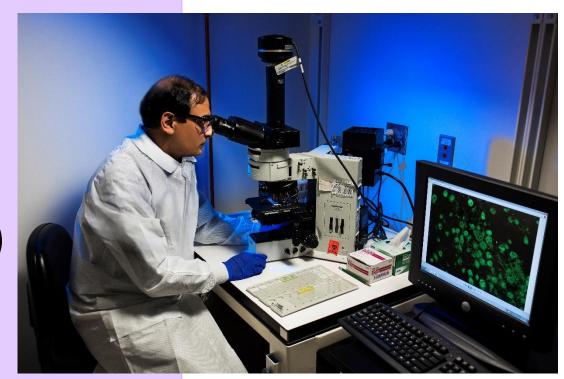


Ibne K. Ali, PhD

Background

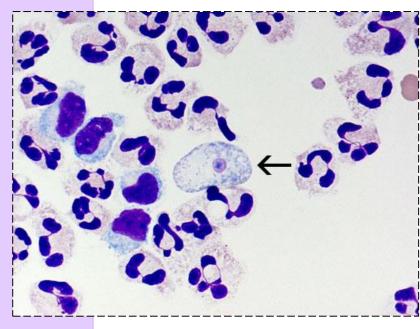
• 4 survivors in U.S.

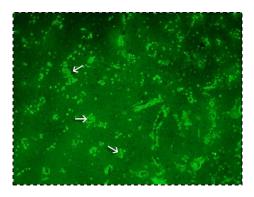
- Rapid diagnosis was a key factor for survival (between 2-3 days)
- CDC national reference lab



Diagnostic methods

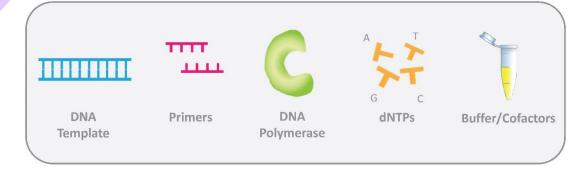
- Microscopy
- ELISA
- Immunohistochemical (IHC) assays
- Indirect immunofluorescence (IIF) assays
- PCR assays

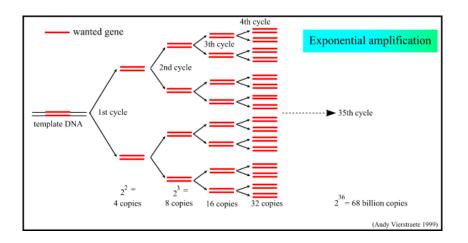




PCR?

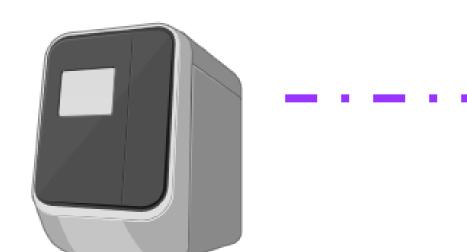
- Polymerase chain reaction
- Amplifies target DNA





Gold standard PCR

Free-living ameba (FLA) real-time PCR



✓ Reasonably fast ~3 hours

✓ Sensitive (LOD: ~1 ameba/reaction)

✓ Specific

✓ Reliable

Why we need a new diagnostic test

FLA real-time PCR disadvantages

- Only available in reference and research laboratories
- Requires a relatively complex DNA extraction step
- Requires expensive real-time PCR instrument
- Real-time PCR and data analysis requires expertise

Not suited in hospital settings

Solution: Point-of-Care (POC) test

Common point-of-care tests



Any test that is performed at or near the patient or generally in a healthcare setting and not in a lab

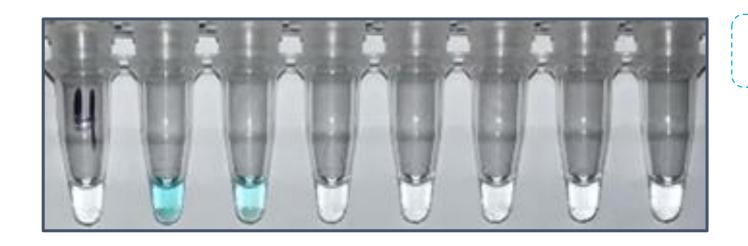
Why LAMP as POC?

