Revolutionizing the practice of medicine through rapid (<1h) DNA-based diagnostics

Michel G. Bergeron OQ, MD, FRCPC

Professor and Chairman, Division of Microbiology and Centre de recherche en Infectiologie of Université Laval, Université Laval and Centre de recherche du CHUL (CHUQ), 2705 Laurier blvd., Québec City, Québec, Canada, G1V 4G2

*Presented 25th September, 2008 at CSCI meeting, Toronto
Dr. Bergeron was the 2008 Henry Friesen Lecturer*

Clin Invest Med 2008; 31 (5): E265-E271.

Abstract

Twenty years ago, I dreamed of using DNA detection for speeding the microbiological identification of microorganisms from two days to less than one hour. This dream is slowly becoming a reality as we were the first to develop and put on the market real-time PCR assays, approved by the United States Food and Drug Administration and Health Canada, for the detection of several pathogens including Group B streptococci, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and *Clostridium difficile*. Since 2000, my team and I have been interested to bring this laboratory revolution to the bedside, by developing a microfluidic centripetal device, a compact disc-like platform that, instead of reading music, reads DNA. This futuristic approach to the management of infectious diseases at point-of-care will undoubtedly necessitate a "change in culture without culture".

Infectious diseases diagnostics, the early years

Since the emergence of mankind, there have been numerous accounts of mysterious diseases caused by 'invisible' agents, their 'invisibility' being unveiled in the 1700s when Antonie van Leeuwenhoek observed 'animalcules' with one of its first microscopes.¹ The second half of the 1900s has witnessed very important progresses in the isolation and cultivation of microor-

ganisms and the understanding that microbes might cause illnesses, culminating in the birth of medical microbiology pioneered by French biologist Louis Pasteur and German physician Robert Koch.^{2,3} This intense scientific discovery period served to establish the *germ theory of disease* and the *Koch's postulates*. From that point in time emerged a wide array of methods and procedures to isolate, cultivate, characterize, and identify microorganisms associated with infectious diseases; applied to medicine, these methods became the silex tools of diagnostic microbiology. Specific cultivation media, staining techniques, serological and immunological tests, electron microscopy, and (semi-)automated systems for microbial identification and antimicrobial susceptibility profile determination were developed since then and gradually introduced in the clinical microbiology laboratories. These technologies are still in use... 113 years after the death of Pasteur!

Compared with other diagnostic disciplines, microbiology is highly dependent on the efficiency of cultivation of pathogens. For most microorganisms, numbers of viable cells sufficient for identification are generally attained within two days, although a few

weeks might be necessary for some pathogens. This slow process results in the overuse of antibiotics (often broad-spectrum) and the development of antimicrobial resistance. I believe that rapid (< 1 hour) diagnostics will, over time, convert our present empirical treatment of infectious diseases into a well informed management where antimicrobial therapy will be used only when required, thus revolutionizing the practice of medicine, infectious diseases still being the first cause of mortality throughout the world.^{4,5}

Going molecular – The advent of the polymerase chain reaction

Time is money, but for infectious diseases, time is life!!! To truly impact on infectious disease control, time constitutes THE critical parameter to improve, to yield useful information within the same time frame as other diagnostic procedures (radiology, biochemistry, haematology), i.e. in less than one hour.⁵ Furthermore, limiting the delay for a correct antimicrobial intervention is an absolute necessity; not only does the population of microorganisms increases with time, but the host immune response also undermines the survival and recovery of the patient.^{4,5} In the last thirty-five years, the empirical approach of prescribing wide-spectrum antimicrobials has significantly contributed to the evolution, emergence, and dissemination of antimicrobial drug resistance mechanisms that further threaten the life of patients while limiting the efficiency of the available drug arsenal.

As stated earlier, a minimum of two days is generally required to yield a diagnostic based on microbiology results, and it is nearly impossible to gain time and restrict the current diagnostic cycle unless more rapid and sensitive procedures are used. In the 1980s, I was observing the fascinating rise of the recombinant DNA technology and in 1984, I sought the opportunity to catch the moving train when I invited Dr Paul H. Roy to join the *Service d'infectiologie* (now the *Centre de recherche en infectiologie*; CRI). Paul, a brilliant scientist, was highly interested in microbial

genetics and antimicrobial resistance mechanisms and was pioneering several technologies for molecular biology and bioinformatics in Québec City. In the next few years, among other things, we developed species-specific hybridisation probes capable of detecting and identifying *Branhamella* (*Moraxella*) *catarrhalis*. In the same period, Kary M. Mullis was inventing and perfecting a method we now know as the polymerase chain reaction (PCR).

