Microfluidic PCR in diagnostic microbiology - an overview.

Veranja Liyanapathirana
PhD Candidate: Dept Micro/CUHK
Joint Graduate Seminar: 20/12/2011

Supervisor: Prof Margaret Ip
Outline

• Laboratory diagnosis of infectious diseases
• Microfluidic PCR
• Applications of microfluidic PCR in diagnostic microbiology
• Issues and future
Diagnostic microbiology

• Laboratory diagnosis of infectious diseases
• Why? - aetiological diagnosis, tailor made treatment, infection control, epidemiology and prevention

• How? – Conventionally, by culture based methods and serology
• Time consuming, requires equipped laboratories, training, man power

• Quest for better tests – Molecular methods
PCR in Diagnostic Microbiology

- “Rapid”, Accurate, Better sensitivity
- “Time consuming” – high thermal mass
- Reagents – expensive, requires specific storage conditions
- Expensive bulky equipment
- Specific laboratory designs – (contamination)
- Trained personnel

Centralized laboratories
? Point of care diagnosis
Microlfuidic systems

- Small volumes of fluids are manipulated precisely in platforms fabricated with micro pumps, valves, etc.

Micro total analysis systems (µTAS)/ Lab-on-a-chip (LOC)

- Applications in diagnostic microbiology

  Lateral flow devices for rapid diagnosis
  - Culture
  - ABST
  - PCR
  - Microarray
  - Sequencing

©http://www.gene-quantification.de/lab-on-chip.html
Microfluidic PCR

First described in 1993 by Northrub et al

Potential advantages over conventional PCR

Faster speed
Less reagent usage
Automation
Complete integration
High throughput
Portability

Microfluidic PCR

- Reaction volumes
  0.45 nl – 50 µl

- Reagents
  Droplet based technology Vs dry reagent

- Heating methods
  Contact Vs non contact

- Heating and cooling rates
  175 °C/s – 2 °C/s

- Materials
  Glass, silicon, polymers

Microfluidic PCR systems

Chips -

• Stationary chamber Vs Continuous flow

Pre PCR processing

• Low levels of organisms responsible and complex nature of samples (inhibitors, other organisms)
• Options available
  • Off chip samples processing – conventional extraction methods
  • On chip samples processing – complicates fabrication separation, lysis, concentration
  • Use of unprocessed samples – overcoming inhibitions by using special Taq polymerases

Post PCR applications

• Options available
  • Off chip Vs On chip
  • Capillary electrophoresis
  • Lateral flow techniques
  • DNA hybridization
  • Real time methods
  • Electrochemical sensing
A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability.


Department of Chemistry, University of Virginia, Charlottesville, VA 22904, USA.
Applications in diagnostic microbiology

- Numerous papers – Dengue, Hep B, MRSA, SARS corona etc
- Initial work concentrates on chip thermal cycling
- Integrated methods Vs isolated use of one component
- Direct detection from patient samples Vs characterization of isolates
- Majority of studies conducted in research settings with spiked samples to represent clinical samples

- Throat swab + antibody-coated magnetic beads + RNA stabilizer in a tube
- Pumped into the device at 60 ml/h
- RNA extraction
- RT PCR Mix injected
- RT PCR
- ssDNA generation
- Detection by hybridization

- Chip dimensions - 1 x 6 cm
- Sample – result time – 3.5 hours (150 min for RTPCR)
- Detection limit ≈ 10 TCID<sub>50</sub>
Microfluidic Platform versus Conventional Real-time PCR for the Detection of *Mycoplasma pneumoniae* in Respiratory Specimens

Elizabeth Wulff-Burchfield\(^1\), Wiley A. Schell\(^2\), Allen E. Eckhardt\(^3\), Michael G. Pollack\(^3\), Zhishan Hua\(^3\), Jeremy L. Rouse\(^3\), Vamsee K. Pamula\(^3\), Vijay Srinivasan\(^3\), Jonathan L. Benton\(^2\), Barbara D. Alexander\(^2\), David A. Wilfret\(^4\), Monica Kraft\(^5\), Charles Cairns\(^6\), John R. Perfect\(^2\), and Thomas G. Mitchell\(^7\)\(*.*

Comparison of real-time PCR results of acute patient NPWs on conventional and microfluidic real-time PCR platforms

<table>
<thead>
<tr>
<th></th>
<th>Conventional real-time PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Microfluidic real-time PCR</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>56</td>
</tr>
</tbody>
</table>
Commercial applications

• At lab on a cartridge level
• Expensive
• Needs bulky equipment, uninterrupted power supply
• Eg – WHO endorsed Xpert MTB/RIF assay

Other systems by Cepheid, Microfluidic systems, Fluidgm etc

Issues

• Integration and fabrication
• Adsorption of reagents and samples by surfaces and evaporation
• Inhibition of PCR by certain products used in fabrication of devices
• Validation for diagnostic use
Future

• Multiplexing
• Not just pathogen detection, but detection of virulence and antibiotic resistance markers
• Organism sensing and detections of biomarkers for infection together
• Point of care application in resource limited setting
Distribution of commercial ventures in microfluidic technologies

http://fluidicmems.com/list-of-microfluidics-lab-on-a-chip-and-biomems-companies/

Disability adjusted life years from infections and parasitic diseases (compiled with WHO 2004 data)


Lab on a chip or chip in a lab?
References

Thank You!