Molecular Diagnosis
Future Directions

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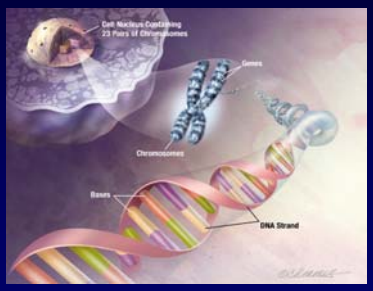
Update on Molecular Diagnostics

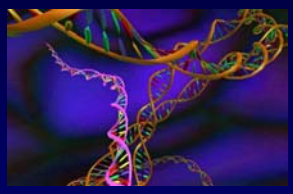
- Detection of target genes of interest
- quantification

- *Infectious diseases*
  - HIV
  - Hepatitis C & B
  - TB / MAC
  - Cytomegalovirus
  - Herpes simplex
  - Varicella zoster
  - CT/GC
  - HPV



Molecular Diagnostics

- Profiling mutations associated with disease outcome
  - Hepatitis C genotype
  - HIV drug resistance genotype
  - Host genetic factors
  - Thrombophilia
  - CyP450 – drug metabolism
  - HLA type



Key developments

- Technology
  - Uptake in diagnostic arena
  - Alternative methods 'other than PCR' – SDA, TMA, LCR, NASBA, bDNA
  - Availability of Analyte Specific Reagents (ASR)
  - Real time or kinetic formats
  - Automation
  - Contamination control

Key developments

- Diagnosis
  - Herpes simplex virus – detection and differentiation
  - Cytomegalovirus – mRNA or qDNA
  - Human papillomavirus (HPV) – ♀♂
  - HCV / HIV primary infection – Tx applications
  - Bacterial sexually transmitted diseases

Key developments

- Monitoring
  - Cytomegalovirus - response to therapy / relapse
  - HIV drug resistance testing
  - Hepatitis C RNA quantification and genotype
  - Improved technology –sensitivity / specificity / efficiency

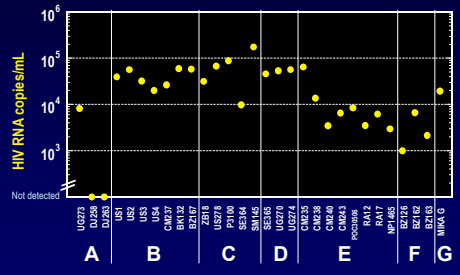
Personalized clinical management

Driving Product Development
Source of error

- **Pre-amplification**
  - Specimen integrity
  - Nucleic acid extraction
  - Reagent preparation
- **Amplification**
  - Conserved target sequence – primer design
  - Equipment – closed tube
  - Contamination control
- **Post-amplification**
  - Volumetric precision / accuracy
  - End point detection – gel, enzymatic, probe hybridisation
  - Real time detection – FRET, Taqman

Uncertainty of measurement

AMPLICOR HIV-1 MONITOR
Performance on Group M Subtypes
Results on 20,000 Virus Particles/mL

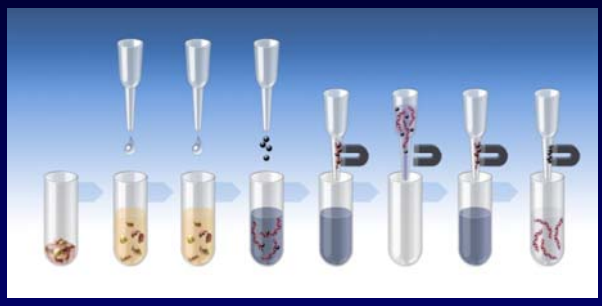


Isolate and Subtype

Pre amplification

- Volumetric errors amplified
- Tedious – manual, repetitive
- Specimen integrity