The immense potential of PCR led us, Marc Ouellette, Paul, and me, to look for ways to exploit this technology for the rapid detection of microbes and possibly of their associated antimicrobial resistance genes.⁶ We, therefore, initiated a molecular diagnostics program aiming at the characterization of genes conserved in evolution, in order to derive amplification primers and eventually specific detection or capture probes. To translate my vision into reality, I founded a small start-up company called Infectio Diagnostic (IDI) Inc. in 1995.

In 2000, as the world overcame a feared computer bug that never was, my team and I really nailed a bug carried in the vagina of women and the first cause of neonatal meningitis, when we demonstrated that it was feasible to detect *Streptococcus agalactiae* (Group B streptococci or GBS) from vaginal-anal swabs of parturient women in less than one hour⁷ (Fig. 1), one of the 10 major discoveries of Québec Science in 2000. This real-time PCR assay was primarily developed on the LightCycler™ instrument of Idaho Technologies, and then adapted to the SmartCycler® of Cepheid, a platform particularly well suited to the burgeoning concept of *stat* microbiology with its random access independent thermal cycling blocks. This assay, transferred to IDI for conversion into a commercial kit, was submitted to regulatory approval by the United States Food and Drug Administration (FDA) and Health Canada. In November 2002, the IDI-StrepB™ assay became the first molecular diagnostics test of its kind ever approved by the FDA, with the mention that it could be used instead of culture-based methods. To further the portfolio of IDI,

FIGURE 1. Results of the conventional PCR assay (Panel A) and the new PCR assay (Panel B) for the detection of Group B Streptococci in combined vaginal and anal specimens from pregnant women. In the conventional PCR assay, the product of group B streptococci-specific amplification is 153 bp, whereas the 252-bp product represents the internal-control amplicon (Panel A). In the new PCR assay, the extent of group B streptococci-specific amplification is measured in terms of the increase in fluorescence during the amplification process (Panel B). In each panel, sample 1 was obtained from a heavily colonized woman; sample 2 was obtained from a lightly colonized woman; sample 3 was obtained from a woman with no colonization; sample 4 was a positive control, to which 10 fg of purified group B streptococcal genomic DNA had been added; and sample B was a negative control, to which no target DNA had been added. In Panel A, lane M shows a 100-bp molecular-size standard. (reprinted from reference 7 with permission)

that became GeneOhm Sciences Canada in 2004 and finally BD Diagnostics - GeneOhm in 2006, we developed other tests for methicillin-resistant *Staphylococcus aureus*⁸ (MRSA: IDI-MRSA™ now BD GeneOhm™ MRSA; Huletsky *et al.*, 2004) and vancomycin-resistant enterococci (VRE: BD GeneOhm™ VanR) that were approved in 2004 and 2006, respectively. The BD GeneOhm™ MRSA also won a USA Medical Device Excellence Award in 2006 and I am extremely proud that these and other molecular diagnostics products could be easily labelled "Made in Québec City", especially since Becton Dickinson has recently inaugurated a brand new fabrication facility in our beautiful city and is now building their world research centre for rapid DNA-based diagnostics. These facilities now offer work to more than 300 highly qualified scientists and research staff. This number will reach 500 in 2010. It is estimated that the impact of CRI's research and discoveries has been more than 500 million dollars in the last ten years.

Medically speaking, MRSA is one of the main threats of the medical system with its increasing and frightening causality of nosocomial infections. Increases in the number of *S. aureus*-related hospitalizations increased over 62%, from 294,570 to 477,927, in the United States between 1999 and 2005. In the meanwhile, MRSA-related hospitalisations doubled, from 127,036 to 278,203.⁹ In recent years, many studies have been conducted to determine the usefulness of admission screening for MRSA, either by culture-based or molecular diagnostic methods. In one of these studies, performed in Southern England, the molecular screening of patients with the IDI-MRSA™ upon admission to a critical care unit permitted significant reduction in the MRSA transmission rate from 13.89 to 4.9 par 1000 patient days.¹⁰ In 1999, it was estimated that treating an MRSA infection while saving lives added more than 27,000\$ in costs per patient.¹¹ Thus, the universal MRSA molecular screening of patients upon admission now constitutes a for-

midable tool to reduce the burden of hospital-acquired infections.

