Accelerate Sepsis Diagnosis by Seamless Integration of DNA Purification and qPCR

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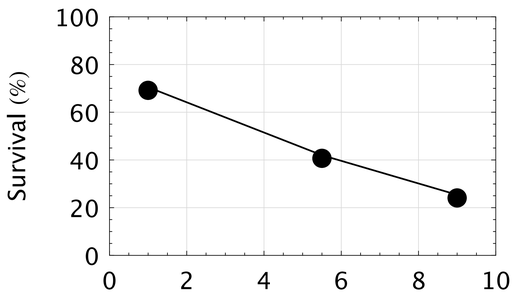
① Sepsis

- bloodstream infection
- 25+ pathogen types [1]
- delayed/ineffective antimicrobial treatment
  → high mortality risk [1]



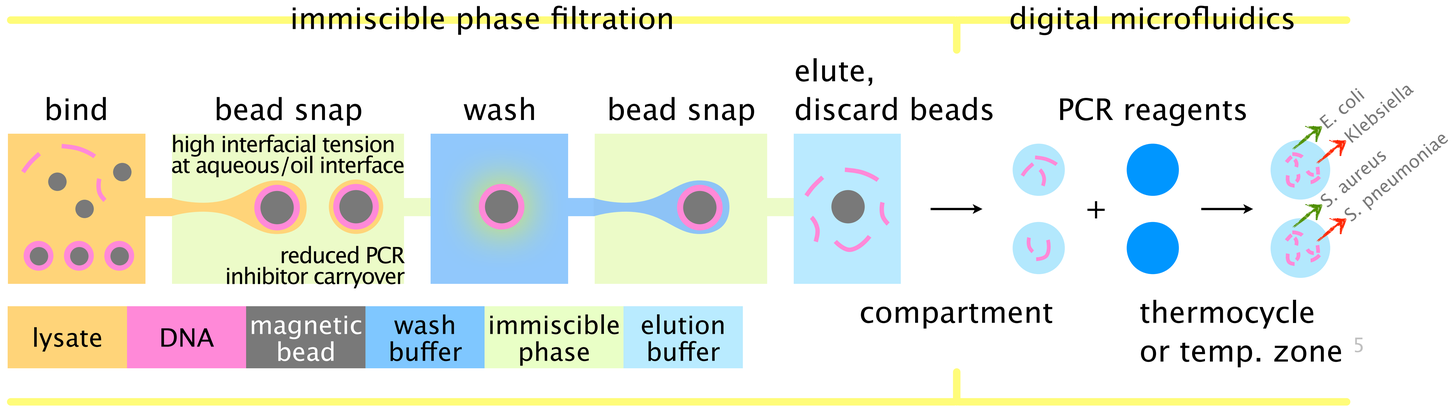
② Traditional workflow

- turnaround: 6 ~ 10 hr [2]
- hands-on: 3 hr [3]
- manual sample transfer between instruments
  → time wasted

step	hr	
mechanical lysis	0.25	manual
chemical lysis DNA purification	1.75 ~ 2.75	manual
qPCR prep	1	manual
qPCR	1.5	
melting curve	0.5	

③ Approach

- chemical lysis to qPCR on one chip
- DNA purification
  by immiscible phase filtration [4]
- followed by qPCR prep, qPCR
  using digital microfluidics



