MOLECULAR DIAGNOSITICS

Outline

- Concept of Molecular Diagnostics
- History of Molecular Diagnostics
- Impact on Human Diseases
- Basis for Molecular Assay
- Novel Biomarkers

Molecular Diagnosis

Molecular diagnosis of human disorders is referred to as the detection of the various pathogenic mutations in DNA and /or RNA samples in order to facilitate detection, diagnosis, sub-classification, prognosis, and monitoring response to therapy.

Molecular Diagnostics

Molecular diagnostics combines laboratory medicine with the knowledge and technology of molecular genetics and has been enormously revolutionized over the last decades, benefiting from the discoveries in the field of molecular biology.

Molecular Diagnostics: Significance

To face the new century, the medical practitioner not only understand molecular biology, but must also embrace the use of this rapidly expanding body of information in his medical practice, whether practicing family medicine, oncology,clinical laboratory, obstetrics and gynecology, pathology, or any other medical specialty.

Molecular Diagnostics: Goal

- To introduce essential concepts in molecular diagnostics that impact on the identification of novel markers of human diseases
- To develop and apply useful molecular assays to monitor disease, determine appropriate treatment strategies, and predict disease outcomes.

History of Molecular Diagnostics

The PCR Revolution

Kary Mullis

1985 41y

Invention of PCR

1993 49y

Received the Noble Prize

Kary B. Mullis, inventor of the Polymerase Chain Reaction

History of Molecular Diagnostics

The PCR Revolution

- PCR markedly decreased need for radioactivity, allowed molecular diagnostics to enter the clinical laboratory.
- PCR either is used for the generation of DNA fragments to be analyzed, or is part of the detection methods