Towards the point of care diagnostic revolution

Real-time PCR is a remarkable technology that has many advantages for molecular diagnostics. Being a closed tube assay, it eliminates most of the contamination of laboratories caused by the carryover of amplification products. However, its capacity for multiple testing is limited by the number of fluorophores that can be analyzed simultaneously, currently 5 or 6. As far back as 2000, we initiated a microarray development program in order to tackle diseases and syndromes associated with multiple pathogens, such as sepsis, respiratory infections, and multiresistance to antibiotics. Our strong comparative genomics program provides the possibility of designing amplification primers for conserved genomic targets and capture probes with the potential of specifically identifying pathogens using a reverse hybridisation format on a glass slide microarray. Aware that we would have to design closed systems, we also incorporated basic microfluidics elements and, for the ultimate goal of detecting pathogens without molecular amplification, we included the possibility of using novel cationic biosensors developed by the team of Dr Mario Leclerc from the Department of Chemistry at Université Laval.¹² The long-term objective of this program is to develop rapid, economical, and adaptable genomic point-of-care (POC) tests that could ultimately surpass real-time PCR assays in speed (<15 minutes) and usefulness. Furthermore, our belief was reinforced by the recognition of molecular diagnostics as the number one biotechnology identified by a panel of world health experts useful to control infectious diseases in developing countries.¹³

I must admit that one of the strengths of the *Centre de recherche en infectiologie* is its multidisciplinary and, over the years, I have assembled a strong team of scientists with different backgrounds. This objective/dream of developing rapid genomic POC

diagnostic tests and tools forced the search of members with complementary expertises to reinforce our program. Marc Madou of the University of California at Irvine, a world leader in microfluidics and biomicroelectromechanical systems (bioMEMS), became a pillar of the expanded research consortium in 2003, shortly followed by Denis Boudreau, an analytical chemist and instrument developer of Université Laval, Teodor Veres and Michel Dumoulin, physicists of the National Research Council (NRC) Industrial Materials Institute of Boucherville, Québec, contributed to the development of devices made of thermoplastics, André Nantel, a world expert in microarrays of the NRC Biotechnology Research Institute of Montréal, Québec and Benoît Simard, a nanoparticle expert of the NRC Steacie Institute for Molecular Sciences of Ottawa, Ontario, searched for novel ways to capture microorganisms and nucleic acids. Altogether, we have been perfecting the essential components of a portable, adaptable and economical centripetally-driven microfluidic platform for the rapid (<1h) detection of microbial nucleic acids. This platform, the microfluidic centripetal device or MCD, is designed to move biological samples and reagent fluids through functional modules and channels by centripetal acceleration, to perform sample preparation, nucleic acids extraction, molecular amplification (PCR or RT-PCR), molecular hybridisation, and sensitive optical detection.

Supported by Genome Canada, Genome Québec, the National Institutes of Health (NIH) and CBRN Research and Technology Initiative (CRTI), we have been able to develop three separate functional devices, a sample preparation MCD, a PCR card, and an hybridisation MCD.^{14,15,16} These devices are now being integrated into one MCD platform that can be adapted for the diagnosis of viral respiratory diseases or of sepsis (Fig. 2).

We are also developing a novel scanning head, heating/cooling elements for molecular amplification and hybridisation, software, and graphic user interface to be incorporated into a diagnostic workstation that will accommodate all plastic MCD devices. Ulti-

FIGURE 2. Schematics of an integrated MCD platform (A) and of its principal microfluidic components (B). The principal elements of the MCD are a cell lysis chamber that contains particles to break open cells and liberate nucleic acids (A), a clarification chamber to pellet cell debris and yield a clear cell lysate compatible with PCR amplification (B), a hybridization buffer chamber (C), a mixing chamber (D), a waste chamber (E), a PCR amplification chamber (F), and a hybridization chamber (G).

mately, the whole MCD system will be a compact micro total analysis system (μ TAS), adapted to most public health infrastructures, and capable of efficient and reliable operation even in tropical conditions (Fig. 3). Specific software will allow us to analyse the data, give the identification of the microbes responsible for the infection and indicate possible management and treatment options.

We are now integrating these technologies on a single MCD to create a lab-on-a-chip or μ TAS capable of detecting the seventeen most important respiratory viruses directly from biological samples. A device like this would be extremely helpful for the management of a respiratory disease outbreak such as the SARS epidemics and would also constitute a powerful tool to minimise the unnecessary use of antibiotics for respiratory infections of viral origin, since a study done in 21 Southern Ontario hospitals in 2005-2006 has revealed that, of 327 patients eventually diagnosed with influenza, 292 (89%) have been prescribed an antibacterial treatment at admission.¹⁷ Globally, I foresee direct applications of multiparametric diagnostic devices for the management of diseases and

syndromes with multiple causes and, indirectly, these devices might contribute to a better control of infectious diseases by rationalizing the utilization of antimicrobial drugs, thereby minimising the selective pressure that has contributed to the emergence of highly feared multiresistant pathogens worldwide.