Simplicity of POC test

Capabilities of the Gold Standard

LAMP Assay

Loop mediated isothermal amplification assay



Results in ~1 hr

Single tube reaction

Colorimetric indicator

No special equipment

N. fowleri LAMP Assay 1:00 Hour IIII Negative **65** c ₿99 c Blue tubes are Add template DNA Heat extract Run assay for positve while to tube containing DNA from CSF negative turn 1 hr at 65 C LAMP mastermix clear

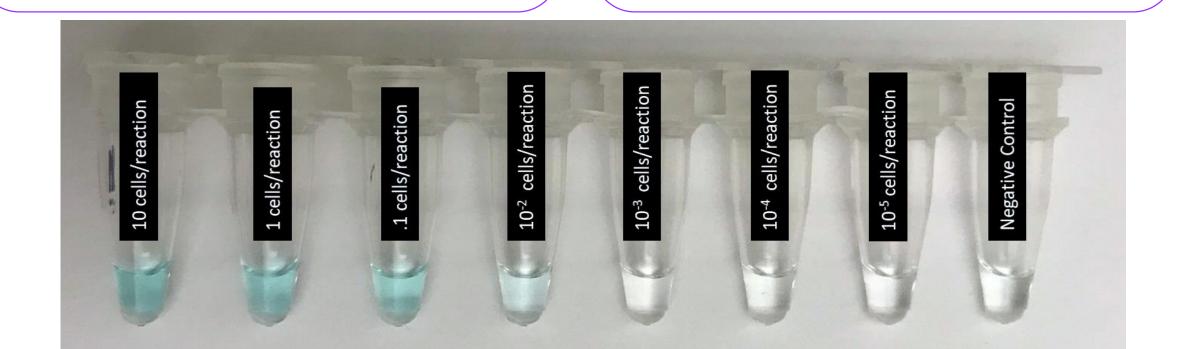


Does it work?

Results: what you can expect from lamp?

Limit of detection: it can detect *N. fowleri* present at the same concentration as FLA real time PCR

Specificity: only cross reacts with *Naegleria Lovaniensis* due to overlap in target region



Sensitivity and specificity: gold standard v. LAMP

Positive FLA real

time PCR

Positive LAMP

Negative LAMP

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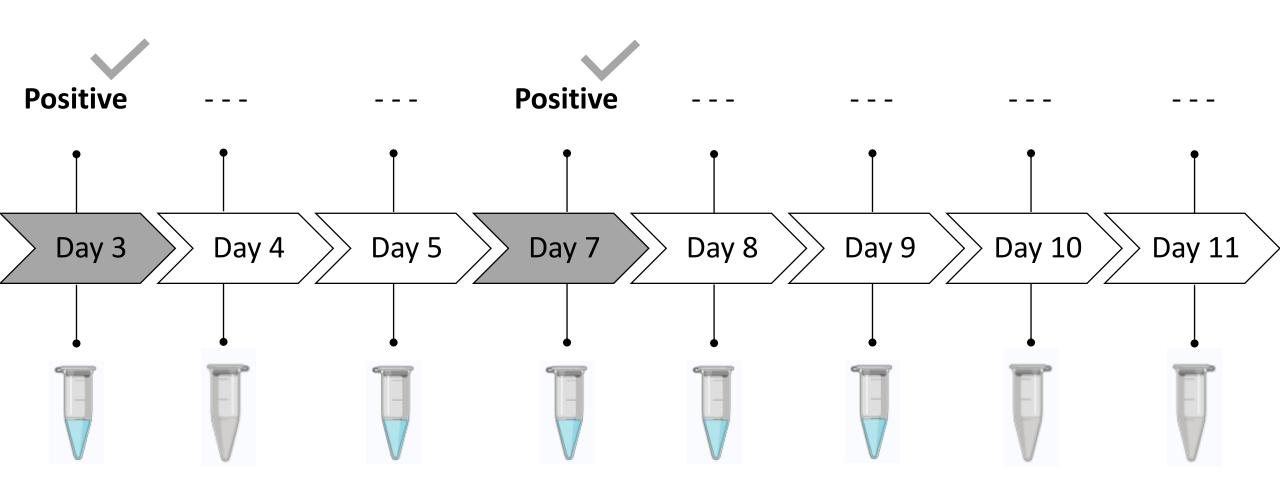
0

