

# TABLE OF CONTENTS

<b>0. Executive Summary .....</b>	<b>23</b>
<b>1. Introduction .....</b>	<b>25</b>
<b>Definitions and scope of the subject .....</b>	<b>25</b>
<b>Historical evolution of molecular diagnostics.....</b>	<b>25</b>
<b>Molecular biology relevant to molecular diagnostics.....</b>	<b>26</b>
DNA .....	26
<i>DNA polymerases.....</i>	26
<i>Restriction endonucleases.....</i>	27
<i>DNA methylation.....</i>	27
RNA .....	28
<i>RNA polymerases.....</i>	28
<i>Non-coding RNAs.....</i>	28
DNA transcription.....	29
Chromosomes .....	29
Telomeres.....	30
Mitochondrial DNA .....	30
Genes.....	31
<i>The genetic code.....</i>	31
<i>Gene expression .....</i>	31
The human genome .....	32
Variations in the human genome.....	32
<i>Variations in DNA sequences .....</i>	32
<i>Single nucleotide polymorphisms .....</i>	33
<i>Genotype and haplotypes.....</i>	33
<i>Complex chromosomal rearrangements .....</i>	33
<i>Insertions and deletions in the human genome.....</i>	33
<i>Large scale variation in human genome .....</i>	34
<i>Variation in copy number in the human genome .....</i>	35
<i>Structural variations in the human genome.....</i>	36
<i>Replication of the DNA helix .....</i>	36
<i>Transposons.....</i>	37
Proteins .....	37
Proteomics.....	38
Monoclonal antibodies .....	38
Aptamers.....	38
<b>Basics of molecular diagnostics .....</b>	<b>38</b>
Tracking DNA: the Southern blot .....	39
Pulsed-field gel electrophoresis.....	39
DNA Probes.....	39
The polymerase chain reaction .....	40
<i>Basic Principles of PCR.....</i>	40
<i>Target selection.....</i>	40
<i>Detection of amplified DNA.....</i>	41
<b>Impact of human genome project on molecular diagnostics.....</b>	<b>41</b>
<i>Mapping and sequencing of structural variation from human genomes.....</i>	42
1000 Genomes Project .....	42
<i>Human Variome Project.....</i>	43
<b>Systems biology approach to molecular diagnostics .....</b>	<b>44</b>
<b>Biomarkers .....</b>	<b>44</b>
<b>Applications of molecular diagnostics.....</b>	<b>45</b>
<b>2. Molecular Diagnostic Technologies .....</b>	<b>47</b>
<b>Introduction .....</b>	<b>47</b>
<b>DNA extraction .....</b>	<b>47</b>
Transrenal DNA .....	47
<b>Sample preparation .....</b>	<b>48</b>
Pressure Cycling Technology .....	48
<b>Membrane immobilization of nucleic acids.....</b>	<b>48</b>
<b>Automation of sample preparation in molecular diagnostics.....</b>	<b>49</b>
ABI PRISM 6700 Automated Nucleic Acid Workstation .....	49
BioRobot technology.....	49
COBAS AmpliPrep System .....	49
GENESIS FE500 Workcell .....	49
GeneMole.....	50
PCR BioCube .....	50
QIASymphony.....	50

Tigris instrument system .....	50
Techniques for sample preparation that are suitable for automation .....	51
<i>Xtra Amp Genomic DNA Extraction</i> .....	51
<i>Extraction of DNA from paraffin sections</i> .....	51
<i>Dynabead technology</i> .....	51
<i>Pressure Cycling Technology</i> .....	52
<i>SamPrep</i> .....	52
Use of magnetic particles for automation in genome analysis .....	52
Companies involved in nucleic acid isolation .....	53
<b>Novel PCR methods</b> .....	<b>54</b>
Addressing limitations of PCR .....	54
Real-time PCR systems .....	54
<i>Dyes used in real-time PCR</i> .....	55
<i>Commercially available real-time PCR systems</i> .....	56
<i>LightCycler PCR system</i> .....	56
<i>LightUp probes based on real-time PCR</i> .....	57
<i>READ™ real-time PCR method</i> .....	57
<i>ViiA™ 7 real-time PCR system</i> .....	57
<i>Applications of real-time PCR</i> .....	57
<i>Limitations of real-time PCR</i> .....	58
<i>Improving the reliability of low level DNA analysis by real-time PCR</i> .....	58
<i>Guidelines for real-time quantitative PCR</i> .....	59
<i>Future applications of real-time Q-PCR</i> .....	59
Reverse transcriptase (RT)-PCR .....	59
<i>Standardized reverse transcriptase PCR</i> .....	60
Single cell PCR .....	61
<i>LATE-PCR</i> .....	61
<i>COLD-PCR</i> .....	61
<i>AmpliGrid-System</i> .....	62
Digital PCR .....	62
Emulsion PCR .....	63
Long and accurate PCR .....	64
Combined PCR-ELISA .....	64
<b>Post-PCR genotyping methods</b> .....	<b>64</b>
High-resolution melt analysis .....	65
<b>Monitoring of gene amplification in molecular diagnostics</b> .....	<b>65</b>
<b>Non-PCR nucleic acid amplification methods</b> .....	<b>65</b>
Linked Linear Amplification .....	65
Multiplex Ligation-Dependent Probe Amplification .....	66
Transcription mediated amplification .....	66
Rapid analysis of gene expression .....	66
WAVE nucleic acid fragment analysis system .....	67
DNA probes with conjugated minor groove binder .....	67
Rolling circle amplification technology .....	68
<i>Gene-based diagnostics through RCAT</i> .....	69
<i>RCAT-immunodiagnosics</i> .....	69
<i>RCAT-pharmacogenomics</i> .....	70
<i>Circle-to-circle amplification</i> .....	70
Ramification amplification method .....	70
Single Primer Isothermal Amplification .....	71
Isothermal reaction for amplification of oligonucleotides .....	71
ICAN (Isothermal and Chimeric primer-initiated Amplification of Nucleic Acids) .....	71
<b>Technologies for signal amplification</b> .....	<b>72</b>
3 DNA dendrimer signal amplification .....	72
Hybridization signal amplification method .....	73
Signal mediated amplification of RNA technology .....	74
Invader assays .....	74
Hybrid Capture technology .....	76
Branched DNA test .....	77
Tyramide signal amplification .....	77
Non-enzymatic signal amplification technologies .....	77
<b>Direct molecular analysis without amplification</b> .....	<b>78</b>
Trilogy™ Platform .....	78
Direct detection of dsDNA .....	79
<b>Multiplex assays</b> .....	<b>80</b>
<b>Fluorescent in situ hybridization</b> .....	<b>80</b>
Modifications of FISH .....	82
Direct visual in situ hybridization .....	82
Direct labeled Satellite FISH probes .....	82
Comparative genomic hybridization .....	83
Primed in situ labeling .....	83

