Microfluidic PCR in diagnostic microbiology - an overview.

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Outline

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

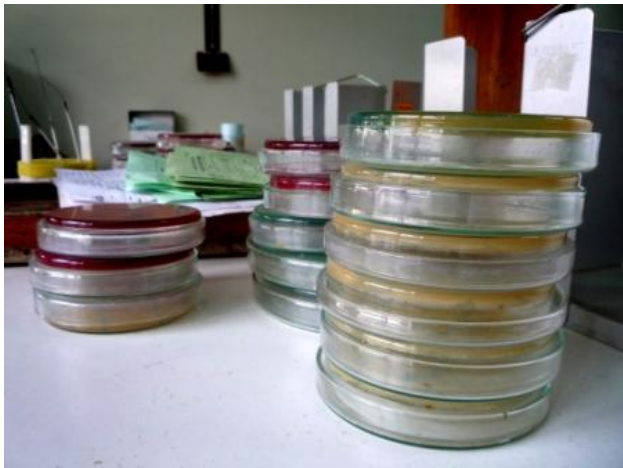
Diagnostic microbiology

- Laboratory diagnosis of infectious diseases
- Why? - aeitiological 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

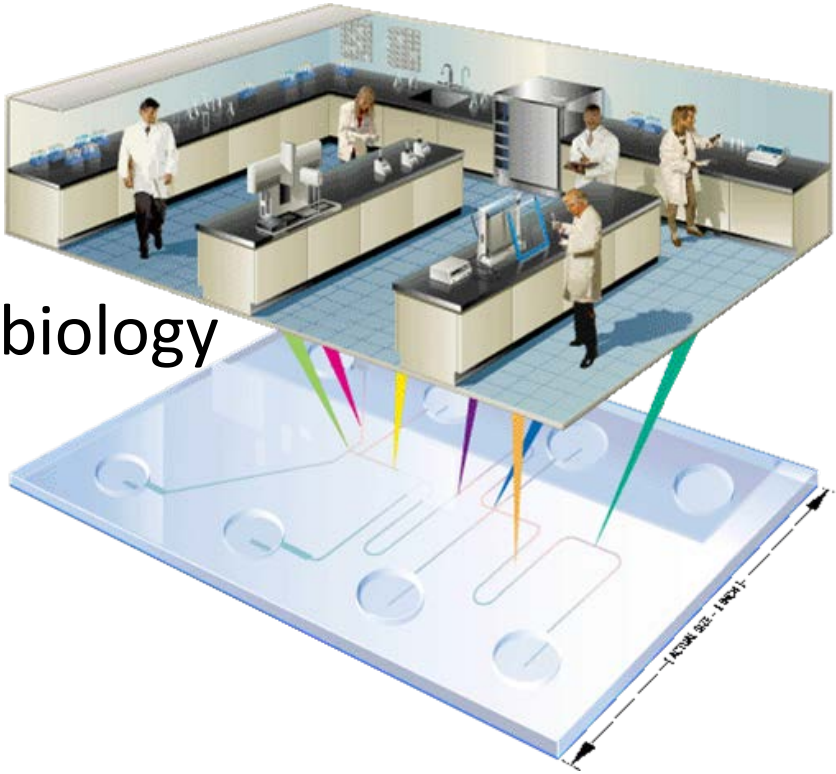
Centralized laboratories
? Point of care diagnosis

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)



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

- Applications in diagnostic microbiology

Lateral flow devises for rapid diagnosis

Culture

ABST

PCR

Microarray

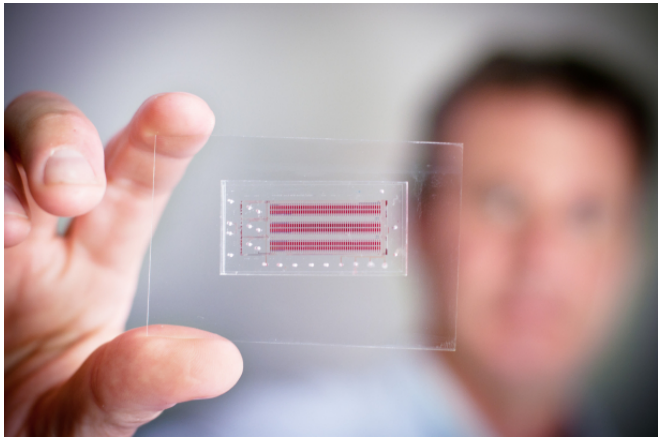
Sequencing

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



(Credit: University of British Columbia) http://news.cnet.com/8301-27083_3-20083814-247/new-lab-on-a-chip-genetic-analysis-resembles-pinball/