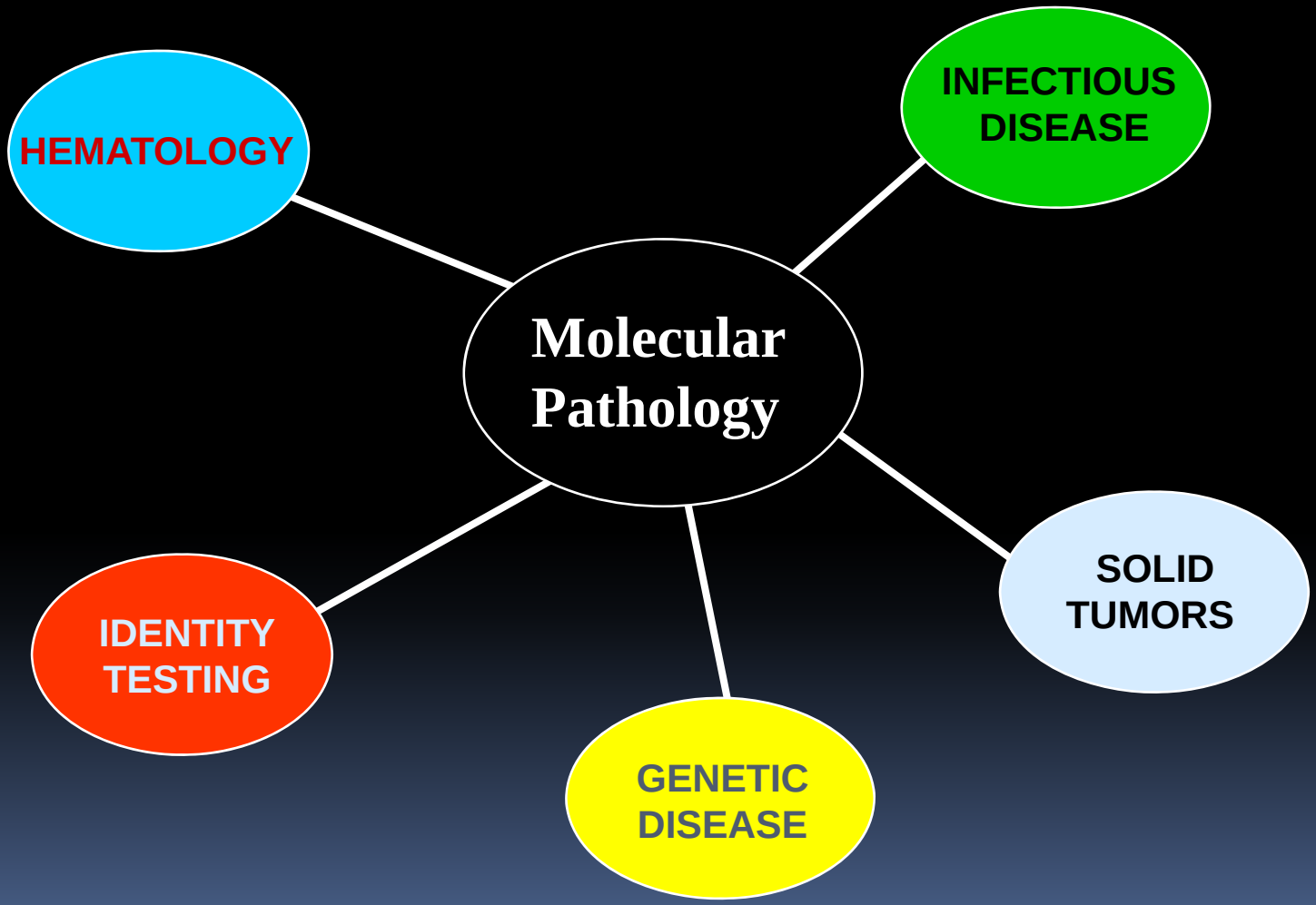
Impact on Human Diseases: Advantage

- Monitor diseases more accurately
  - Allows for early treatment and better patient care
- Determine most appropriate treatment
  - Reduces or eliminates unnecessary and inadequate treatment
  - Yields greater cost effectiveness
- Reduce patient morbidity and mortality

Impact on Human Diseases: Practical application

- Diagnostic-Identity of a disease
- Prognostic-Outcome of a disease
- Predictive-Possibility of a disease
- Therapeutic-Response of a disease to treatment

Impact on Human Diseases



Molecular Technologies in the Clinical Laboratory

- **Amplification Techniques**
  - **PCR** polymerase chain reaction, Real Time PCR
  - **LCR** ligase chain reaction
  - **NASBA** nucleic-acid sequence-based amplification
- **DNA Sequencing**

Molecular Technologies in the Clinical Laboratory

- **Hybridization Techniques**
  - **Southern hybridization Blot**
  - **Northern hybridization Blot**

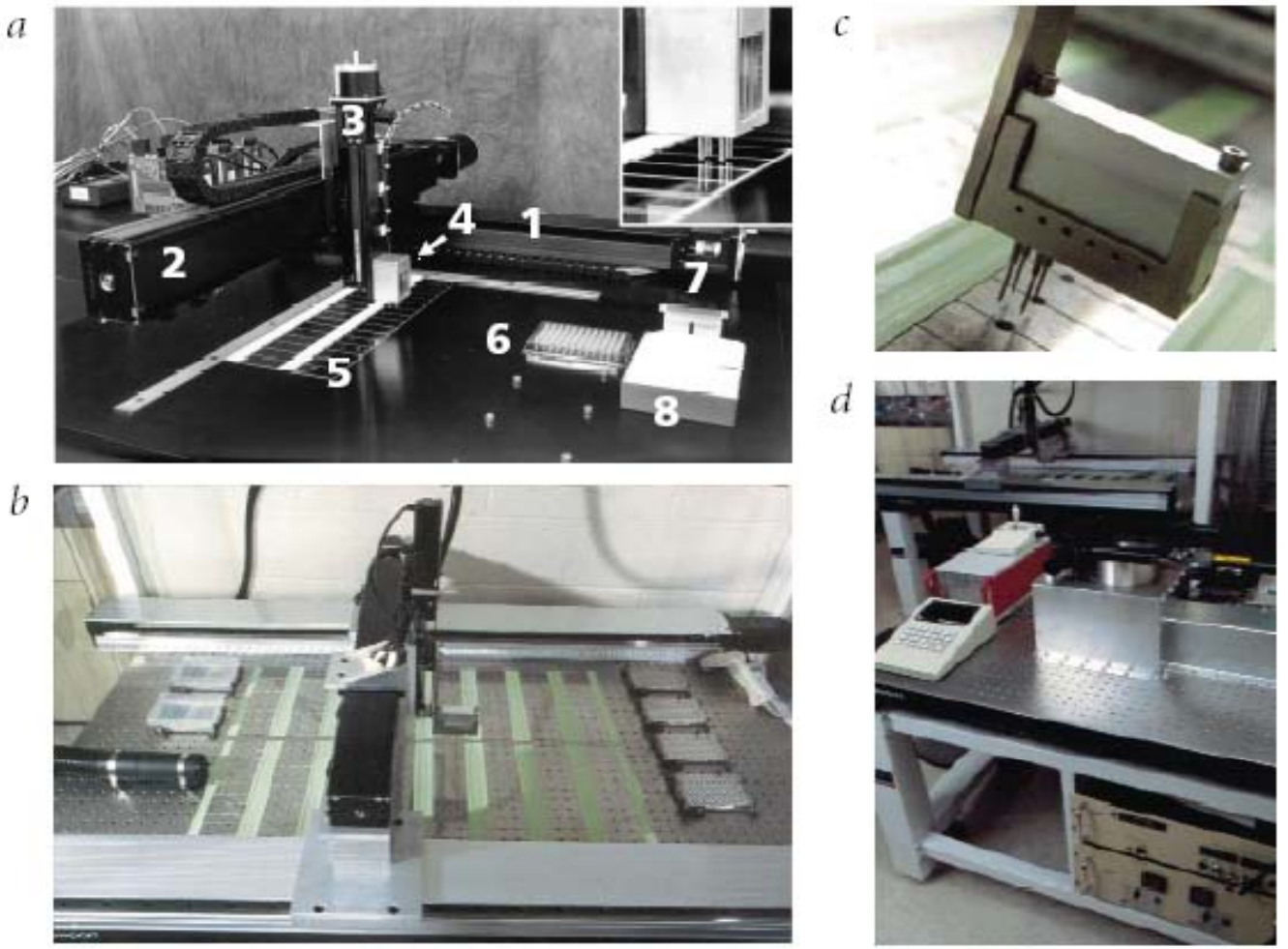
- **Electrophoretic Methods**
  - **SSCP** (single-strand conformation polymorphism)
  - **DGGE** (denaturing gradient gel electrophoresis)