Interphase FISH .....	83
FISH with telomere-specific probes .....	83
Multicolor FISH .....	84
Automation of FISH.....	84
Companies involved in FISH diagnostics.....	84
<b>RNA diagnostics.....</b>	<b>85</b>
Branched-chain DNA assay for measurement of RNA .....	86
Cycling probe technology .....	87
Invader RNA assays .....	87
Linear RNA amplification .....	87
Non-isotopic RNase cleavage assay .....	88
Nucleic acid sequence-based amplification .....	88
Q Beta replicase system .....	90
Solid Phase Transcription Chain Reaction .....	90
Transcriptome analysis .....	90
Visualization of mRNA expression in vivo .....	91
MicroRNA diagnostics .....	91
<i>Real-time PCR for expression profiling of miRNAs.....</i>	<i>91</i>
<i>Microarray vs quantitative PCR for measuring miRNAs .....</i>	<i>92</i>
<i>Use of LNA to explore miRNA.....</i>	<i>92</i>
<i>Nuclease Protection Assay to measure miRNA expression.....</i>	<i>93</i>
<i>Microarrays for analysis of miRNA gene expression .....</i>	<i>93</i>
<i>Modification of in situ hybridization for detection of miRNAs .....</i>	<i>94</i>
<b>Whole genome amplification.....</b>	<b>94</b>
Companies that provide technologies for whole genome amplification.....	94
<i>QIAGEN's Repli-G system.....</i>	<i>95</i>
<i>GenomePlex™ Whole Genome Amplification .....</i>	<i>95</i>
<b>DNA sequencing.....</b>	<b>96</b>
Companies involved in sequencing .....	97
Applications of next generation sequencing in molecular diagnostics .....	98
<b>Genome-wide approach for chromatin mapping .....</b>	<b>99</b>
<b>Mitochondrial sequencing .....</b>	<b>99</b>
Identification of unknown DNA sequences .....	99
<b>Optical mapping.....</b>	<b>100</b>
<b>Gene expression analysis.....</b>	<b>100</b>
Gene expression profiling on whole blood samples .....	101
Gene expression patterns of white blood cells.....	101
Gene expression profiling based on alternative RNA splicing .....	102
MAUI (MicroArray User Interface) hybridization .....	102
Monitoring in vivo gene expression by molecular imaging .....	103
Serial analysis of gene expression (SAGE).....	103
Single-cell gene expression analysis .....	103
T cell receptor expression analysis .....	104
Tangerine™ expression profiling .....	104
Whole genome expression array .....	104
Ziplex™ system .....	105
Companies involved in gene expression analysis.....	105
<b>Peptide nucleic acid technology.....</b>	<b>106</b>
Use of PNA with fluorescence in situ hybridization .....	107
PNA and PCR.....	108
Use of PNA with biosensors .....	108
PNA-based PD-loop technology .....	109
PNA-DNA hybrid quadruplexes.....	109
Companies involved in PNA diagnostics.....	109
<b>Locked nucleic acids .....</b>	<b>110</b>
<b>Zip Nucleic Acids.....</b>	<b>110</b>
<b>Electrochemical detection of DNA .....</b>	<b>111</b>
Mediated nucleic acid oxidation.....	111
Detection of hybridized nucleic acid with cyclic voltametry .....	112
Electrochemical detection based on Toshiba's CMOS technology.....	112
Concluding remarks on electrochemical DNA detection .....	112
<b>Bead-based assay platforms .....</b>	<b>113</b>
<b>Scorpions™ technology.....</b>	<b>114</b>
The Scorpions reaction .....	114
Applications of Scorpions .....	114
<b>3. Biochips, Biosensors, and Molecular Labels .....</b>	<b>115</b>
<b>Introduction to biochip technology.....</b>	<b>115</b>
<b>Applications of biochips in diagnostics .....</b>	<b>115</b>
GeneChip.....	116
<i>GeneChip Human Genome Arrays .....</i>	<i>117</i>

<i>AmpliChip CYP450</i> .....	117
Electronic detection of nucleic acids on microarrays.....	117
Microchip capillary electrophoresis.....	118
Strand displacement amplification on a biochip.....	118
Rolling circle amplification on microarrays.....	118
<i>LiquiChip-RCAT</i> .....	118
Fast PCR biochip.....	119
Multiplex microarray-enhanced PCR for DNA analysis.....	119
Multiplexed Molecular Profiling.....	119
Universal DNA microarray combining PCR and ligase detection reaction.....	120
Genomewide association scans.....	120
Whole genome chips/microarrays.....	121
Transposon insertion site profiling chip.....	121
Standardizing the microarrays.....	122
Companies involved in developing biochip technology for diagnostics.....	122
Future of biochip technology for molecular diagnostics.....	123
<b>Microfluidic chips.....</b>	<b>124</b>
Fish-on-chip.....	124
Lab-on-a-chip.....	124
LabCD.....	125
Micronics' microfluidic technology.....	125
Microfluidic automated DNA analysis using PCR.....	125
Microfluidic chips integrated with RCAT.....	125
Microfluidic chips integrated with PET.....	126
Companies developing microfluidic technologies.....	126
<b>Biosensor technologies.....</b>	<b>127</b>
Classification of biosensor technologies.....	128
DNA-based biosensors.....	128
<i>DNA hybridization biosensor chips</i> .....	129
<i>PCR-free DNA biosensor</i> .....	129
<i>DNA based biosensor to detects metallic ions</i> .....	129
Genetically engineered B lymphocytes.....	129
Biosensors immunoassays.....	130
PNA (peptide nucleic acid)-based biosensors.....	130
Protein-based biosensors.....	130
Antibody biosensors.....	130
Cell-based biosensors (cytosensors).....	131
<i>Multicell biosensors</i> .....	131
<i>Microbial biosensors</i> .....	132
Optical biosensors.....	132
<i>Surface plasmon resonance technology</i> .....	132
<i>Label-free optical biosensor</i> .....	133
Microsensors using with nano/microelectronic communications technology.....	133
Electrochemical sensors.....	134
<i>Enzyme electrodes for biosensing</i> .....	134
<i>Conductometric sensors</i> .....	134
<i>Electrochemical genosensors</i> .....	134
<i>Electrochemical nanobiosensor</i> .....	135
Bioelectronic sensors.....	135
Phototransistor biochip biosensor.....	136
Ribozyme-based sensors.....	136
<i>RiboReporters</i> .....	136
Concluding remarks and future prospects of biosensor technology.....	137
Companies developing biosensors for molecular diagnostics.....	138
<b>Molecular labels and detection.....</b>	<b>139</b>
Detection technologies for molecular labels.....	139
Fluorescence and chemiluminescence.....	140
<i>Fluorescence technologies for label detection</i> .....	140
<i>Companies with fluorescence and chemiluminescence products</i> .....	141
Molecular beacons.....	142
The Green fluorescent protein.....	143
Multiphoton detection radioimmunoassay.....	143
Multi-pixel photon counter.....	144
Enzyme labels and detection by fluorescence.....	144
Phase-sensitive flow cytometry.....	144
Microtransponder-based DNA diagnostics.....	145
Laboratory Multiple Analyte Profile.....	146
Multiple labels.....	146
<b>Protein-DNA chimeras for detection of small numbers of molecules.....</b>	<b>147</b>
<b>Single molecule detection.....</b>	<b>147</b>
Atomic force microscopy.....	147

Capillary electrophoresis .....	147
Confocal laser scanning .....	147
Spectrally resolved fluorescence lifetime imaging microscopy .....	148
<b>Molecular imaging .....</b>	<b>148</b>
Basic research in molecular imaging .....	149
Devices for molecular imaging .....	149
Molecular imaging in clinical practice .....	149
Challenges and future prospects of molecular imaging .....	150
Companies involved in molecular imaging .....	150
<b>Nanobiotechnology for molecular diagnostics.....</b>	<b>150</b>
Magnetic nanoparticles .....	151
Gold nanoparticles .....	152
Quantum dot technology.....	152
Nanotechnology on a chip .....	154
Nanogen's NanoChip .....	154
Fullerene photodetectors for chemiluminescence detection on microfluidic chip.....	154
Diagnostics based on nanopore technology .....	155
Nanosensors .....	155
<i>Detection of cocaine molecules by nanoparticle-labeled aptasensors.....</i>	<i>155</i>
<i>Nanosensors for glucose monitoring .....</i>	<i>156</i>
<i>PEBBLE nanosensors .....</i>	<i>156</i>
<i>Quartz nanobalance biosensor .....</i>	<i>156</i>
Cantilever arrays .....	156
Resonance Light Scattering technology .....	157
DNA nanomachines for molecular diagnostics .....	158
Nanobarcodes technology for molecular diagnostics .....	158
Qdot nanobarcode for multiplexed gene expression profiling .....	158
Role of nanobiotechnology in improving molecular diagnostics.....	159
Companies involved in nanomolecular diagnostics.....	159
Concluding remarks about nanodiagnostics .....	162
Future prospects of nanodiagnostics.....	162