Microfluidic PCR

- Reaction volumes

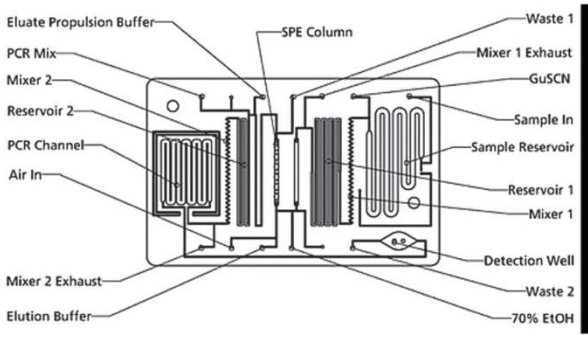
  0.45nl – 50 µl
- Reagents

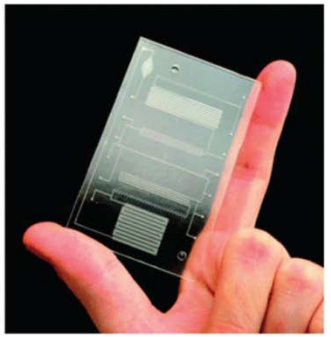
  Droplet based technology Vs dry reagent
- Heating methods

  Contact Vs non contact
- Heating and cooling rates

  175 ^{o}C/s – 2^{o}C/s
- Materials

  Glass, silicon, polymers



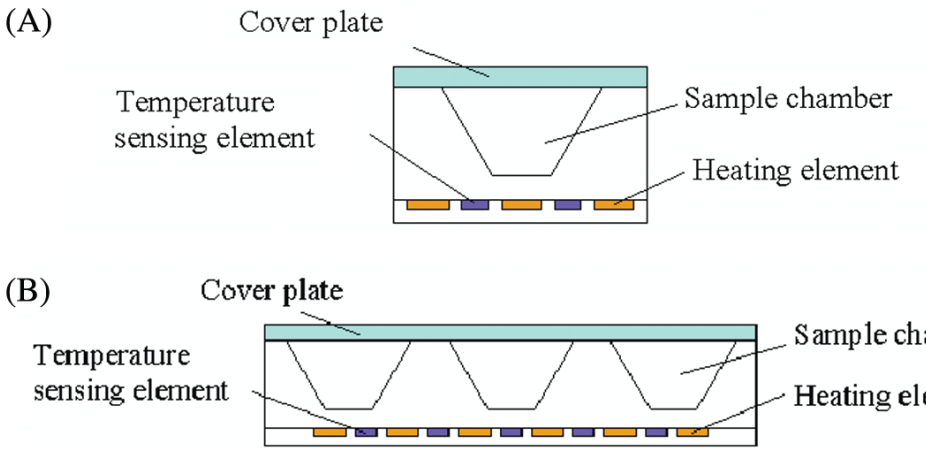


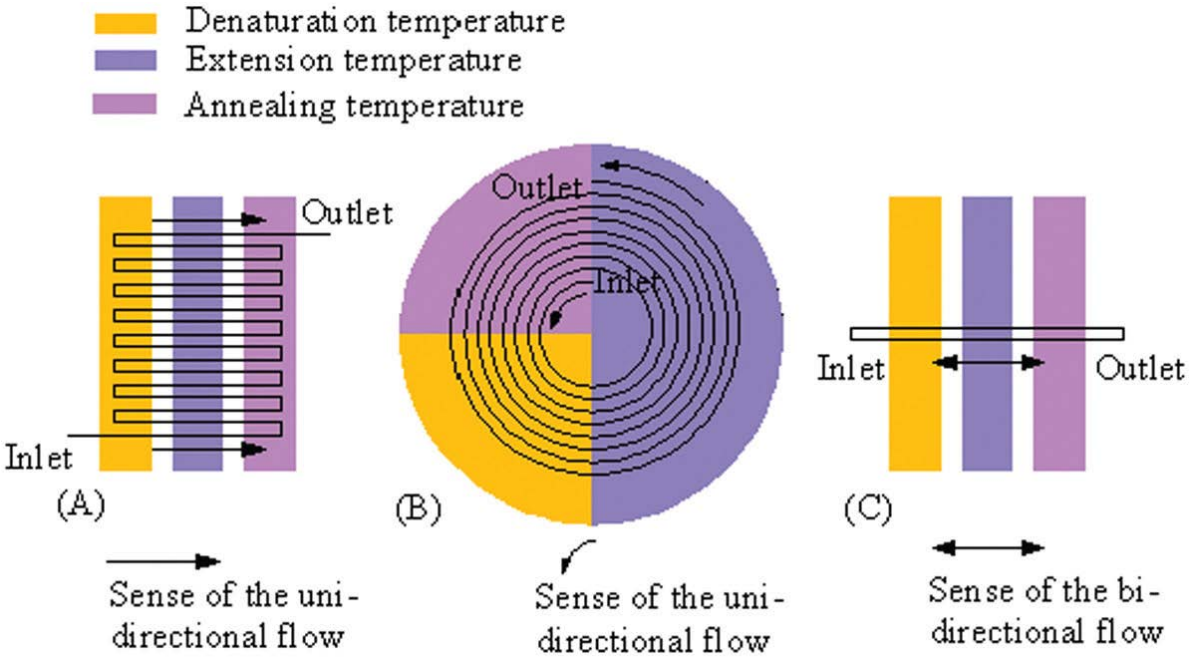
©Mark D, et al. Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. Chem Soc Rev. 2010 Mar;39(3):1153-82