Conclusions and perspectives

Recalling on a molecular diagnostics (ad)venture of almost 25 years, I must admit that significant progress has been made towards the realization of a vision that will revolutionize the management of infectious diseases. The 'need for speed' has been my leitmotiv for years and providing a diagnostic answer on the timescale of other diagnostic procedures is a major achievement we can all be proud of. The next step is to bring infectious diseases diagnostics even closer to the patient, ideally at bedside, in the doctor's office, in dispensaries, or even in households, similarly to glucose testing for diabetes. Technologically, we have made huge leaps towards truly helpful diagnostic devices, but we are confronted by several administrative and procedural hurdles that arise from the medical

FIGURE 3. The integrated MCD or μ TAS for the diagnostics of viral respiratory infections and its wireless workstation.

system itself. Indeed, a little prevention and better rapid diagnostics might allow the health system to save lots of time, energy, public funds, and most importantly, human lives and it is highly paradoxical that so little funds, in fact less than 3,5% of all private and public money, are available for the research, development, and health implementation of rapid and efficient diagnostic tools compared to therapeutics.

It is imperative that we operate "*A change in culture, without (microbial) culture!*"

Acknowledgments

The author acknowledges the contribution of the many students, scientists, and of Université Laval that helped to achieve such a dream, of Luc Bissonnette who helped with this paper, and the financial support of the Canadian Institutes for Health Research (CIHR), Genome Canada, Génome Québec, Fonds de recherche en santé du Québec (FRSQ), Canadian Department of National Defence, CBRN Research and Technology Initiative (CRTI), Canadian Foundation for Innovation, National Institutes of Health, and BD Diagnostics - GeneOhm.

References

1. Barton HA. Introduction to cave microbiology: a review for the non-specialist. *J Cave Karst Studies* 2006;68:43-54.
2. Bull AT, Quayle JR. New dimensions in microbiology. *Phil Trans R Soc Lond B* 1982;297:447-57.
3. Ullmann A. Pasteur-Koch distinctive ways of thinking about infectious diseases. *Microbe* 2007;2:383-7.
4. Boissinot M, Bergeron MG. Toward rapid real-time molecular diagnostic to guide smart use of antimicrobials. *Curr Opin Microbiol* 2002;5:478-82.
5. Picard FJ, Bergeron MG. Rapid molecular theranostics in infectious diseases. *Drug Discov Today* 2002;7:1092-1100.
6. Bergeron MG, Ouellette M. Preventing antibiotic resistance through rapid genotypic identification of bacteria and of their antibiotic resistance genes in the clinical microbiology laboratory. *J Clin Microbiol* 1998;36:2169-72.
7. Bergeron MG, Ke D, Ménard C et al. Rapid detection of Group B streptococci in pregnant women at delivery. *N Engl J Med* 2000;343:175-9.
8. Huletsky A, Giroux R, Rossbach V et al. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* 2004;42:1875-84.
9. Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerg Infect Dis* 2007;13:1840-6.
10. Cunningham R, P Jenks, J Northwood et al. Effect on MRSA transmission of rapid PCR testing of patients admitted to critical care. *J Hosp Infect* 2007;65:24-8.
11. Abramson MA, Sexton DJ. Nosocomial methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* primary bacteremia: at what costs? *Infect Control Hosp Epidemiol* 1999;20:408-11.
12. Ho HA, Doré K, Boissinot M et al. Direct molecular detection of nucleic acids by fluorescence signal amplification. *J Am Chem Soc* 2005;127:12673-6.
13. Daar AS, Thorsteinsdóttir H, Martin DK et al. Top ten biotechnologies for improving health in developing countries. *Nat Genet* 2002;32:229-32.
14. Kido H, Mimic M, Smith D et al. A novel, compact disk-like centrifugal microfluidics system for cell lysis and sample homogenization. *Colloids Surf B Biointerfaces* 2007;58:44-51.
15. Jia G, Siegrist J, Deng C et al. A low-cost, disposable card for rapid polymerase chain reaction. *Colloids Surf B Biointerfaces* 2007;58:52-60.
16. Peytavi R, Raymond FR, Gagné D et al. Microfluidic device for rapid (<15 min) automated microarray hybridization. *Clin Chem* 2005;51:1836-44.
17. McGeer A, Green KA, Plevneshi A et al. Antiviral therapy and outcomes of influenza requiring hospitalization in Ontario, Canada. *Clin Infect Dis* 2007;15:1568-75.

Correspondence to:

Michel G. Bergeron, O.Q., MD, FRCPC
 Département de biologie médicale (Microbiologie)
 Faculté de médecine and Centre de recherche en
 infectiologie of l'Université Laval and Centre de recherche
 du CHUL (CHUQ)
 2705 Laurier blvd.
 Québec City (Québec) CANADA G1V 4G2
 Email: Michel.G.Bergeron@crchul.ulaval.ca