#### **4. Proteomic Technologies for Molecular Diagnostics ..... 165**

<b>Introduction .....</b>	<b>165</b>
<b>Proteomic technologies .....</b>	<b>165</b>
Biomarkers of disease .....	165
<i>Proteomic tools for biomarkers .....</i>	<i>165</i>
<i>Search for biomarkers in body fluids.....</i>	<i>166</i>
Captamers with proximity extension assay for proteins.....	166
Cyclical amplification of proteins .....	166
Detection of misfolded proteins by ELISA with exponential signal amplification .....	167
Diagnostics based on designed repeat proteins .....	167
Differential Peptide Display .....	167
Light-switching excimer probes.....	168
MALDI-TOF Mass Spectrometry.....	168
Molecular beacon aptamer .....	169
Molecular beacon assay .....	169
Proteomic patterns.....	169
Real-time PCR for protein quantification.....	171
<b>Protein biochip technologies.....</b>	<b>171</b>
ProteinChip .....	172
LabChip for protein analysis .....	173
TRINECTIN proteome chip.....	173
Protein chips for antigen-antibody interactions molecular diagnostics .....	173
Microfluidic devices for proteomics-based diagnostics .....	174
Nanotechnology-based protein biochips/microarrays .....	174
<i>Nanoparticle protein chip .....</i>	<i>174</i>
<i>Protein nanobiochip.....</i>	<i>174</i>
<i>Protein biochips based on fluorescence planar wave guide technology .....</i>	<i>175</i>
New developments in protein chips/microarrays .....	175
<i>Antibody microarrays .....</i>	<i>176</i>
<i>Aptamer-based protein biochip .....</i>	<i>176</i>
<i>Multiplexed Protein Profiling on Microarrays.....</i>	<i>176</i>
<i>Proteomic pattern analysis .....</i>	<i>177</i>
<i>Single molecule array.....</i>	<i>177</i>
<i>Viral protein chip .....</i>	<i>177</i>
Commercial development of protein chips for molecular diagnostics .....	178
Proteome Identification Kit.....	179
<b>Laser capture microdissection (LCM).....</b>	<b>179</b>
LCM technology .....	179
Applications of LCM in molecular diagnostics .....	180

<b>Proteomic diagnosis of CNS disorders.....</b>	<b>180</b>
Cerebrospinal fluids tests based on proteomics .....	180
Urine tests for CNS disorders based on proteins in urine .....	181
Diagnosis of CNS disorders by examination of proteins in the blood.....	181
Diagnosis of CNS disorders by examination of proteins in tears.....	182
Role of proteomics in the diagnosis of Alzheimer's disease .....	183
Role of proteomics in the diagnosis of Creutzfeldt-Jakob disease.....	183
Future prospects of use of proteomics for diagnosis of CNS disorders .....	183
<b>Concluding remarks on the use of proteomics in diagnostics.....</b>	<b>183</b>

## **5. Molecular Diagnosis of Genetic Disorders ..... 185**

<b>Introduction .....</b>	<b>185</b>
<b>Cytogenetics .....</b>	<b>186</b>
FISH with probes to the telomeres .....	186
Single copy FISH probes .....	186
Comparative genomic hybridization .....	187
<b>Use of biochips in genetic disorders.....</b>	<b>187</b>
Representational oligonucleotide microarray analysis.....	188
SignatureChip®-based diagnostics for cytogenetic abnormalities .....	188
<b>Diagnosis of genomic rearrangements by multiplex PCR .....</b>	<b>188</b>
<b>Quantitative fluorescent PCR .....</b>	<b>188</b>
<b>Mutation detection technologies.....</b>	<b>189</b>
PCR-based methods for mutation detection .....	190
<i>Cleavase Fragment Length Polymorphism .....</i>	<i>190</i>
<i>Direct dideoxy DNA sequencing .....</i>	<i>190</i>
<i>Digital Genetic Analysis (DGA).....</i>	<i>190</i>
<i>Fluorescence-based directed termination PCR.....</i>	<i>191</i>
<i>Heteroduplex analysis .....</i>	<i>191</i>
<i>Restriction fragment length polymorphism .....</i>	<i>192</i>
<i>Single-stranded conformation polymorphism (SSCP) analysis .....</i>	<i>192</i>
<i>TaqMan real-time PCR .....</i>	<i>193</i>
Non-PCR methods for mutation detection.....	193
<i>Arrayed primer extension.....</i>	<i>193</i>
<i>BEAMing (beads, emulsion, amplification, and magnetics).....</i>	<i>193</i>
<i>ELISA-protein truncation test .....</i>	<i>194</i>
<i>Enzymatic mutation detection.....</i>	<i>194</i>
<i>Specific anchor nucleotide incorporation .....</i>	<i>194</i>
<i>Conversion analysis for mutation detection .....</i>	<i>195</i>
Biochip technologies for mutation detection.....	195
<i>Combination of FISH and gene chips .....</i>	<i>195</i>
<i>Haplotype Specific Extraction .....</i>	<i>196</i>
<b>Technologies for SNP analysis .....</b>	<b>196</b>
DNA sequencing .....	197
Electrochemical DNA probes.....	198
<i>Use of NanoChip for detection of SNPs.....</i>	<i>198</i>
Single base extension-tag array.....	198
Laboratory Multiple Analyte Profile .....	198
SNP genotyping with gold nanoparticle probes.....	199
PCR-CTPP (confronting two-pair primers).....	199
Peptide nucleic acid probes for SNP detection .....	199
SNP genotyping on a genome-wide amplified DOP-PCR template .....	200
Pyrosequencing .....	200
Reversed enzyme activity DNA interrogation test .....	201
Smart amplification process version 2 .....	201
Zinc finger proteins .....	202
UCAN method (Takara Biomedical).....	202
<b>Biochip and microarray-based detection of SNPs.....</b>	<b>202</b>
SNP genotyping by MassARRAY .....	202
Electronic dot blot assay .....	202
Biochip combining BeadArray and ZipCode technologies .....	203
SNP-IT primer-extension technology .....	203
OmniScan SNP genotyping.....	204
Affymetrix SNP genotyping array .....	204
Concluding remarks on SNP genotyping.....	204
Limitations of SNP in genetic testing.....	204
Haplotyping versus SNP genotyping .....	205
Companies involved in developing technologies/products for SNP analysis .....	205
<b>Role of copy number variations in genetic diagnostic testing .....</b>	<b>206</b>
CNVs in various diseases .....	207
<i>CNVs in genetic epilepsy syndromes.....</i>	<i>207</i>
<i>CNVs associated with schizophrenia .....</i>	<i>207</i>