Microfluidic PCR systems

Chips -

- Stationary chamber Vs Continuous flow





©Zhang C, Xing D. Miniaturized PCR chips for n ucleic acid amplification and analysis: latest advances and future trends. Nucleic Acids Res. 2007;35(13):4223-37.

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

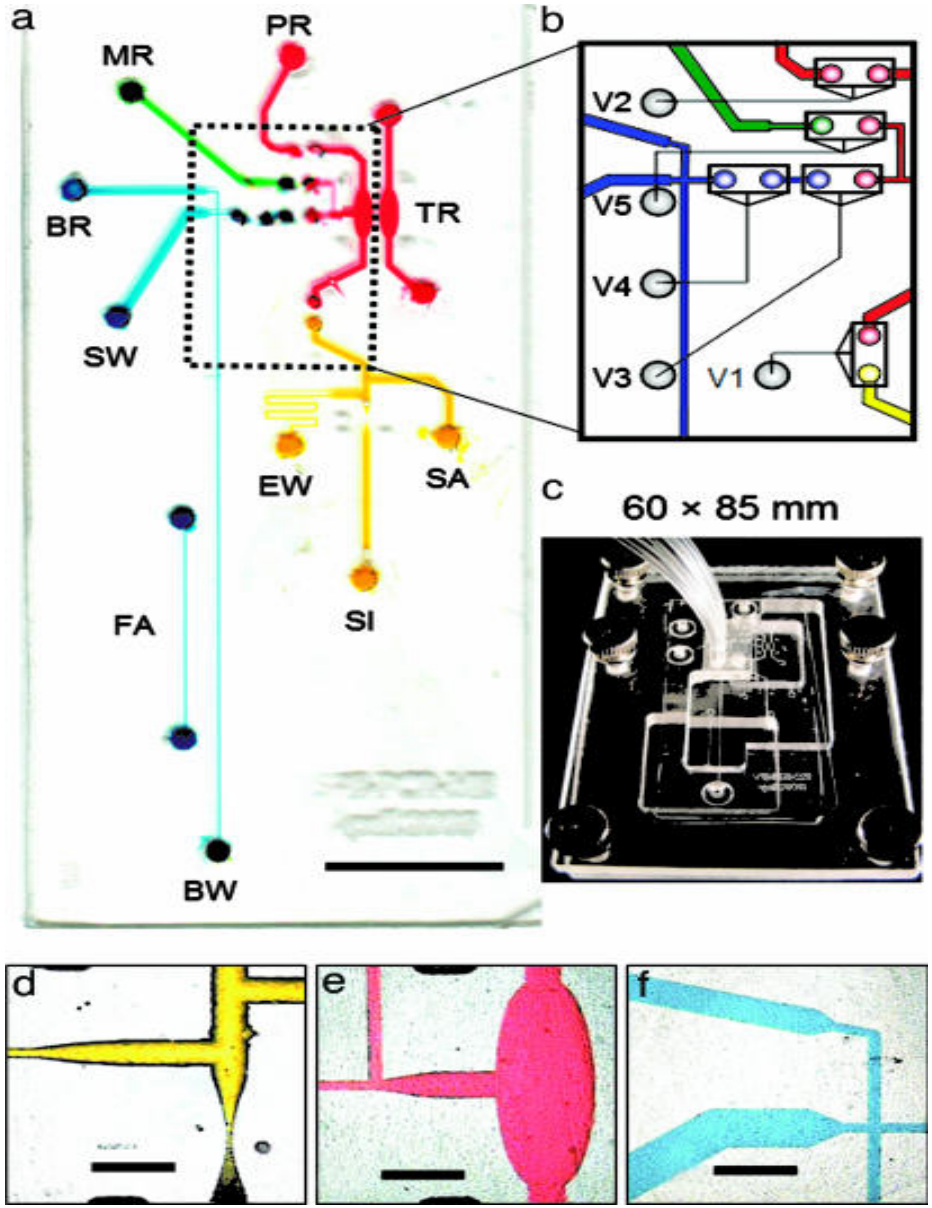
- Options available
  Off chip Vs On chip
  Capillary electrophoresis
  Lateral flow techniques
  DNA hybridization
  Real time methods
  Electrochemical sensing