Molecular Technologies in the Clinical Laboratory

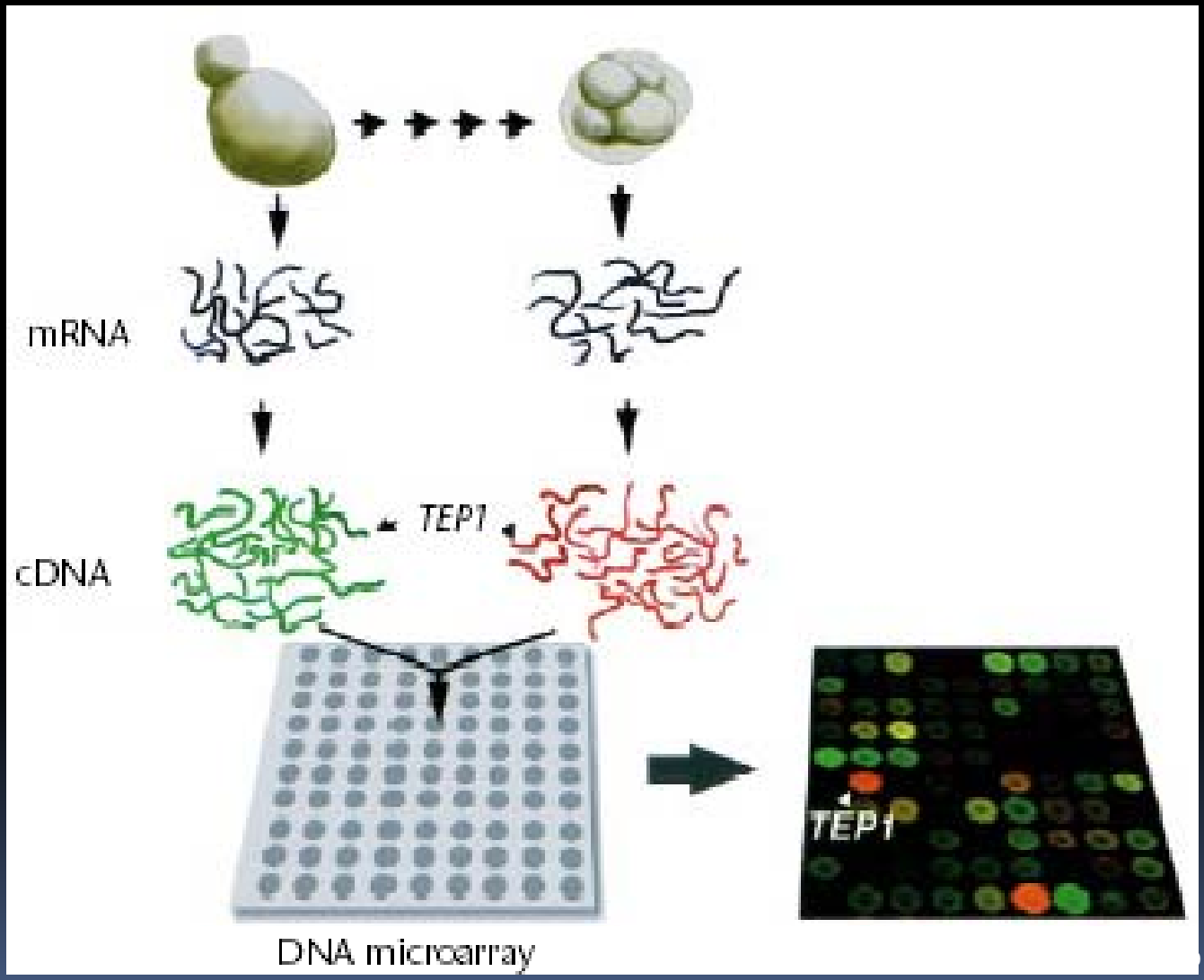
- **Biochip Technology**
  - **DNA micro-array**
  - **Protein micro-array**