<i>CNVs associated with autism</i> .....	207
Methods for determination of CNVs .....	208
<i>Wellcome Trust Case Control Consortium CNV typing array</i> .....	209
Study of rare variants in pinpointing disease-causing genes .....	209
<b>Prenatal DNA diagnosis</b> .....	<b>210</b>
Amniocentesis .....	210
Chorionic villus sampling .....	210
Separating fetal cells in maternal blood for genetic diagnosis .....	210
Antenatal screening for Down's syndrome .....	210
Fetal DNA in maternal blood .....	211
Molecular methods for prenatal diagnosis .....	212
<i>aCGH for prenatal diagnosis</i> .....	212
<i>BAC HD Scan test</i> .....	212
<i>FISH for prenatal diagnosis</i> .....	212
<i>PCR for prenatal diagnosis</i> .....	213
<i>Plasma DNA sequencing to detect fetal chromosomal aneuploidies</i> .....	213
In vivo gene expression analysis of the living human fetus .....	213
Noninvasive prenatal diagnosis of monogenic diseases .....	214
<i>Digital relative mutation dosage</i> .....	214
<i>Massively parallel plasma DNA sequencing</i> .....	214
Applications of prenatal diagnosis .....	214
Diagnosis of congenital infections .....	216
Diagnosis of eclampsia .....	216
Use of transrenal DNA for prenatal testing .....	216
<b>Preimplantation genetic diagnosis</b> .....	<b>216</b>
Technologies for preimplantation genetic diagnosis (PGD) .....	217
<i>PCR for preimplantation genetic diagnosis</i> .....	217
<i>FISH for preimplantation genetic diagnosis</i> .....	217
<i>Microarrays for preimplantation genetic diagnosis</i> .....	218
Conditions detected by preimplantation genetic diagnosis .....	218
The future of preimplantation genetic diagnosis .....	219
<b>Companies involved in prenatal/preimplantation diagnosis</b> .....	<b>219</b>
<b>Cystic fibrosis</b> .....	<b>220</b>
Detection of CFTR gene mutations .....	220
CFTR technologies of various companies .....	221
<i>Genzyme's CF gene sequencing</i> .....	222
<i>CF Plus™ Tag-It Cystic Fibrosis Kit</i> .....	222
<i>Asuragen's bead array test</i> .....	222
<i>The Ambry CF Test</i> .....	223
<i>Biochip for CF diagnosis</i> .....	223
<i>Identification of CF variants by PCR/Oligonucleotide Ligation Assay</i> .....	224
<i>SensiGene (SEQUENOM) CF carrier screening test</i> .....	224
<i>Serum proteomic signature for CF using antibody microarrays</i> .....	224
Guidelines for genetic screening for CF .....	224
<b>Congenital adrenal hyperplasia</b> .....	<b>225</b>
<b>Primary immunodeficiencies</b> .....	<b>225</b>
<b>Hematological disorders</b> .....	<b>226</b>
Hemoglobinopathies .....	226
<i>Sickle cell anemia</i> .....	226
<i>Thalassemia</i> .....	227
<i>Paroxysmal nocturnal hemoglobinuria</i> .....	227
Hemophilia .....	227
Hereditary hemochromatosis .....	228
<b>Polycystic kidney disease</b> .....	<b>228</b>
<b>Hereditary metabolic disorders</b> .....	<b>229</b>
Lesch-Nyhan Syndrome .....	229
Gaucher's Disease .....	229
Acute Intermittent Porphyria .....	229
Phenylketonuria .....	230
Hereditary periodic fever .....	230
<b>Achondroplasia</b> .....	<b>230</b>
<b>Molecular diagnosis of cardiovascular disorders</b> .....	<b>231</b>
Coronary Heart Disease .....	232
Cardiomyopathy .....	232
<i>Familial Hypertrophic Cardiomyopathy</i> .....	232
<i>Idiopathic dilated cardiomyopathy</i> .....	233
Cardiac Arrhythmias .....	233
<i>Long Q-T Syndrome</i> .....	233
<i>Familial atrial fibrillation</i> .....	233
<i>Idiopathic ventricular fibrillation</i> .....	234
Congestive heart failure .....	234

Hypertension.....	234
Disturbances of blood lipids.....	235
<i>Familial dyslipoproteinemias</i> .....	235
<i>Hypercholesterolemia</i> .....	235
Thrombotic disorders.....	235
<i>Factor V Leiden mutation</i> .....	236
<i>Pulmonary embolism</i> .....	236
<b>Molecular diagnosis of eye diseases</b> .....	<b>237</b>
Molecular diagnosis of retinitis pigmentosa.....	237
Genetic screening for glaucoma.....	237
<b>Role of molecular diagnostics in rheumatoid arthritis</b> .....	<b>238</b>
<b>Molecular diagnosis of neurogenetic disorders</b> .....	<b>238</b>
Alzheimer's disease.....	240
Charcot-Marie Tooth disease.....	240
Down syndrome.....	241
Duchenne and Becker muscular dystrophy.....	241
eNOS gene polymorphisms as predictor of cerebral aneurysm rupture.....	242
Fragile X syndrome.....	242
Huntington disease.....	242
Hereditary neuropathy with liability to pressure palsies.....	243
Mitochondrial disorders affecting the nervous system.....	243
Parkinson's disease.....	244
Pompe's disease.....	245
Spinal muscular atrophy.....	245
Triple repeat disorders.....	245
<b>Genetic testing for disease predisposition</b> .....	<b>246</b>
Direct-to-consumer genetic tests.....	246
<b>6. Molecular Diagnosis of Infections</b> .....	<b>249</b>
<b>Introduction</b> .....	<b>249</b>
<b>Molecular techniques for the diagnosis of infections</b> .....	<b>249</b>
Antibody-enhanced microplate hybridization assays.....	250
Bacteriophage-based methods for detection of bacteria.....	250
Biosensors for detection of microorganisms.....	251
<i>Ibis T5000™ Biosensor System</i> .....	251
DNA enzyme immunoassay.....	251
DNA biochip/microarray in diagnosis of infections.....	251
DNA-based typing methods.....	252
<i>Restriction fragment length polymorphism analysis</i> .....	252
<i>Ribotyping</i> .....	253
<i>Random amplified polymorphic DNA</i> .....	253
<i>Combinatorial DNA melting assay</i> .....	253
Electrochemical detection of pathogens.....	253
High resolution melt analysis for diagnosis of infections.....	254
Ligase chain reaction.....	254
Mass spectrometry for microbial identification.....	254
Metagenomic pyrosequencing.....	254
Multiplex PCR for detection of infections.....	255
<i>Dual priming oligonucleotide for multiplex PCR</i> .....	256
<i>LightCycler® SeptiFast Test</i> .....	256
<i>Multiplex amplified nominal tandem repeat analysis</i> .....	257
<i>VYOO® Sepsis Test</i> .....	257
NASBA for detection of microorganisms.....	257
Nucleic acid probes.....	257
Neutrophil CD11b expression as a diagnostic marker.....	258
Optical Mapping.....	258
PNA-FISH for diagnosis of infections.....	258
Quantitative reverse-transcription PCR for bacterial diagnostics.....	259
Rupture event scanning.....	259
Real-time single-molecule imaging of virus particles.....	259
Single-strand conformational polymorphism.....	259
SmartGene platform for identifying pathogens based on genetic sequences.....	260
Tessera array technology.....	260
<b>Applications, advantages and limitations of molecular diagnostics</b> .....	<b>260</b>
Molecular diagnostics versus other microbial detection technologies.....	260
Advantages of nucleic acid-based diagnostics in infections.....	261
Drawbacks of nucleic acid-based diagnostics in infections.....	261
Nanotechnology for detection of infectious agents.....	262
<b>Bacterial and fungal infections</b> .....	<b>262</b>
Mycobacterium tuberculosis.....	264
<i>Conventional diagnosis of tuberculosis</i> .....	264

