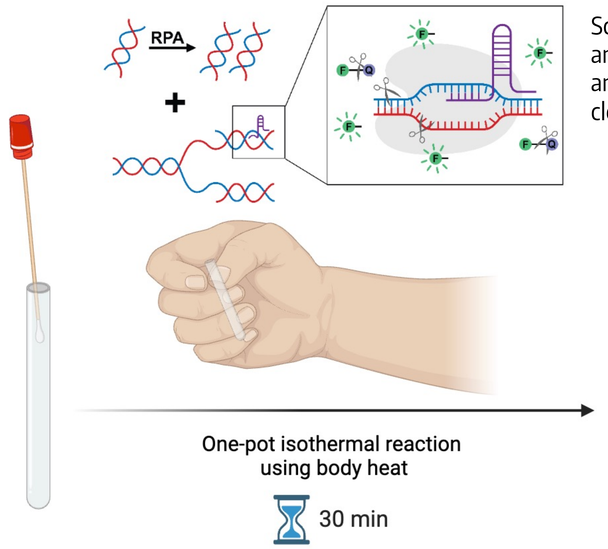
**Quick Results**
Visible in 30 min

**Body Heat Driven**
No equipment needed

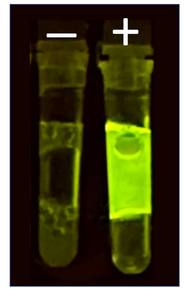
**Simple Assay**
User friendly

## Materials and Method

### Simple workflow from patient urine samples to analysis using software.




Schema of a **single-pot** reaction of **isothermal** amplification by **RPA** (recombinase polymerase amplification) and target-activated **Cas12a** trans-cleavage of reporter substrates by CRISPR Cas12a.




Examples of images of reaction tubes after 30 minutes under the blue light are captured by cell phone and analyzed using our in-house python software

## References

**[1]** TM Lanzieri, et. al. "Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries". Int J Infect Dis. 2014. doi: 10.1016/j.ijid.2013.12.010

**[2]** S Manicklal, et. al. "The silent global burden of congenital cytomegalovirus". Clin Microbiol Rev. 2013. doi: 10.1128/CMR.00062-12.

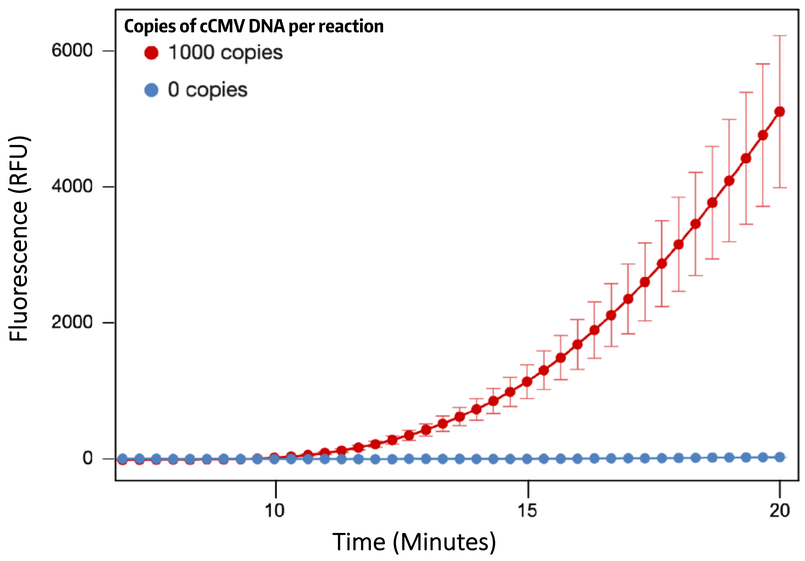
**[3]** US Centers for Disease Control and Prevention. "Cytomegalovirus (CMV) and Congenital CMV Infection". April 28th, 2020.

**[4]** MR Schleiss. "Congenital cytomegalovirus infection: update on management strategies". Curr Treat Options Neurol. 2008. doi: 10.1007/s11940-008-0020-2.

**[5]** KB Fowler. "Congenital cytomegalovirus infection: audiologic outcome" Clin Infect Dis. 2013. doi: 10.1093/cid/cit609.

