

BLOOC Advancing Transfusion Medicine 


Applications of Microtechnology in Blood Banking and Transfusion Medicine

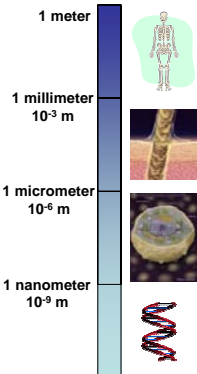
Jason Acker MBA PhD
 University of Alberta, Department of Laboratory Medicine and Pathology
 Canadian Blood Services,
 Research and Development

 Frontiers in Transfusion Medicine: Blood Bank Without Borders, November 14, 2009. 

Presentation Outline


- Nano vs Microtechnology
- Microfabrication & Microfluidics
- Application of Microfluidics in Transfusion Medicine
- Blood Group Genotyping
- Future Impact on Blood Banking






1 meter
 1 millimeter 10^{-3} m
 1 micrometer 10^{-6} m
 1 nanometer 10^{-9} m

“Think of a person that’s 6 feet tall (1,830,000,000 nanometers). A hair from that person’s head is around 50,000 nanometers wide: if you could split that hair into 50,000 separate strands, each would be a nanometer wide. A molecule of DNA is only 2.5 nanometers wide.” www.mos.org




Nanotechnology


Technology development at the atomic, molecular, or macromolecular range of approximately 1-100 nanometers that is able to create structures, devices, and systems that have novel properties



Nanotechnology Research




“Carbon Monoxide Man”
 CO on platinum



www.almaden.ibm.com

On-going international research focuses on areas such as:

- molecular scale development
- supramolecular nanoscale assembly
- materials and interfacial chemistry

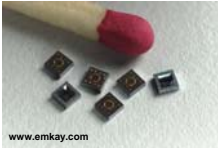




news.softpedia.com



MicroTechnology



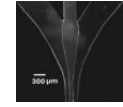
microtechnology: objects with dimensions in the micrometer to millimeter range

Microfluidics: Miniature Pipes

electricity



water

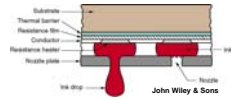


The Microfluidic Advantage

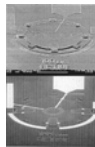
- smaller
 - small device size; portable
- faster
 - high SA / V; rapid reaction rates
 - high throughput
- cheaper
 - low reagent consumption
 - batch / parallel processing
 - disposable chips

Commercial Devices

Consumer Products – ink-jet print heads, magnetic RW heads, CDs and DVDs



Automotive Sensors – engine control, airbag sensor, power steering, AWD, ABS, stability control, cruise control



Medical Applications – blood pressure sensors, drug delivery, microarrays, single, cell processing, sensors



Laboratory Application of Microtechnology

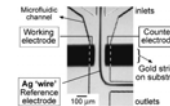


Building Small



- small feature size
- precise geometrical control
- reduced material consumption
- batch processing; scalability
- integration of components
- ➔ **low cost**

Microfluidic Components

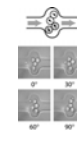
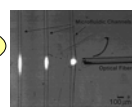
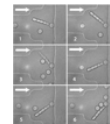


valves

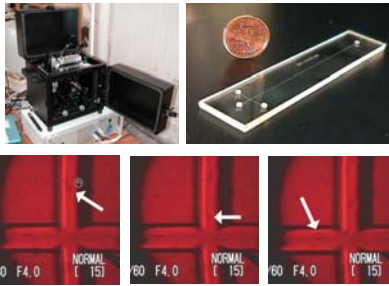
sensors

pumps

mixers



Microtechnology: Lab-on-a-chip

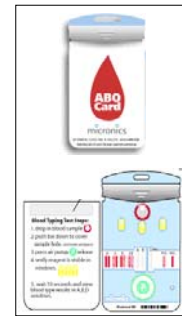


Separation of RBC from Whole Blood

Blood Serology & Analysis



RBC and platelet counts,
supernatant hemoglobin
concentration, WBC
differential analysis