<i>Microscopic Observation Drug Susceptible Assay for tuberculosis</i> .....	264
<i>Molecular diagnostics for tuberculosis</i> .....	265
<i>Combined tuberculin testing and ELISpot<sup>PLUS</sup> assay</i> .....	266
<i>Biomarkers for tuberculosis</i> .....	267
<i>Diagnosis of drug-resistant M. tuberculosis infection</i> .....	267
<i>Cost-effectiveness of PCR in tuberculosis screening</i> .....	268
<i>Other mycobacteria</i> .....	268
Chlamydial infections .....	268
<i>Neisseria gonorrhoeae</i> .....	270
Bacteria associated with bacterial vaginosis .....	270
Streptococcal infections .....	270
<i>Group B Streptococci</i> .....	270
<i>Streptococcus pyogenes and Streptococcus dysgalactiae</i> .....	271
<i>Pseudomonas aeruginosa</i> .....	271
<i>Helicobacter pylori</i> .....	272
Lyme disease .....	272
Mycoplasmas .....	273
Fungal infections .....	273
<i>Aspergillus</i> .....	274
<i>Candida species</i> .....	274
<b>Viral infections</b> .....	<b>274</b>
HIV/AIDS .....	275
<i>Diagnosis of HIV</i> .....	275
<i>Neonatal screening of infants of HIV-positive mothers</i> .....	276
<i>Screening of cadaveric tissue donors</i> .....	277
<i>Detection of HIV provirus</i> .....	277
<i>Resolution of indeterminate Western blot</i> .....	277
<i>Global Surveillance of HIV-1 genetic variations</i> .....	277
<i>Genotyping for drug-resistance in HIV</i> .....	278
<i>Phenotyping as predictor of drug susceptibility/resistance in HIV</i> .....	279
<i>Tests used for quantification of HIV</i> .....	279
<i>Conclusions about HIV genotyping</i> .....	280
Hepatitis viruses .....	281
<i>Hepatitis A virus</i> .....	281
<i>Hepatitis B virus</i> .....	281
<i>Hepatitis C virus</i> .....	282
<i>Detection and quantification of HCV RNA</i> .....	283
<i>Quantification of HCV RNA levels as a guide to antiviral therapy</i> .....	283
<i>Electrochemical DNA chip for diagnosis of HCV</i> .....	284
<i>HCV Genotyping as a guide to therapy</i> .....	284
Enteroviruses .....	284
Adenoviruses .....	285
Rhinoviruses .....	286
Herpes viruses .....	286
<i>Herpes simplex virus</i> .....	286
<i>Genital and neonatal herpes simplex</i> .....	286
<i>Human cytomegalovirus infections</i> .....	287
<i>Epstein-Barr virus</i> .....	287
Human papilloma virus .....	288
<i>Molecular diagnostics for HPV</i> .....	288
Detection of encephalitis viruses .....	289
<i>West Nile and St. Louis encephalitis</i> .....	289
<i>Venezuelan equine encephalitis virus</i> .....	289
<b>Protozoal infections</b> .....	<b>290</b>
Amebiasis .....	290
Cryptosporidium parvum .....	290
Malaria .....	290
Neurocysticercosis .....	291
Pneumocystis carinii .....	291
Toxoplasmosis .....	291
<b>Infections of various systems</b> .....	<b>292</b>
CNS infections .....	292
<i>Molecular diagnosis in bacterial meningitis</i> .....	292
<i>Molecular diagnosis in herpes simplex encephalitis</i> .....	292
<i>Diagnosis of transmissible spongiform encephalopathies</i> .....	293
Molecular diagnosis of respiratory viruses .....	294
<i>SARS-associated coronavirus</i> .....	294
<i>Influenza viruses</i> .....	295
<i>Avian influenza</i> .....	297
<i>H1N1 influenza</i> .....	301
Gastrointestinal infections .....	303

Periodontal infections .....	304
Diagnosis of urinary infections by a biosensor.....	305
Role of molecular diagnostics in septicemia .....	305
<b>Limitations and needs of diagnostics for infections .....</b>	<b>306</b>
Differentiation between live and antibiotic-killed bacteria .....	307
Cell-based methods for identifying pathogenic microorganisms.....	307
<i>Cell-based virus assays</i> .....	307
<i>Cell-based detection of host response to infection</i> .....	307
<b>Role of molecular diagnostics in hospital acquired infections .....</b>	<b>308</b>
Detection of hospital-acquired bacterial infections .....	308
<i>Detection of methicillin-resistant S. aureus</i> .....	308
<i>Detection of vancomycin-resistant enterococci</i> .....	309
<i>Detection of hospital-acquired C. difficile</i> .....	309
<i>Bacterial genome sequencing in antimicrobial resistance</i> .....	310
Detection of hospital-acquired viral infections .....	310
<i>Molecular diagnosis of BK virus</i> .....	310
<i>Diagnosis of hospital-acquired rotavirus gastroenteritis</i> .....	311
<b>Molecular diagnostics and the microbiome .....</b>	<b>311</b>
Human Microbiome Project.....	311
Application of metagenomics to study of the microbiome .....	312
MicroBiome Analysis Center .....	312
<b>Concluding remarks and future prospects of diagnosis of infections .....</b>	<b>312</b>
Rapid point-of-care diagnosis of infection .....	313
Diagnosis of viruses using protein fingerprinting .....	315
QIAplex PCR multiplex technology.....	315
<b>Companies involved in molecular diagnosis of infectious diseases .....</b>	<b>316</b>

## **7. Molecular Diagnosis of Cancer ..... 319**

<b>Introduction .....</b>	<b>319</b>
<b>Cancer genomics.....</b>	<b>319</b>
Cancer genes .....	320
Oncogenes.....	320
Tumor Suppressor Genes.....	320
<i>p53</i> .....	321
<i>p16</i> .....	322
CNVs in cancer .....	322
Viruses and cancer.....	323
<i>Detecting viral agents in cancer</i> .....	323
<b>Conventional cancer diagnosis.....</b>	<b>324</b>
<b>Molecular techniques for cancer diagnosis.....</b>	<b>325</b>
Genome analysis at the molecular level .....	326
Mutation detection at molecular level .....	327
Expression profiling of tumor cells sorted by flow cytometry .....	327
MicroRNA expression profiling for cancer diagnostics .....	327
Biomarkers in cancer .....	328
<i>Circulating nucleosomes in serum of cancer patients</i> .....	329
<i>Detection of DNA methylation</i> .....	329
<i>eTag assay system for cancer biomarkers</i> .....	331
<i>HAAH as a biomarker for cancer</i> .....	331
<i>LigAmp for detection of gene mutations in cancer</i> .....	332
<i>Mitochondrial DNA as a cancer biomarker</i> .....	332
<i>Oncoproteins as biomarkers for cancer</i> .....	332
<i>Sequencing-based approaches for detection of cancer biomarkers</i> .....	333
Molecular fingerprinting of cancer .....	333
Fluorescent in situ hybridization.....	334
Genetic analysis of cancer.....	334
<i>Comparative genomic hybridization in cancer diagnostics</i> .....	334
<i>Loss of heterozygosity</i> .....	335
<i>Digital karyotyping</i> .....	335
<i>Gene expression profiles predict chromosomal instability in tumors</i> .....	335
PCR Techniques .....	336
<i>Realtime quantitative PCR for diagnosis of cancer</i> .....	336
<i>Cold-PCR</i> .....	337
Antibody-based diagnosis of cancer.....	337
<i>Monoclonal antibodies for diagnosis of cancer</i> .....	337
<i>Recombinant antibodies as a novel approach to cancer diagnosis</i> .....	337
Combined immunological and nucleic acid tests .....	338
<i>Combination of MAbs and RT-PCR</i> .....	338
<i>Immunobead RT-PCR</i> .....	338
Assays for determining susceptibility to cancer .....	338
Gene expression profiling in cancer .....	338