Negative FLA real time PCR

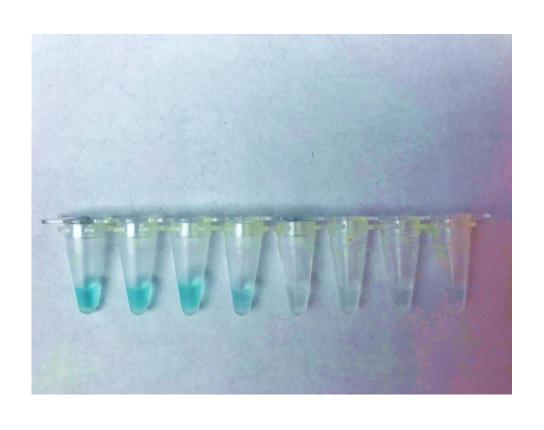
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17

FLA real-time PCR

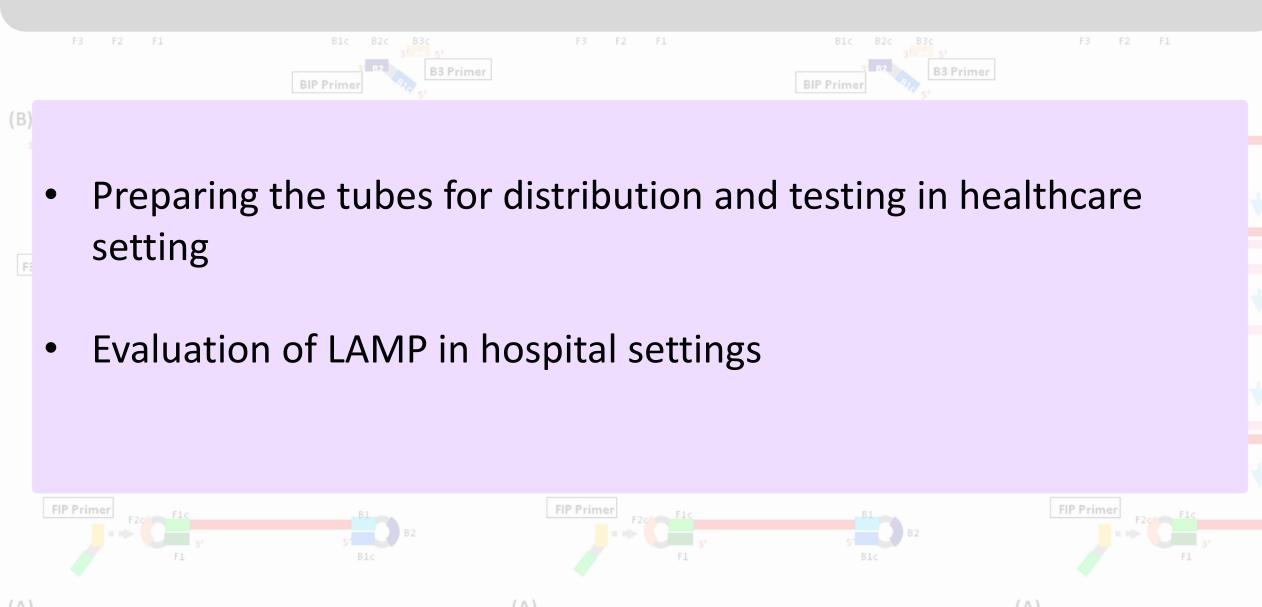


Benefits



- * Time efficient
- Crude extraction method
- Easy to read
- Portable
- Inexpensive

Ongoing work



Acknowledgements

- Jordan Smelski and Kyle Lewis Foundations
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- CDC Biotechnology Core Facility

***images created with BioRender @biorender.com

***All other images from Google