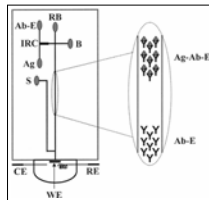


Microfluidic Immunoassays

Anal. Chem. 2005, 77, 5022-5027

Electrochemical Enzyme Immunoassays on Microchip Platforms

Joseph Wang, Alfredo Salinas, Marika Piskunov, Chaitali, and Alberto Escamez
Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003

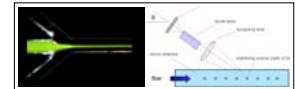


- glass chip; 50 μm channels
- off-chip amperometric detector, reservoirs and electronics
- IgG / anti-IgG-AP
- electrophoretic separation and detection of Ab-Ag
- 2.5 x 10⁻¹⁶ g/mol sensitivity
- HBsAg: 10⁻¹² g/mL

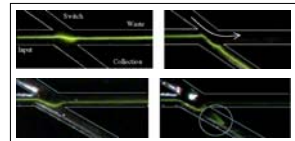
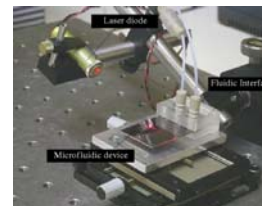
Flow Cytometry / Cell Sorting

Development of a microfluidic device for fluorescence activated cell sorting

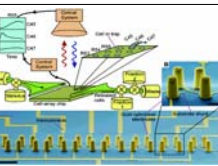
Jon Kruger, Alton Singh, Alan O'Hall, Carl Jackson, Alan Morrison, and Peter Wilson
Research Institute for Health Care, University of Alberta, Edmonton, Alberta, Canada
Department of Chemical Engineering, University of Alberta, Edmonton, Alberta, Canada



hydrodynamic streaming and detection

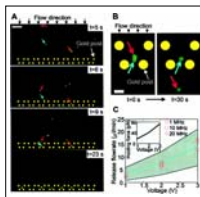
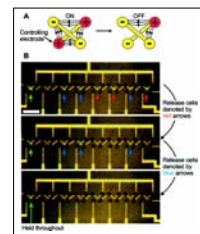


hydrodynamic switching



A Microfabrication-Based Dynamic Array Cytometer

Joel Voldman, Marika L. Gray, Michael Toner, and Martin A. Schmidt
Department of Electrical Engineering and Computer Science and Division of Health Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Center for Engineering in Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114

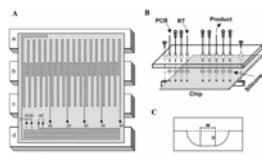


Nucleic Acid Amplification

Anal. Chem. 2005, 77, 2004-2010

Microfabricated Device for DNA and RNA Amplification by Continuous-Flow Polymerase Chain Reaction and Reverse Transcription-Polymerase Chain Reaction with Cycle Number Selection

James J. Good, Thomas S. Chong, and Christopher J. Beaudoin
Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada
Department of Chemical Engineering, University of Alberta, Edmonton, Alberta, Canada
Department of Electrical and Computer Engineering, University of Alberta, Edmonton, Alberta, Canada



- 4 x 4.5 cm glass chip
- 40 cycle-PCR (15.3 μL)
- = 95°C (denature), 58°C (primer anneal), 72°C (extension)
- RT (5 μL)
- = 43°C, 95°C (inactivation)
- 21 nL/s; 13 s / cycle
- continuous flow
- = syringe pump + manual
- electrophoresis / EB

- **integrated continuous flow RT and PCR**
- **DNA + RNA amplification**
 - = **no observed non-specific amplification products**
- **multiple sample processing**
 - = **no cross contamination**
- **reusable**
- **β -globin gene amplification from 200 μ L whole blood**

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An Integrated PCR Chip

- **plastic chip (60 x 100 x 2 mm) with integrated electronics and microarray sensor**
 - = **on-board magnetic bead cell capture**
 - = **disposable pumps, mixers, valves**
 - = **PCR & DNA hybridization**
- **bacterial detection**
- **SNP detection of HFE-C**

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Blood Group Genotyping

- molecular basis of most blood group systems known
- genotyping of patients and donors is becoming more routine with a number of techniques and equipment available including available on the market
- BloodGen, CBGG, AABB, ISBT & others developing standards for genotyping

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Human Platelet Antigens

- alloimmunization against human platelet antigens (HPA) can result in:
 - platelet transfusion refractoriness
 - neonatal alloimmune thrombocytopenia
 - post-transfusion purpura.
- molecular basis of a majority of HPA antigens have been resolved
 - most are identified as a single nucleotide polymorphisms
- CBS routinely genotypes apheresis platelet donors for HPA-1
 - genotyping of HPA-1 to -6 and 15 is also performed on occasion

Indian Ped 41 (8): 797-805.

