Nobuyuki Futai[1]
Ashwin Ramachandran[2]
Catherine Hogan[3]
Kanagavel Murugesan[3]
Niaz Banaei[3]
Juan G. Santiago[2]

Cell-free DNA enrichment for rapid and lower-cost molecular diagnostics for tuberculosis using isotachophoresis

1 Department of Mechanical Engineering, Shibaura Institute of Technology, Tokyo, JAPAN
2 Department of Mechanical Engineering, Stanford University, CA, USA
3 Department of Pathology, Stanford University, CA, USA

Introduction

- PCR-based detection of pathogen-derived cell-free DNA (cfDNA) in plasma is promising as a non-sputum-based biomarker assay for tuberculosis (TB) diagnosis. It will replace expensive and invasive sampling required for extrapulmonary TB, pediatric TB, and other patient populations in resource-limited countries.
- To increase the sensitivity of cfDNA detection while maintaining specificity, enrichment and purification of pathogen-derived cfDNA is required. However, a simple, rapid, and efficient enrichment and purification method from plasma still remains a challenge.
- Isotachophoresis (ITP) can rapidly extracts and purifies cfDNA from the sample contains plasma proteins and other PCR inhibitors in less than 60 min.

Major Challenges in ITP of Plasma Samples Include:

A) High concentration of ions (Na^+, $HCO3^-$ etc) prevents electrolyte interface formation.

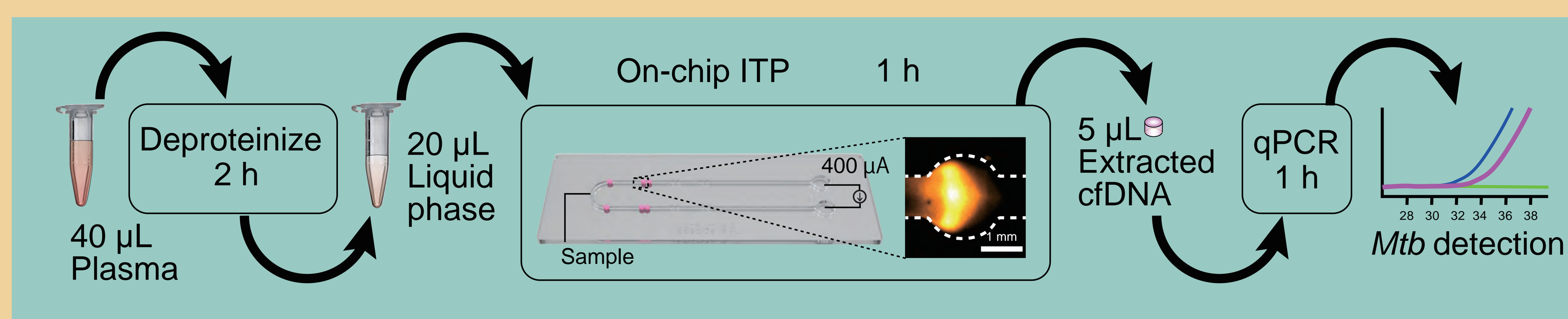
B) Proteins and peptides that alter the electrophoretic mobility of DNA and that act as spacer molecules.

▶ We have developed a rapid and automated assay system that uses ITP-based purification and qPCR that we can detect cell-free DNA (cfDNA) markers for M. tuberclosis from small amount of raw plasma in a short amount of time.

