

## New Diagnostic Methodologies

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### Objectives

At the end of the lecture, attendees should be capable of understanding the technologies currently in use in the diagnostic Molecular Biology Lab, as well as to discuss their potential uses, advantages and limitations. The attendee should also be able to discuss the potential uses of some newly developed techniques.

### Summary

Conventional Culture based diagnostic methods have severe limitations when the microorganisms are slow growing, fastidious, or require special culture conditions. The same applies for serology technologies when the host is immunocompromised or shows an early primary infection. All these limitations may be overcome by the increased use of the ever evolving diagnostic molecular methodologies.

All pathogenic microorganisms have potentially unique DNA signatures that may be used to establish infection aetiology and to quantify the micro-organism load, thus allowing the precise evaluation of therapeutic response. Alternatively, the presence of specific genes or mutations may be tested to assess the existence of drug resistance genotypes.

The Diagnostic Molecular Biology techniques are fast evolving, providing ever more speed and ease of use, while maintaining the exceedingly high levels of specificity and sensitivity that have always been its landmark.

Techniques for manual, semi-automated or fully automated nucleic-acid extractions and purification from all kinds of clinically relevant biological specimens will be discussed. Similarly, techniques for the amplification, detection and characterization of nucleic acids will be discussed, with particular emphasis on the real-time assays and its advantages and limitations. Also, sequencing methods will be presented, with particular emphasis on the new generation methods and their tremendous parallel processing capacity. Examples of applications with tremendous potential to revolutionize the clinical microbiology lab will be addressed.

Finally, the presentation will focus on the challenges that only now are being addressed efficiently such as the need to bring the Molecular Biology tests to “core-lab” facilities or to “emergency labs”, and the need to ask many Molecular Biology questions in parallel, within a short amount of time, and with a very small amount of sample. Solutions such as Septifast, 16S rRNA amplification/sequencing, microchips, Bead based parallel hybridization, in chip-PCR and massively parallel sequencing will be discussed.

### Recommended reading

- 1) Limitations of conventional microbiology diagnostic methods
  - Zaidi N., et. Al The Role of Molecular Biology and Nucleic Acid Technology in the Study of Human Infection and Epidemiology. **Arch Pathol Lab Med.** (2003) 127:1098–1105
- 2) Nucleic acid preparation
  - Albinana-Gimenez A. Comparison of methods for concentrating human adenoviruses, polyomavirus JC and noroviruses in source waters and drinking water using quantitative PCR. **J Virol. Meth.** 158 (2009) 104–109