microarray

Fig. 1 ***a***, Penn microarray robot. The X-, Y-, Z- axes are labesled 1, 2, and 3, respectively. The key component of the arrayer is the print-head, containing pens (4). Microscope glass slides are placed on the slide station (5). Samples are prepared and arrayed from 96-well sample plates (6). The pins are cleaned between sample acquisitions at the washing (7) and drying (8) stations. ***b***, AECOM microarray robot. The table configuration shown contains 160 slides with four microtitre plates, two wash stations and the dryer. The print-head (***c***) shows four of the possible twelve pen tips in use. ***d***, AECOM laser scanner. Visible are the optical table, power supplies for lasers and PMT cooling, the Ludl stage, and lasers. The 20× microscope objective is inside the ludl stage while lenses, mirrors and other optics are enclosed in the metal casing. PMTs are to the right and outside the photo.



Cheung et al. 1999


mRNA
cDNA
TEP1
TEP1
DNA microarray

Basis for Molecular Assay: Pathogenesis

Diagnostic

- **Distinguishing variants of human disease based on presence of specific molecular markers (chromosome translocations in Burkitt's lymphoma: *c-myc*)**

Basis for Molecular Assay: Pathogenesis

Prognostic

- Prediction of likely patient outcomes based on presence of specific molecular markers (gene mutations predicting clinical course in cancer)

Basis for Molecular Assay: Pathogenesis

Therapeutic

- **Prediction of response to specific therapies based on presence of specific molecular markers (gene mutations predicting poor drug sensitivity in lung cancer: *p53, k-ras*)**

Basis for Molecular Assay: Molecular biology

Genetic Lesions in Human Disease

- Identification of genetic markers
- Identification of disease-related genes
- Molecular targets for assay development

Conclusion

- As many as 5000 disease have direct genetic causes
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- High sensitivity and increased specificity for most tests adds diagnostic utility

- Potential for simple standardized procedures an automation

- Increased number of techniques for infectious diseases and tumor diagnostics

- Prices are falling

Conclusion

The ultimate goal of the molecular diagnostics is to provide molecular information that will combine with and complement information related to patient history and symptomology, clinical laboratory results, histopathological findings, and other diagnostic information to provide a more sensitive, precise, and accurate determination of disease diagnosis and/or guidance toward appropriate and effective treatment options.