Methods

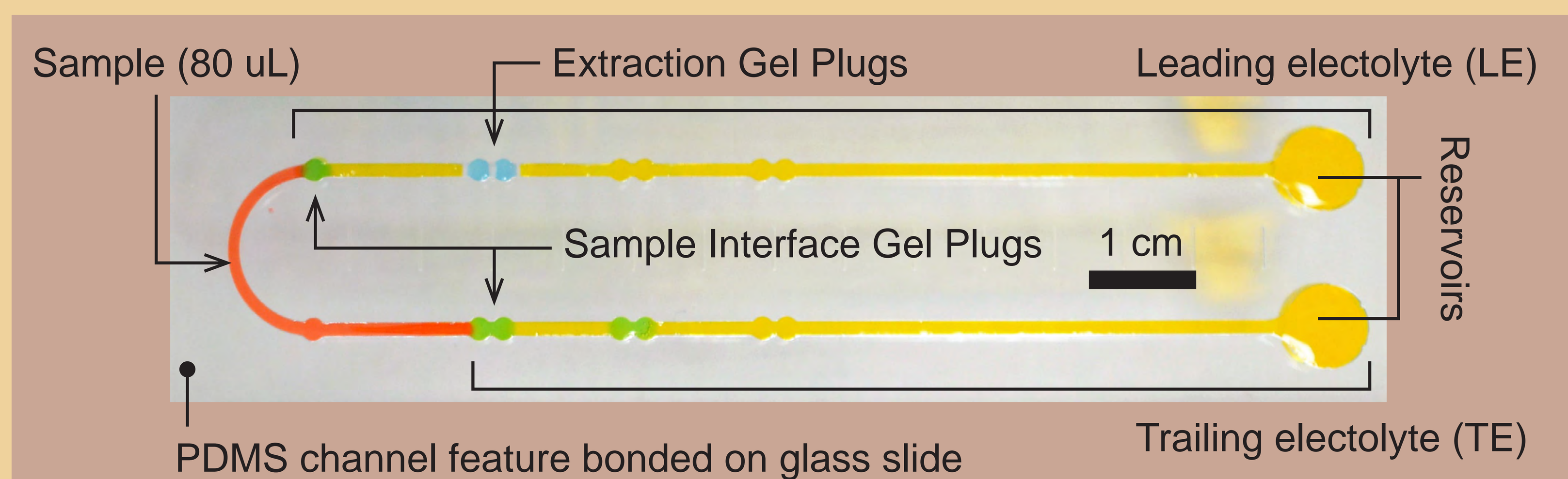
Assay Overview: microfluidic ITP with qPCR

- Detect trace target cfDNA using 40 microliters of plasma.
- A microfluidic device that uses ITP to enrich cfDNA within 60 min including setup time, followed by qPCR (~1 h).
- Safe deproteinization by heat and Proteinase-K digestion.