- 3) Nucleic acid amplification
  - NASBA:  
Compton-J, Nucleic Acid Sequence Based Amplification. **Nature** (1991) 350:91-92
  - LAMP  
Notomi-T et al. Loop-Mediated Isothermal amplification of DNA. **Nucleic Acids Research** (2000) 28:i-vii  
Seki-M et al. Loop-Mediated Isothermal amplification Method targeting the LytA Gene for Detection of streptococcus pneumoniae. **J.Clin.Microb.**(2005) 43(4):1581-1586
  - OLA  
Nickerson D. et al. Automated DNA diagnostics using an ELISA-based oligonucleotide ligation assay. **PNAS** (1990) 87: 8923-8927
  - SDA  
Walker GT et al. Isothermal in vitro amplification of DNA by a restriction enzyme/DNA polymerase system. **PNAS** (1992) 89:392-396
  - PCR  
Mullis K et al. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. **Cold Spring Harb.Symp.Quant.Biol.**(1986)51:263-73  
Saiki RK Analysis of enzymatically amplified beta-globin and HLA-DQ alpha with oligonucleotide probes. **Nature**(1986) 324(6093):163-166
- 4) Nucleic acid detection and characterization
  - Amorim ML et al. Changes in the Clonal Nature and Antibiotic Resistance Profiles of Methicillin-Resistant Staphylococcus aureus Isolates Associated with Spread of the EMRSA-15 Clone in a Tertiary Care Portuguese Hospital **J.Clin.Microb.** (2007) 45: 2881–2888
  - Goldenberg O. et al. Use of Denaturing High-Performance Liquid Chromatography for Rapid Detection and Identification of Seven Candida Species. **J.Clin.Microb.** (2005) 43: 5912–5915
  - Espy MJ et al. Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing. **Clinical Microbiology Reviews**, (2006) 19:165–256
  - Tsourkas A & Bao-G. Shedding light on health and disease using molecular beacons. **Briefings In Functional Genomics And Proteomics** (2003) 1: 372-384
- 5) Challenges and trends
  - Rossney AS. Et al. Evaluation of the Xpert Methicillin-Resistant Staphylococcus aureus (MRSA) Assay Using the GeneXpert Real-Time PCR Platform for Rapid Detection of MRSA from Screening Specimens. **J.Clin.Microb.** (2008) 46:3285-3290
  - Pabbaraju-K et al. Comparison of the Luminex xTAG Respiratory Viral Panel with In-House Nucleic Acid Amplification Tests for Diagnosis of Respiratory Virus Infections. **J.Clin.Microb.** (2008) 46:3056-3062
  - Palacios-G et al. MassTag Polymerase Chain Reaction for Differential Diagnosis of Viral Hemorrhagic Fevers. **Emerging Infectious Diseases** (2006) 12:692-695
  - Morrison-T et al. Nanoliter high throughput quantitative PCR. **Nucl.Acids.Res.**(2006) 34:e123
  - Walser et al. Novel method for high-throughput colony PCR screening in nanoliter-reactors. **Nucl.Acids.Res.**(2009) 37:e57
  - Stedtfeld-RD et al., Development and Experimental Validation of a Predictive Threshold Cycle Equation for Quantification of Virulence and Marker Genes by High-Throughput Nanoliter-Volume PCR on the OpenArray Platform. **Applied And Environmental Microbiology** (2008) 74: 3831–3838
  - Mancini-N et al. Molecular diagnosis of sepsis in neutropenic patients with haematological malignancies. **J. Medical Microb.** (2008) 57:601-604
  - Reier-Nilsen T. Comparison of broad range 16S rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: a case control study. **BMC Pediatrics** (2009) 9:5

- Nakamura S. et al. Direct Metagenomic Detection of Viral Pathogens in Nasal and Fecal Specimens Using an Unbiased High-Throughput Sequencing Approach. **PLOS One** (2009) 4: e4219
- Andersson AF. et al. Comparative Analysis of Human Gut Microbiota by Barcoded Pyrosequencing. **PLOS One** (2008) 3: e2836
- Nakamura S. et al. Metagenomic Diagnosis of Bacterial Infections. **Emerging Infectious Diseases** (2008) 14:1784-1786
- Rosera G. et al. Massively parallel pyrosequencing highlights minority variants in the HIV-1 env quasispecies deriving from lymphomonocyte sub-populations. **Retrovirology** (2009) 6:15
- Hoffmann C. et al. DNA bar coding and pyrosequencing to identify rare HIV drug resistance mutations. **Nucleic Acids Res.**(2007) 35:e91
- Wang C. et al. Characterization of mutation spectra with ultra-deep pyrosequencing: Application to HIV-1 drug resistance. **Genome Res.** (2007) 17:1195-1201

**Recommended reading for the small group tutorial session:  
“Rapid Diagnostic, hands on experience”**

- Fontana C et al. *Acinetobacter baumannii* in intensive care unit: A novel system to study clonal relationship among the isolates. **BMC Infectious Diseases** (2008) 8: 79
- Archimbaud C et al. Impact of Rapid Enterovirus Molecular Diagnosis on the Management of Infants, Children, and Adults with Aseptic Meningitis **J. Medical Virol.** (2009) 81: 42-48