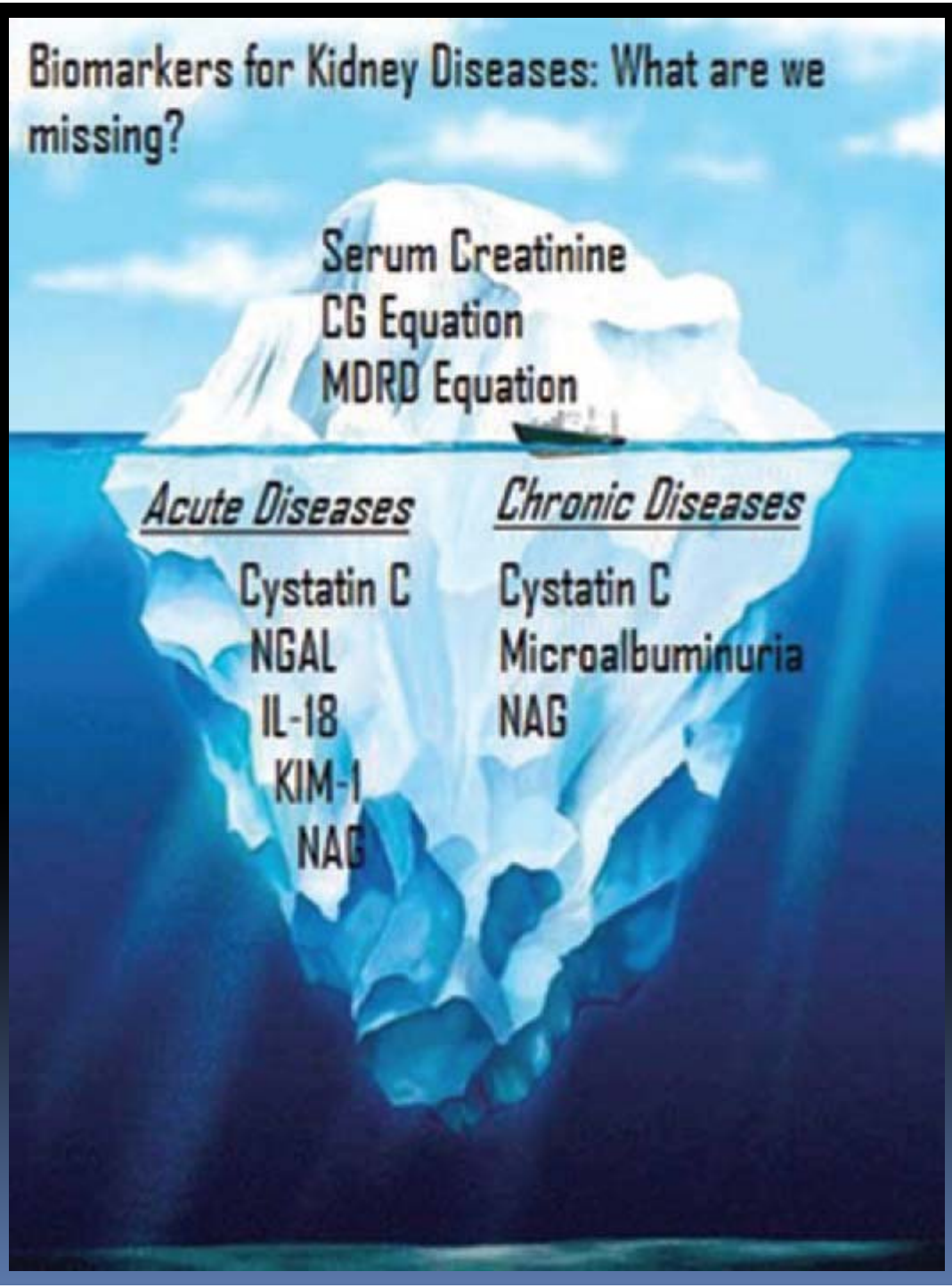
NOVEL BIOMARKERS

CARDIAC BIOMARKERS

- AST
- LD (LDH)
- CK (CPK)
- CK-MB
- Troponin-I/T
- Myoglobin
- Homocystein
- hsCRP
- Choline
- sCD40 Ligand
- Ischemia Modified Albumin
- Myeloperoxidase
- Oxidized LDL
- Lipoprotein-Associated Phospholipase A2(LpPLA2)
- Pregnancy-Associated Plasma Protein A

Novel Biomarkers of kidney injury

- Neutrophil gelatinase-associated lipocalin
- N-acetyl-b-D-glucosaminidase
- Kidney injury molecule-1
- Interleukin-18
- Urine cystatin C


Biomarkers for Kidney Diseases: What are we missing?
Serum Creatinine
CG Equation
MDRD Equation
Acute Diseases
Cystatin C
NGAL
IL-18
KIM-1
NAG
Chronic Diseases
Cystatin C
Microalbuminuria
NAG