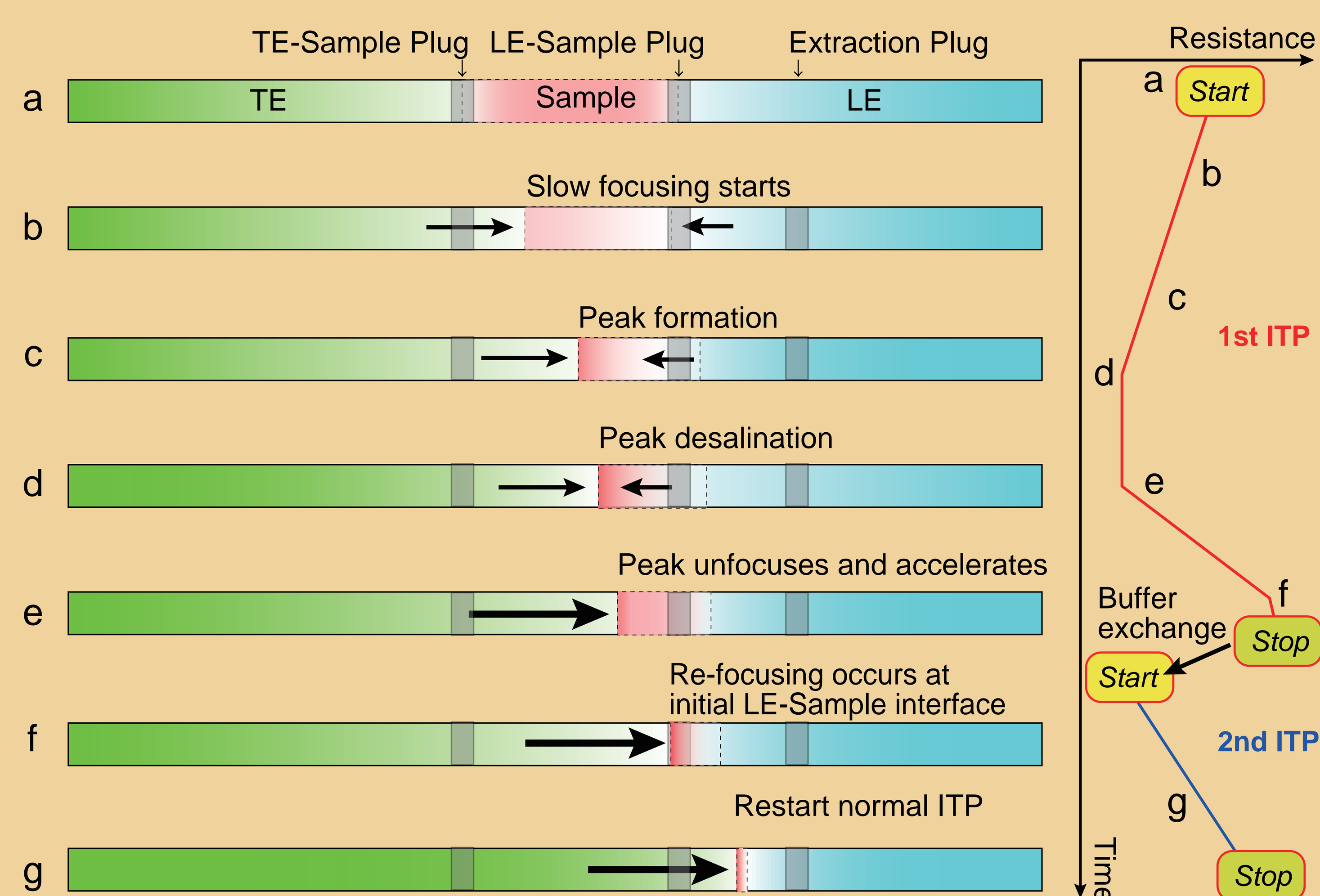
Microfluidic ITP Device

- Softlithographic PDMS open channel with agarose gel plugs for sharp sample-LE/TE interfaces and easy extraction.
- Large channel for pH buffering capacity and heat dissipation.



ITP peak 'jump' and 2-stage ITP

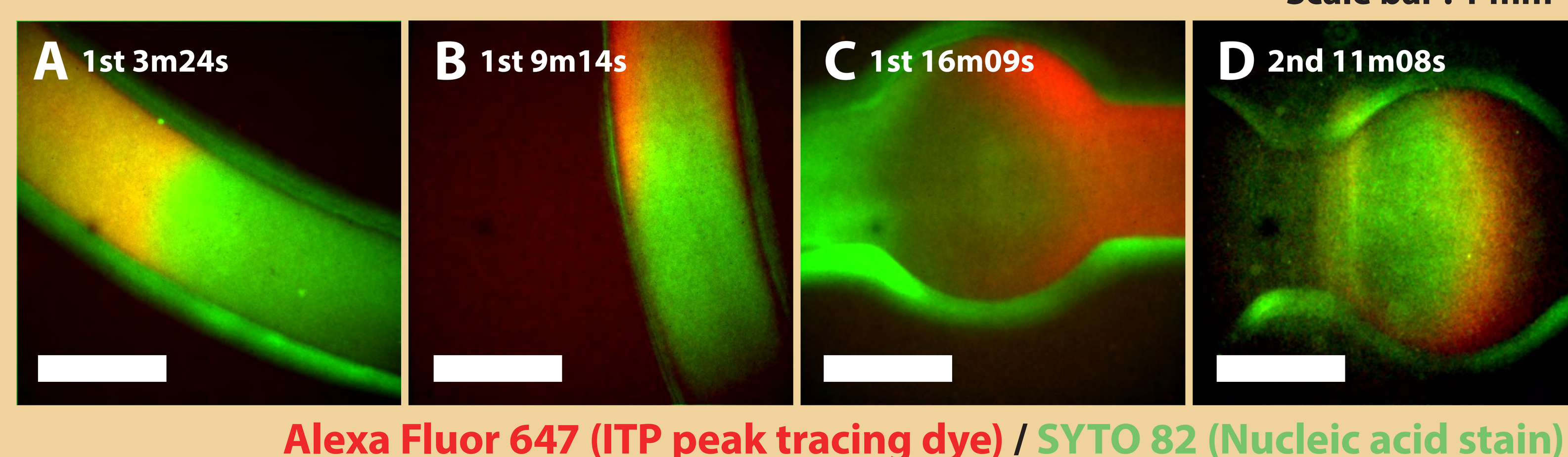
- During 1st ITP, Na^+/$Counter^+$ interface that migrates toward TE slows down DNA focusing, and desalination occurs.
- When Na^+/$Counter^+$ and TE^-/$HCO3^-$ interfaces meet, DNA start unfocusing but migrating about 2x faster.
- LE-Sample plug captures DNA that refocuses due to sharp concentration gradient of $Counter^+$.
- Since DNA has been captured in gel, ITP can be restarted after buffer exchange. The 2nd ITP is a 'normal' ITP.