Proc Natl Acad Sci U S A. 2006 Dec 19;103(51):19272-7. Epub 2006 Dec 11.

A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability.

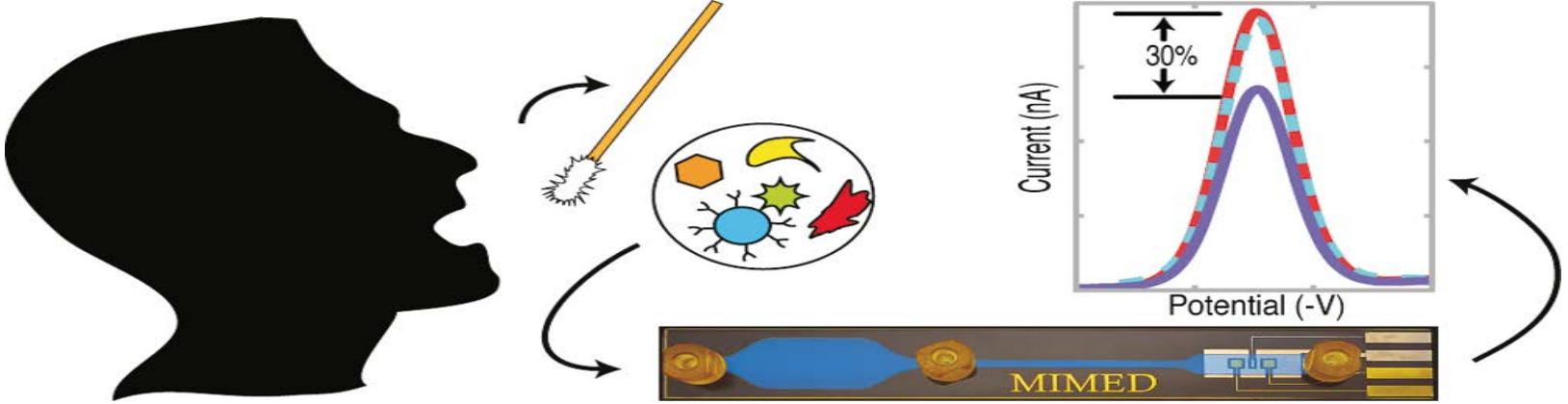
Easley CJ, Karlinsey JM, Bienvenue JM, Legendre LA, Roper MG, Feldman SH, Hughes MA, Hewlett EL, Merkel TJ, Ferrance JP, Landers JP.

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



Ferguson BS *et al*. Genetic analysis of H1N1 influenza virus from throat swab samples in a microfluidic system for point-of-care diagnostics. *J Am Chem Soc.* 2011 Jun 15;133(23):9129-35.

- 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 $TCID_{50}$

Diagn Microbiol Infect Dis. 2010 May ; 67(1): 22–29. doi:10.1016/j.diagmicrobio.2009.12.020.