- HPA-1 is an epitopes on the integrin $\alpha_{IIb}\beta_3$
- T197C polymorphism

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Purpose

To demonstrate that microfluidic platforms may be used for all steps necessary to determine the HPA-1 genotype from whole blood samples by PCR-RFLP analysis

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HPA Genotyping Approach

1st Stage PCR

⇒

2nd Stage PCR

⇒

forward: 5'-ATA GTT CTG ATT GCC GGA CTT CTC-3'

reverse: 5'-GAT TCT GGG GCA CAG TTA TCC-3'

MspI

114 bp amplicon

- multiplex PCR
- 12 SNP targets
- 20 PCR cycles
- HPA-1 specific
- 30 PCR cycles

1...ctccaaagta cggpptaacc ttttagctat tggpaaatgg tggpccctgc agpagaatgag gactgacacac agttttgatt gttatctta tttttggct tctgctaac

111 agpccctgcg tctggctctc tctggctctc agtgcgagga gactgctgag aagpataaac gttccctgag aacctctgag gttccctgag gttccctgag agactctgag

HPA 1 SNP ↓

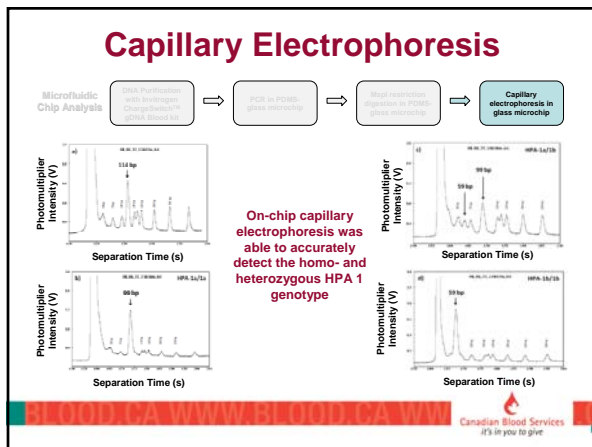
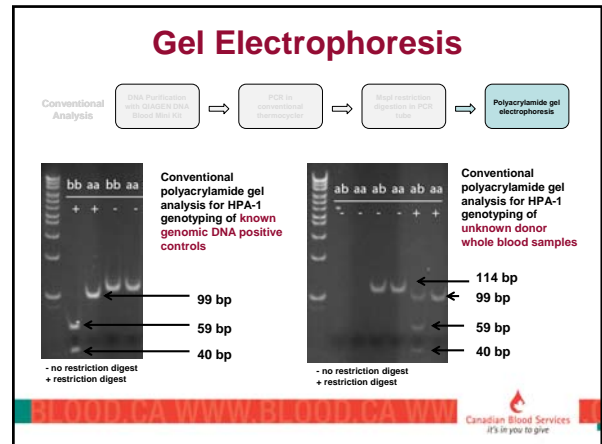
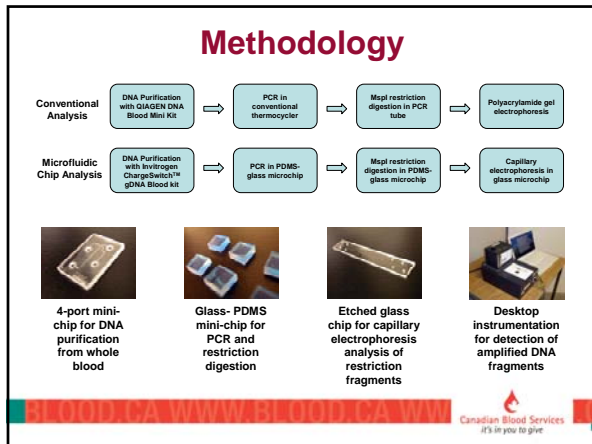
aggcctgccc tcgggctca HPA 1a

aggcctgccc no site HPA 1b

aggcctgccc tcgggctca MspI

- MspI restriction site introduced into forward primer to verify digestion
- > HPA1a = 99 bp & 15 bp
- > HPA1b = 59 bp & 40 bp