<i>Microarrays for gene expression profiling in cancer</i> .....	339
<i>Serial analysis of gene expression (SAGE)</i> .....	339
<i>DNA tags for finding genes expressed in cancer</i> .....	340
<i>Suppression subtractive hybridization</i> .....	340
Measurement of telomerase activity .....	340
Detection of cancer cells in blood of patients with solid tumors .....	341
Epithelial aggregate separation and isolation .....	342
Proteomic technologies for the molecular diagnosis of cancer .....	343
<i>Proteomic technologies for tumor biomarkers</i> .....	343
<i>Affibodies as contrast agents for imaging in cancer</i> .....	343
<i>Aptamer-based technology for protein signatures of cancer cells</i> .....	344
<i>Aptamers for combined diagnosis and therapeutics of cancer</i> .....	344
<i>Automated image analysis of nuclear protein distribution</i> .....	345
<i>Laser capture microdissection in oncology</i> .....	345
<i>Layered expression scanning</i> .....	346
<i>Membrane-type serine protease-1</i> .....	346
<i>Survivin and molecular diagnosis of cancer</i> .....	346
Biochip/microarrays for cancer diagnosis .....	346
<i>Role of DNA microarrays in gene expression profiling</i> .....	347
<i>Biochip detection of FHIT gene</i> .....	348
Nanobiotechnology for early detection of cancer .....	348
<i>Detection of nanoparticle self assembly in tumors by MRI</i> .....	348
<i>Differentiation between normal and cancer cells by nanosensors</i> .....	348
<i>Magnetic nanoparticle probes</i> .....	349
<i>Quantum dots for early detection of cancer</i> .....	349
Molecular imaging of cancer .....	350
<i>In vivo tumor illumination by adenoviral-GFP</i> .....	350
<i>PET for in vivo molecular diagnosis of cancer</i> .....	350
<i>Xenon-enhanced MRI</i> .....	351
<i>Optical systems for in vivo molecular imaging of cancer</i> .....	351
Detection of micrometastases.....	351
<b>Molecular diagnosis of cancers of various organs .....</b>	<b>352</b>
Brain tumors .....	352
<i>Molecular diagnostic methods for brain tumors</i> .....	352
<i>Glioblastoma multiforme</i> .....	353
<i>Circulating microvesicles as biomarkers of glioblastoma</i> .....	353
<i>Combination of neuroimaging and DNA microarray analysis in GBM</i> .....	353
<i>Medulloblastoma</i> .....	354
<i>Multigene predictor of outcome in GBM</i> .....	354
<i>Oligodendroglioma</i> .....	354
<i>Advantages and limitations of molecular diagnosis of brain tumors</i> .....	355
Breast cancer .....	355
<i>Breast cancer genes</i> .....	356
<i>Circulating nucleic acid biomarkers of breast cancer</i> .....	357
<i>Molecular diagnostic tests for breast cancer</i> .....	357
<i>Mouse ESC-based assays to evaluate mutations in BRCA2</i> .....	359
<i>Genomic profiles of breast cancer</i> .....	360
<i>Role of molecular diagnostics in management of breast cancer</i> .....	361
<i>Tests for prognosis of breast cancer</i> .....	365
<i>Prediction of recurrence in breast cancer for personalizing therapy</i> .....	366
Cervical cancer .....	368
Colorectal cancer .....	369
<i>Detection of familial adenomatous polyposis coli</i> .....	369
<i>Detection of CRC at precancerous state</i> .....	369
<i>Detection of circulating tumor cells in colorectal cancer</i> .....	369
<i>Diagnosis of hereditary nonpolyposis colorectal cancer</i> .....	370
<i>Diagnosis of colorectal cancer from DNA in stools</i> .....	370
<i>Early diagnosis of colorectal cancer from blood samples</i> .....	371
<i>Guanylyl cyclase C tests for colorectal cancer</i> .....	371
<i>Minimally invasive screening for colorectal cancer</i> .....	371
Gastric cancer .....	372
Head and neck cancer .....	372
<i>Nanobiochip sensor technique for analysis of oral cancer biomarkers</i> .....	372
<i>ProteinChip for diagnosis of head and neck cancer</i> .....	373
Hematological malignancies .....	373
<i>Chromosome translocations</i> .....	373
<i>Flow cytometry in diagnosis of leukemia</i> .....	373
<i>Gene chip technology</i> .....	373
<i>Laboratory assessment of leukemia</i> .....	374
<i>Molecular probes</i> .....	375
<i>Minimal residual disease</i> .....	375

<i>Screening of gene mutations in chronic myeloproliferative diseases</i> .....	376
Lung cancer .....	376
Melanoma .....	378
Ovarian cancer .....	378
<i>Mutation of genes</i> .....	379
<i>Relevance of genetic testing to management of ovarian cancer</i> .....	379
<i>Serum biomarkers for early detection of ovarian cancer</i> .....	380
<i>Biomarkers of ovarian cancer</i> .....	380
<i>Concluding remarks on testing for ovarian cancer</i> .....	380
Pancreatic cancer.....	381
Prostate cancer.....	381
<i>Early detection of prostate cancer recurrence by nanotechnology</i> .....	382
<i>Gene expression analysis of prostate cancer</i> .....	382
<i>Huntingtin Interacting Protein 1</i> .....	382
<i>Integrative genomic and proteomic profiling of prostate cancer</i> .....	383
<i>LCM for diagnosis of prostate cancer</i> .....	383
<i>PCA3 gene detection in urine</i> .....	383
<i>PCR assay for assessing silencing of protein cadherin 13 gene</i> .....	384
<i>Prostate biopsy for detection of prostatic intraepithelial neoplasia</i> .....	384
<i>Screening of multiple SNPs for risk of prostate cancer</i> .....	385
<i>Semen testing for prostate cancer biomarkers</i> .....	385
<i>Serum-protein fingerprinting in prostate cancer</i> .....	386
Thyroid cancer.....	386
<i>Gene expression biomarkers of thyroid cancer</i> .....	386
<i>Multiple endocrine neoplasia type 2B as risk factor for thyroid cancer</i> .....	387
<i>miRNA expression profiling in thyroid cancer</i> .....	387
Urinary bladder cancer .....	387
<b>Role of molecular diagnostics in the management of cancer</b> .....	<b>388</b>
Risk assessment and prevention of cancer .....	388
Role of molecular diagnosis in the design of future cancer therapies .....	388
Molecular classification of cancer.....	389
Determination of cancer prognosis .....	389
<i>Prognosis by tumor classification</i> .....	389
<i>Prognosis by cancer gene expression</i> .....	389
Selection of anticancer drugs based on molecular diagnosis .....	390
Integrated genome-wide analysis of cancer for diagnosis and therapy .....	390
<b>Personalized therapy for cancer patients</b> .....	<b>391</b>
Pharmacogenetics and cancer therapy .....	391
Molecular diagnostics as an aid to selection of cancer therapy .....	392
Drug resistance in cancer.....	392
<b>Role of organizations in molecular diagnosis of cancer</b> .....	<b>393</b>
Role of NCI in molecular diagnosis of cancer.....	393
<i>Molecular profiling of cancer</i> .....	393
<i>Cancer Genome Atlas</i> .....	393
<i>Cancer Genetic Markers of Susceptibility Project</i> .....	394
<i>Support for future research in molecular diagnosis of cancer</i> .....	394
Role of the International Cancer Genome Consortium.....	394
<b>Future prospects of molecular diagnosis of cancer</b> .....	<b>395</b>
<b>Companies involved in molecular diagnosis of cancer</b> .....	<b>396</b>