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

		Conventional real-time PCR	
		Positive	**Negative**
Microfluidic real-time PCR	Positive	2	0
	Negative	1	56

Commercial applications

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

  Other systems by Cepheid ,

  Microfluidic systems,

  Fluidgm etc

Boehme CC et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 2010 Sep 9;363(11):1005-15

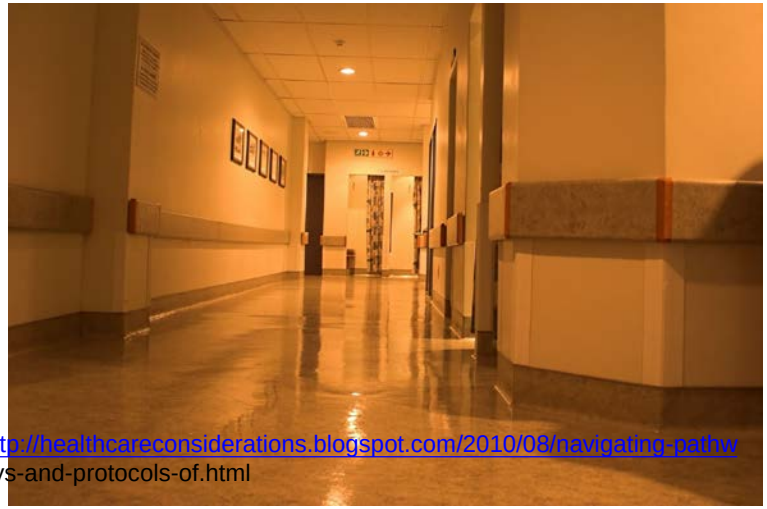
Issues

- Integration and fabrication
- Adsorption of reagents and samples by surfaces and evaporation
- Inhibition of PCR by certain products used in fabrication of devises
- 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

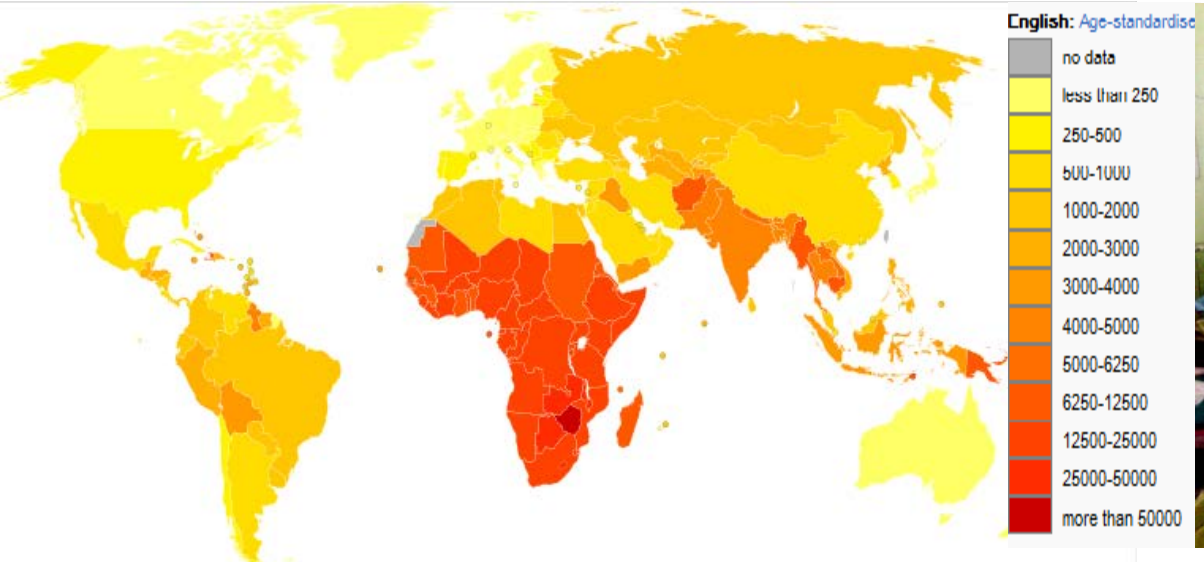
Distribution of commercial ventures in microfluidic technologies



http://healthcareconsiderations.blogspot.com/2010/08/navigating-pathways-and-protocols-of.html

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)



http://en.wikipedia.org/wiki/File:Infectious_and_parasitic_diseases_world_map_-_DALY_-_WHO2004.svg#filelinks

Lab on a chip or chip in a lab?

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Thank You!