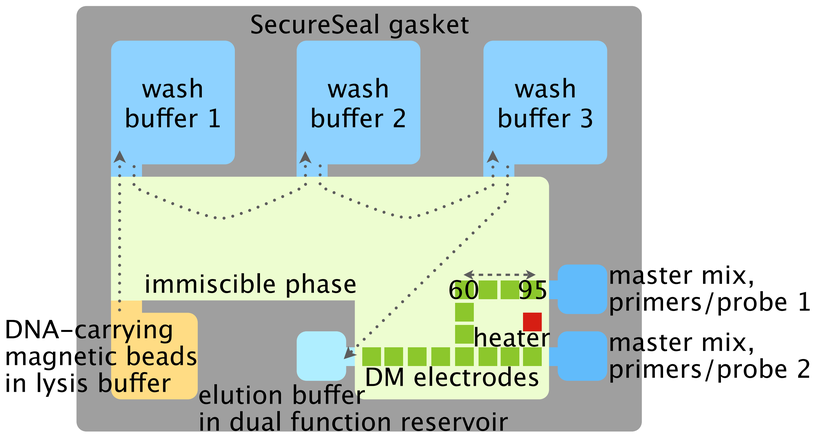
- multiplex detection:
  compartment eluent into droplets
  → multiplex qPCR in each droplet
- improved detection limit:
  small eluent volume
  → high [DNA] in droplets

④ Device

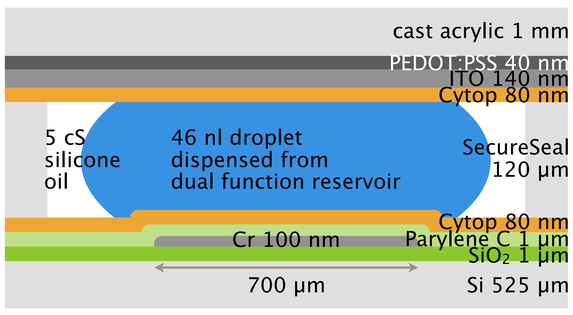
beads transported by an external magnet

droplets transported by digital microfluidic actuation

- top-down view



- side view



ITO/PEDOT/acrylic at 100 °C

- 3 Ω/□, w/o PEDOT: high Ω/□
- thermal expansion mismatch
  → cracked ITO electrode

SecureSeal vs. SU-8

- laser-cut → halved fab time
- superior sealing

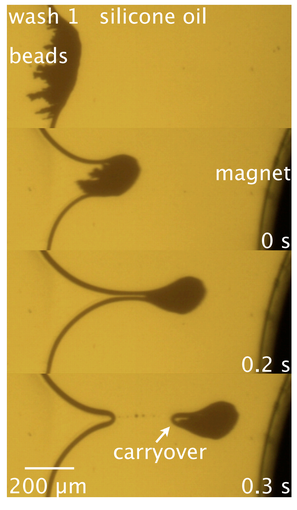
⑤ Preliminary results

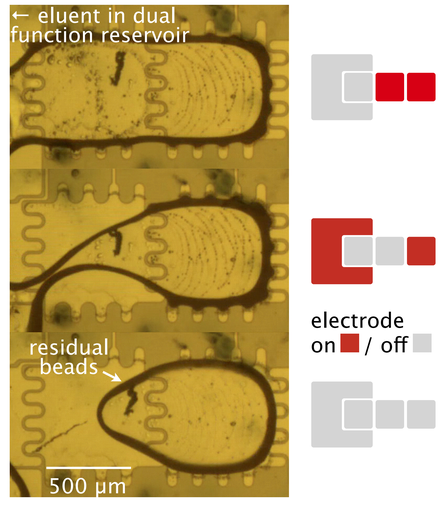
immiscible phase purification

- DNA retention: 47 ~ 88%
  relative to benchtop protocol
- purification power
  = [inhibitor in]/[inhibitor out]
  40x lower bound
  10^2 ~ 10^3x/wash achievable

eluent compartmentation

- eluent from upstream purification stage
- concentrated dsDNA in droplet:
  2.5 ng/µl = 13x [DNA in]
- minimum residual beads





⑥ Significance

- prior immiscible phase filtration demo: 1 qPCR [6]
- integration with digital microfluidics:
  - inline auto qPCR prep
  - eluent compartmentation
    → multiple qPCR

1. *Crit. Care Med.* **2006,** *34,* 1589
2. *Crit. Care* **2012,** *16,* 404
3. *J. Med. Microbiol.* **2008,** *57,* 601
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5. *Anal. Chem.* **2010,** *82,* 2310
6. *Angew. Chem. Int. Ed.* **2008,** *47,* 3900