## **8. Molecular Diagnostics in Biopharmaceutical Industry & Healthcare ... 401**

<b>Introduction</b> .....	<b>401</b>
<b>Molecular diagnostics in biopharmaceutical industry</b> .....	<b>401</b>
Molecular diagnostic technologies and drug discovery .....	401
Molecular diagnostics and pharmacogenetics .....	402
Molecular toxicology .....	403
<i>Gene expression studies for toxicology</i> .....	404
<i>Toxicogenomics</i> .....	404
<i>Toxicoproteomics</i> .....	404
Mitochondrial assays .....	406
MetaChip .....	406
Molecular diagnostics and pharmacogenomics .....	406
Applications molecular diagnostics in gene therapy .....	407
<i>Use of PCR to study biodistribution of gene therapy vectors</i> .....	408
<i>PCR for verification of the transcription of DNA</i> .....	408
<i>In situ PCR for direct quantification of gene transfer into cells</i> .....	408
<i>Detection of retroviruses by reverse transcriptase (RT)-PCR</i> .....	409
<i>Assessment of safety issues of gene transfer</i> .....	409
<i>Quantitative PCR for monitoring the effectiveness of gene therapy</i> .....	409
<i>Use of FISH for analysis of adeno-associated viral vector integration</i> .....	409
<i>Monitoring of gene expression by green fluorescent protein</i> .....	409

Detection of microbial contamination in biopharmaceutical manufacturing .....	410
<i>Role of PCR in detecting contamination</i> .....	410
<i>Systems for rapid detection of contaminants</i> .....	410
<i>Contamination of biopharmaceuticals with prions</i> .....	411
DNA tagging for control and tracing of drug distribution channels .....	411
<b>Molecular diagnostics for organ transplantation .....</b>	<b>411</b>
Tissue typing .....	411
Commercial products for transplant molecular diagnostics .....	413
Post-cardiac transplant patient monitoring for rejection .....	415
<b>Application of molecular diagnostics in blood transfusion .....</b>	<b>416</b>
Molecular diagnostics for testing transfusion compatibility .....	416
Transmission of infections in blood transfusion .....	416
Molecular tests for screening of blood supply for viruses .....	417
Commercial molecular diagnostic technologies for blood screening .....	417
<i>Bridge amplification technology</i> .....	418
<i>COBAS AmpliScreen HCV and HIV Assays</i> .....	418
<i>INACTINE</i> .....	418
<i>NucliSens Extractor system</i> .....	419
<i>Pall's enhanced Bacteria Detection System</i> .....	419
<i>PCR combined with algorithm method</i> .....	420
<i>Prions detection in human blood</i> .....	420
<i>PRISM® automated system</i> .....	420
<i>Procleix HIV-1/HCV Assay</i> .....	421
<i>West Nile virus detection in human blood</i> .....	421
Limitations of molecular diagnostics for blood screening .....	421
<b>Molecular epidemiology .....</b>	<b>422</b>
Molecular epidemiology of genetic diseases .....	423
<i>Role of CNVs in study of genetic epidemiology</i> .....	423
<i>Monogenic versus polygenic disorders</i> .....	423
<i>Critical issues facing genetic epidemiology</i> .....	424
Molecular epidemiology of infectious diseases .....	424
<i>Methods and purposes</i> .....	424
<i>Emerging infections</i> .....	425
<i>Human vs. non-human infections</i> .....	425
<i>Genetics and susceptibility to infectious disease</i> .....	426
Molecular epidemiology of cancer .....	426
<i>Molecular epidemiology of p53 gene mutations</i> .....	426
<i>Molecular epidemiology of link between virus and cancer</i> .....	427
<i>Molecular epidemiology and cancer prevention</i> .....	427
<i>SNPs and molecular epidemiology</i> .....	427
<b>Molecular diagnostics for identification of food-borne pathogens .....</b>	<b>427</b>
Introduction .....	427
Molecular diagnostic methods used in food-borne infections .....	428
Limitations of use of molecular probes in food analysis .....	429
Detection of Listeria-contaminated foods .....	429
<i>Optical biosensor for detection of Listeria</i> .....	429
<i>MicroSEQ® Listeria Detection Kit</i> .....	430
Detection of Salmonella .....	430
<i>MicroSEQ® Salmonella Detection Kit</i> .....	430
<i>E. Coli</i> detection .....	430
<i>MicroSEQ® E. Coli Detection Kit</i> .....	430
<i>DuPont Bax system</i> .....	431
Companies with technologies for food pathogen detection .....	431
Transmissible spongiform encephalopathies (TSEs) .....	432
<i>Molecular diagnosis of TSEs</i> .....	432
<i>Companies involved in developing molecular diagnostics for TSEs</i> .....	435
<b>Detection of genetically modified organisms in food .....</b>	<b>436</b>
<b>Molecular diagnostics for detection of doping in sports .....</b>	<b>436</b>
Screening of synthetic glucocorticosteroids in human urine .....	437
Detection of gene doping .....	437
<b>Role of molecular diagnostics in future healthcare .....</b>	<b>437</b>
Translation of genomic research into genetic testing for healthcare .....	438
Molecular diagnostics and disease management .....	438
Role of genetic biomarkers in disease management .....	439
Role of molecular diagnostics in personalized medicine .....	439
Integrated healthcare .....	439
<i>Screening</i> .....	439
<i>Early diagnosis</i> .....	440
<i>Prevention</i> .....	440
<i>Therapy based on molecular diagnosis</i> .....	440
<i>Monitoring of therapy</i> .....	440

<i>Advantages and limitations of integrated healthcare</i> .....	441
<i>Commercially available systems for integrated healthcare</i> .....	441
Combination of diagnostics and therapeutics .....	442
<i>Companion diagnostics</i> .....	442
<i>Companies involved in companion diagnostics</i> .....	442
Point-of-care diagnosis .....	444
<i>Technologies for point-of-care diagnosis</i> .....	445
<i>Biochips for point-of-care diagnosis</i> .....	446
<i>Advantages versus disadvantages of point-of-care diagnosis</i> .....	447
<i>Companies developing point-of-care diagnosis</i> .....	448
The impact of molecular diagnostics on clinical laboratory practice .....	450
<b>9. Molecular Diagnostics in Forensic Medicine and Biological Warfare ....</b>	<b>451</b>
<b>Application of molecular diagnostics in forensic medicine .....</b>	<b>451</b>
Technologies .....	451
<i>Extraction of DNA from forensic samples</i> .....	451
<i>Mitochondrial DNA (mtDNA) analysis</i> .....	452
<i>Polymorphic Alu insertions</i> .....	452
<i>Single Nucleotide Polymorphisms (SNP) analysis</i> .....	453
<i>Short tandem repeat (STR)</i> .....	453
<i>Fluorescent detection systems</i> .....	454
<i>ABO genotyping</i> .....	454
<i>DNA analysis for identification of ancient or historical specimens</i> .....	454
Applications .....	454
<i>Applications in criminology</i> .....	455
<i>Identification of remains of military personnel</i> .....	456
<i>Identification of remains of victims of mass disasters</i> .....	456
<i>Parentage testing</i> .....	458
<i>Gender determination</i> .....	459
Companies developing molecular diagnostics for forensic science .....	459
<b>Molecular detection of biological warfare agents .....</b>	<b>460</b>
Introduction to biological warfare agents .....	460
Role of PCR in the diagnosis of biological warfare agents .....	461
<i>Multiplex PCR microarray assay to detect bioterror pathogens in blood</i> .....	462
Laboratory diagnosis of Anthrax .....	462
Challenges in diagnosis of biological warfare agents .....	463
US government efforts for detection of biological warfare agents .....	463
<i>The US Army Medical Research Institute of Infectious Diseases</i> .....	463
<i>Homeland Security Advance Research Projects Agency</i> .....	464
<i>Handheld Isothermal Silver Standard Sensor</i> .....	465
Commercial development of diagnostic devices for biological agents .....	465
<i>Companies developing diagnostic devices for biological agents</i> .....	465
<i>Biodefense microarray</i> .....	467
<i>Identification of genetic markers of individual pathogens</i> .....	468
<i>Microbial Identification System based on OptiChip™</i> .....	468
<i>Hand-Held Advanced Nucleic Acid Analyzer</i> .....	468
<i>Nanogen's portable detection device</i> .....	469
<i>Nanode Array Sensor Microchips</i> .....	469
<i>MicroChemLab</i> .....	469
<i>BioThreat Alert Test Strip</i> .....	470
<i>Benchtop living cell biosensor</i> .....	470
<i>BioForce NanoArray sensor technology</i> .....	470
<i>QTL handheld biosensor</i> .....	471
<i>Analyte 2000 biosensor</i> .....	471
<i>Airborne bacterial spore detection technology</i> .....	471
<i>Destruction and detection of anthrax by lysin</i> .....	471
<i>Biosensor based on mass spectrometry of microorganisms's RNA</i> .....	472
<i>Bead ARray Counter</i> .....	472
<i>ProteinChip-based detection of bioterrorism agents</i> .....	472
<i>TIGER biosensor</i> .....	473
<i>The PathAlert™ Detection System</i> .....	473
<i>VereThreat™</i> .....	473
Concluding remarks about biodefense applications of diagnostics .....	473
<b>10. References .....</b>	<b>475</b>