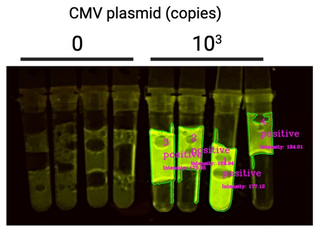
## Results and Discussion

### Assay chemistry evaluated on the qPCR machine at 37 °C




### Application on body temperature

- 0 and 1000 copies of CMV DNA were distinguishable by **naked eye** and **in-house software** after 25-30 minutes.




```
Number of contours: 4
Contour 1 intensity: 177.12 – positive
Contour 2 intensity: 162.94 – positive
Contour 3 intensity: 175.35 – positive
Contour 4 intensity: 124.01 – positive
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- Longer reaction time is needed due to the **variability of body temperature from individuals and the fluctuation** of body temperature within the palm.
- We aim to **prevent false positives and false negatives by personalizing the reaction time according to individual body temperatures**. Include a body temperature measuring unit, automatically providing the guide for the required reaction time based on user's body temperature.

## Conclusions

- We have successfully demonstrated the development of **a new isothermal amplification for cCMV DNA detection**.
- The rapid detection and equipment-free operation hold great promise for cCMV diagnostics in low-resource settings.

## Acknowledgments

**Collaborators**: Drs. Jennifer Slyker, Bhavna Chohan, and Grace John-Stewart for their clinical insights from Global WACh.

**Fund**:

- 2022 Thrasher Early Career Award (PI: Dr. Panpradist) and 2023 UW ITHS Catalyst Award (PI: Dr. Panpradist).
- Ana Kim thanks 2022 Mary Gates Research Scholarship. Ana Kim, Lesley Chan, and Xuanchang Hu thank 2023 Undergraduate Conference Travel grants.

Visit the website using the QR code on the top right corner to download poster after the BMES conference ends.

# Older Drafts:

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**Comments**

- Overall this looks beautiful and I only had to make minor changes! Good job!
- I changed overall fonts to encode (UW font) as it has so many options for boldness (semi, light, thin, black, bold, etc).
- I added the affiliations. Please help me doublecheck.
- In the icon panel, there was the watermark on the middle icon so I redrew the new icon. Hope it looks okay.
- One request for correction - contact (barcode) was originally linked to Ana's linkedin. Usually this should be for corresponding authors (PI or otherwise senior postdoc, etc).
- If we want to add for presenting authors – we should have for Ana, Lesley, and Xuanchang but then it will be very crowded.
- Instead: I recommend putting it on the back of your name tag so it is even easier for people to scan and add you on their linkedin.

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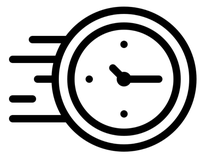

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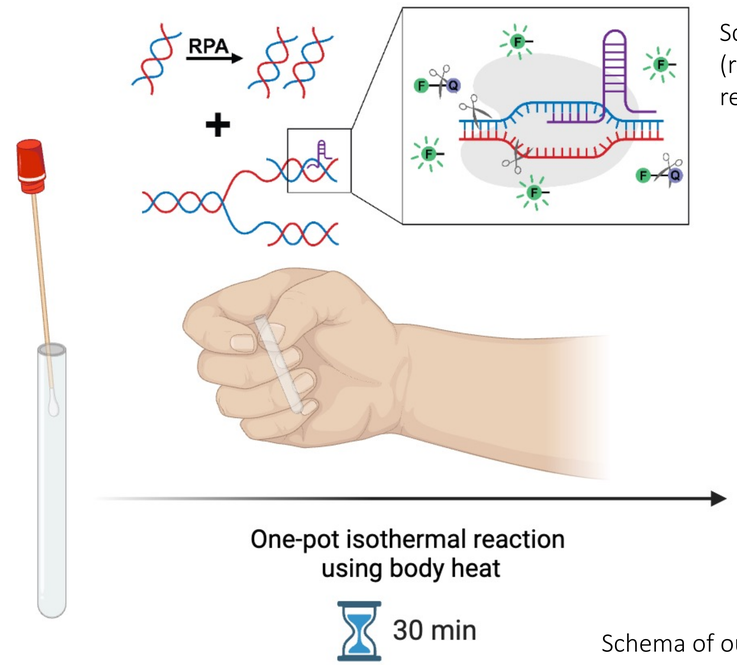


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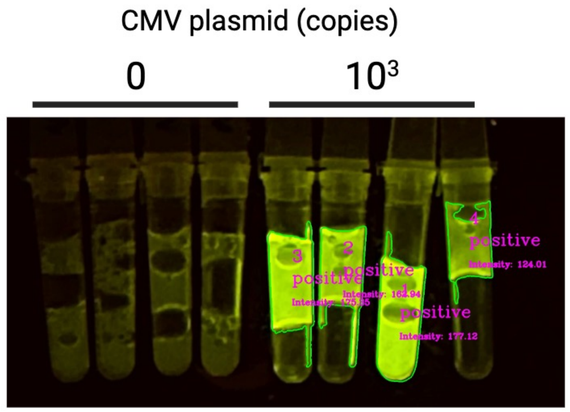


Schema of our developing simple workflow from patient samples to software detection.

## Results and Discussion

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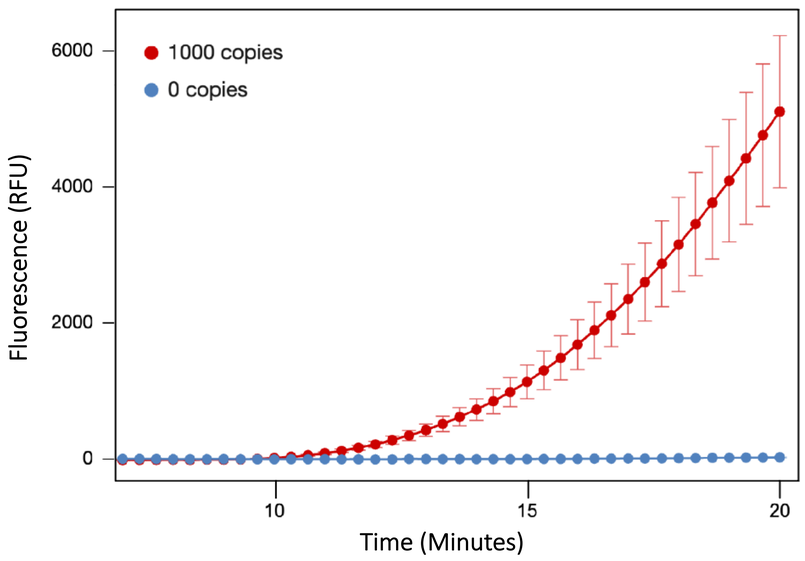


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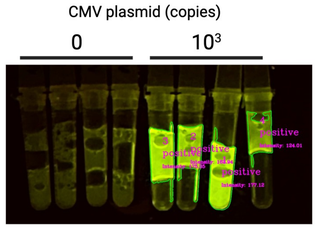


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