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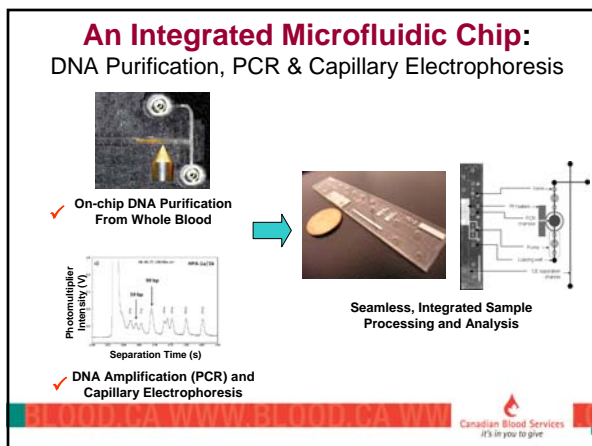


Summary of Blinded Analysis

Sample ID	00	02	06	09	24	29	32	35	38	40	43	44	45	46	78	80	81	83	98	
Conventional Analysis	ab	aa	aa	ab	aa	ab	aa	ab	aa	aa	ab	aa	aa	aa	ab	aa	ab	aa	ab	aa
On-chip Analysis	ab	aa	ab	ab	aa	ab	aa	ab	aa	aa	aa	ab	aa	aa	ab	aa	ab	ab	ab	aa

- concordance between the conventional and on-chip methods was achieved in 95% (18/19) of samples tested
 - likely due to human error during sample preparation for DNA purification

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- ## Summary of Research
- whole blood specimens can undergo complete blood genotyping analysis on microfluidic chip-based platform
 - comparison against conventional methods shows that the chip-based method is reliable and amenable for clinical applications
 - platforms are well suited for complete integration and automation
 - would lead to significant improvements in testing time, cost, and portability
- Canadian Blood Services
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Current Research Activities at University of Alberta

- integration of DNA isolation, PCR & CE on chip
 - sensitivity, specificity, robustness
- real-time PCR and melt curve analysis of HPA-1 to -5, 15
 - prototype chip and instrument are currently undergoing testing
- PCR of RhD for HDFN investigation

Current Limitations of Microfluidic Technology

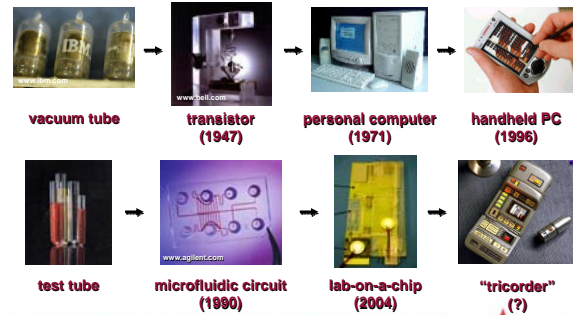
- **technology**
 - component integration (pumps, valves, sensors)
 - surface tension, air bubbles, evaporation
 - clogging of microchannels
 - lack of efficient pumps and fluid flow monitoring
 - mass production of complex chips
- **application**
 - validation of specificity and sensitivity of sensors
 - calibration and standardization of devices
 - user interfaces
- **cost** (~\$100-500 / chip)

Impact on Blood Banking

- **multiplexing: 1 or 2 chips for all testing**
 - consolidation of NAT, serology & bacterial detection
 - quality control testing (Hb, 2,3-DPG, plt activation)
 - on-chip confirmatory testing
- **integrated, automated testing**
 - reduced instrumentation & staffing
 - simplified data collection & handling
- **modular platform**
 - rapid adoption of new tests for emerging threats
- **point-of-care, point-of-contact testing**
 - real-time, pre-donation donor exclusion



The Future of Blood Testing...



Acknowledgements