Results

2-stage ITP

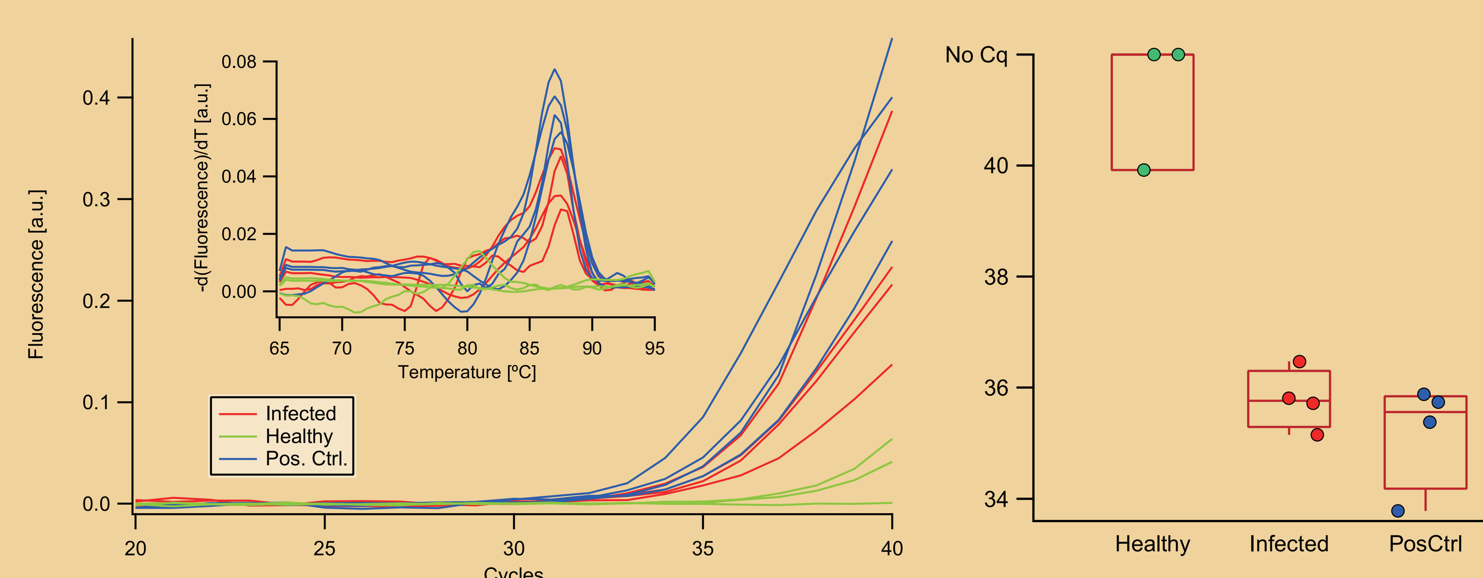
Scale bar : 1 mm



Alexa Fluor 647 (ITP peak tracing dye) / SYTO 82 (Nucleic acid stain)

- A) Slow ITP focusing starts. DNA is not yet well focused.
- B) ITP peak starts to unfocus and shows rounded velocity profile. DNA follows the tail of the ITP peak.
- C) ITP peak refocused at the initial Sample/LE interface. Large amount of DNA(-peptide comlex) was left behind the ITP peak.
- D) After 2nd ITP, both ITP peak and DNA band were sharpened.

qPCR of Mtb DNA



- qPCR of IS6110 sequence from plasma from healthy donors and from an infected patient, and Mtb gDNA fragment as Pos. Ctrl),
- Cq of infected plasma was 35.79±0.41, close to the positive control (35.20±0.72). Healthy plasma showed Cq larger than 39.

Conclusions

- We have developed a robust millifluidic isotachophoretic (ITP) method to enrich and purify cfDNA from human plasma using a reusable elastomeric chip with simple sample loading and extraction of DNA focused by ITP.
- A selective focusing of pathogen-derived cfDNA with ITP was confirmed with quantification cycle (Cq) values obtained by off-chip quantitative PCR of IS6110 repetitive sequence in M. tuberculosis genome.