## Tables

Table 1-1: Landmarks in development of molecular technology and its application to diagnosis .....	25
Table 1-2: Applications of molecular diagnostics .....	45
Table 2-1: Companies with products for nucleic acid isolation .....	53
Table 2-2: Some commercially available real-time PCR systems .....	56
Table 2-3: Applications of real-time PCR .....	58
Table 2-4: A selection of companies with commercially available FISH diagnostics .....	85
Table 2-5: Selected companies with RNA diagnostic tests .....	86
Table 2-6: Companies involved in whole genome amplification .....	94
Table 2-7: Companies involved in sequencing .....	97
Table 2-8: Comparison of methods of identification of unknown DNA sequences .....	100
Table 2-9: Classification of methods of gene expression analysis .....	100
Table 2-10: A selection of companies with gene expression technologies .....	106
Table 2-11: Companies involved in developing PNA diagnostics .....	109
Table 2-12: Companies with bead-based diagnostic assay platforms .....	113
Table 3-1: Applications of biochip technology in relation to molecular diagnostics .....	115
Table 3-2: Companies developing whole genome chips/microarrays .....	121
Table 3-3: Companies involved in biochips for molecular diagnostics .....	122
Table 3-4: Companies developing microfluidic technologies.....	126
Table 3-5: Biosensor technologies with potential applications in molecular diagnostics .....	128
Table 3-6: Important applications of biosensors .....	137
Table 3-7: Companies involved in application of biosensors in molecular diagnostics .....	138
Table 3-8: Selected labels for nucleic acid detection.....	139
Table 3-9: Selected companies with fluorescence and chemiluminescence products .....	141
Table 3-10: Companies involved in molecular beacon manufacture and research .....	142
Table 3-11: Selected companies involved in molecular imaging .....	150
Table 3-12: Nanotechnologies with potential applications in molecular diagnostics .....	151
Table 3-13: Companies developing nanomolecular diagnostics .....	159
Table 4-1: Applications of protein biochips/microarrays .....	171
Table 4-2: Companies involved in developing diagnostic applications of protein biochips.....	178
Table 4-3: Disease-specific proteins in the cerebrospinal fluid of patients.....	180
Table 5-1: Mutation detection technologies .....	189
Table 5-2: Technologies for SNP analysis .....	196
Table 5-3: A sampling of companies involved in technologies for SNP genotyping .....	205
Table 5-4: Application of preimplantation genetic diagnosis in monogenic disorders .....	218
Table 5-5: Companies involved in prenatal/preimplantation diagnostics.....	219
Table 5-6: CFTR genotyping in cystic fibrosis – companies and technologies.....	221
Table 5-7: X-linked immunodeficiency disorders .....	225
Table 5-8: Genes that cause cardiovascular diseases .....	231
Table 5-9: Available molecular diagnostics for neurogenetic diseases .....	238
Table 5-10: Companies offering genetic screening tests directly to consumers .....	247
Table 6-1: Molecular techniques for the diagnosis of infections .....	249
Table 6-2: Bacteria and fungi that can be detected by recombinant DNA tests.....	263
Table 6-3: Viruses that can be detected by recombinant DNA methods .....	275
Table 6-4: Companies with molecular diagnostics for avian influenza virus H5N1 .....	297
Table 6-5: Companies with molecular diagnostics for influenza virus H1N1 .....	301
Table 6-6: Companies developing POC tests for the diagnosis of infections .....	314
Table 6-7: Selected companies involved in molecular diagnosis of infections .....	316
Table 7-1: Estimated new cases of cancer in the US at most involved organs - 2008.....	319
Table 7-2: Tumor suppressor genes, their chromosomal location, function, and associated tumors ....	321
Table 7-3: Viruses linked to human cancer .....	323
Table 7-4: A classification of molecular diagnostic methods in cancer .....	325
Table 7-5: Desirable characteristics of biomarkers for cancer .....	328
Table 7-6: Approved monoclonal antibodies for cancer diagnosis .....	337
Table 7-7: Methods for comparison of gene-expression profiling in tumor specimens.....	339
Table 7-8: Impact of in vivo molecular imaging of cancer on oncology practice.....	351
Table 7-9: Molecular diagnostic tests for breast cancer.....	357
Table 7-10: Companies developing cancer molecular diagnostics .....	396
Table 8-1: Applications of molecular diagnostics in the biopharmaceutical industry .....	401
Table 8-2: Molecular diagnostic technologies for drug discovery .....	402
Table 8-3: Molecular diagnostic technologies used for pharmacogenetic studies.....	402
Table 8-4: Companies with novel molecular toxicology technologies .....	403
Table 8-5: Applications of molecular diagnostics in gene therapy .....	408
Table 8-6: Companies involved in transplant molecular diagnostics.....	413
Table 8-7: Companies involved in molecular diagnostics of blood transfusions.....	417
Table 8-8: Pathogenic bacteria in food and targets for molecular diagnostic probes.....	428
Table 8-9: Companies involved in molecular diagnostics for food-borne infections .....	431
Table 8-10: Testing for harmful prions in brain tissue from dead cattle.....	433
Table 8-11: Companies involved in developing molecular diagnostics for TSEs .....	435
Table 8-12: Companies involved in companion diagnostics.....	443

Table 8-13: Applications of point-of-care diagnosis .....	444
Table 8-14: Companies developing point-of-care diagnostic tests .....	448
Table 9-1: Forensic and legal applications of molecular diagnostics. ....	454
Table 9-2: Molecular technologies used for forensic applications .....	459
Table 9-3: Classification of biological and chemical agents used as weapons of mass destruction .....	460
Table 9-4: Biological warfare agents that can be identified by PCR methods.....	461
Table 9-5: Companies developing detection devices for biological warfare agents .....	465

## **Figures**

Figure 1-1: Relation of molecular diagnostics to other technologies.....	46
Figure 2-1: Rolling circle amplification technology.....	68
Figure 2-2: A schematic view of the Invader operating system .....	76
Figure 2-3: Principle of fluorescent in situ hybridization .....	80
Figure 2-4: Repli-G system of Qiagen.....	95
Figure 2-5: DNA sequencing process .....	96
Figure 2-6: Electrochemical detection of DNA.....	112
Figure 2-7: Elements of a Scorpions primer .....	114
Figure 3-1: Affymetrix GeneChip technology .....	116
Figure 3-2: Basic principle of a biosensor.....	127
Figure 3-3: Surface plasmon resonance (SPR) technology.....	133
Figure 6-1: Use of DNA chips in diagnosing microbial infections.....	252
Figure 6-2: High throughput DNA pyrosequencing for pathogen discovery .....	255