MagNA Pure LC
Isolation Principle - DNA

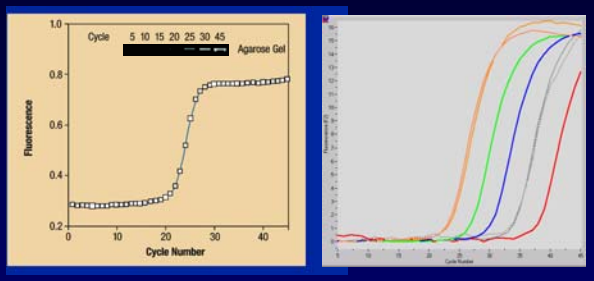


Post amplification & detection
Endpoint detection

- Volumetric error
- Time to result
- Calibration issues
- Result calculations

Kinetic / real time product detection

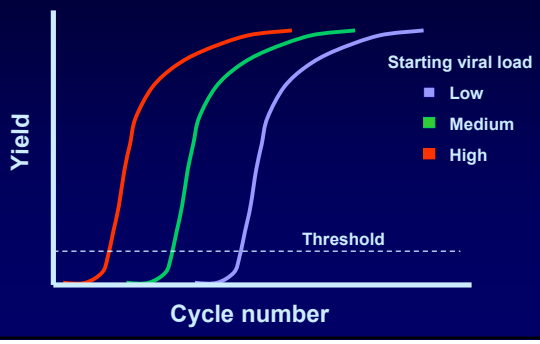
Monitoring in Real time



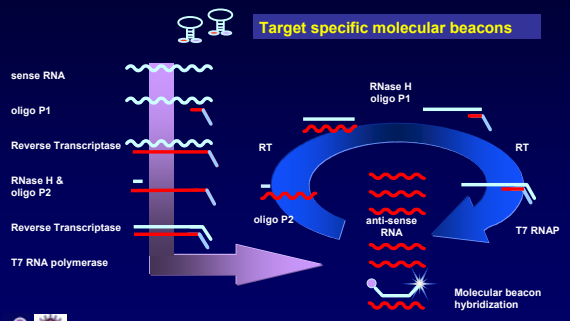
Agarose Gel Blotting FRET

Real Time PCR

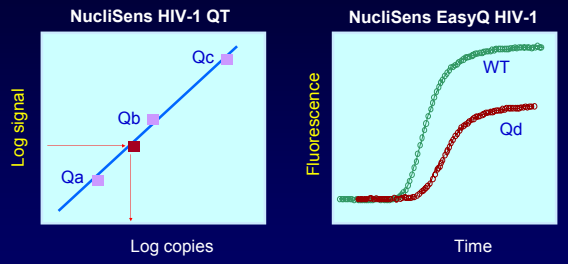
Real Time analysis



Real-time Detection in NASBA



NASBA Calibrators & Quantitation



End-point measurement (WT and 3 calibrators)

Kinetic measurement (WT and 1 calibrator)

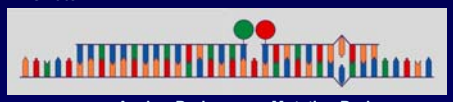
Real Time Target Amplification

Mutation detection and product analysis

Mutation Detection Probe Design

perfect match

mismatch



Anchor Probe Mutation Probe

T_m of Mutation Probe approx. 5 °C lower than T_m of Anchor Probe

Variation in Product T_m

T_m varies by GC composition

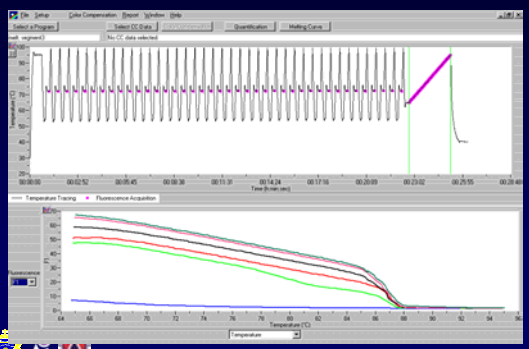
Low GC High GC

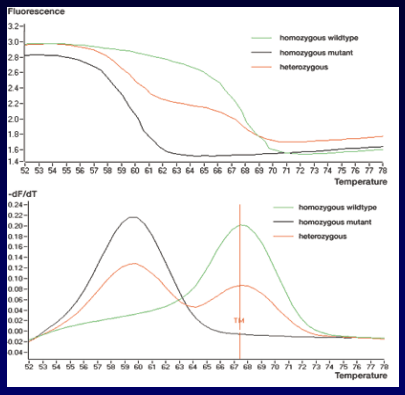
temperature

T_m is influenced by:

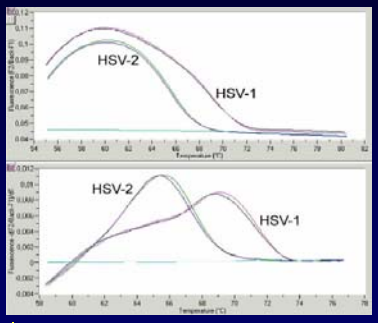
- Salt concentration
- $MgCl_2$ concentration
- SYBR Green I concentration

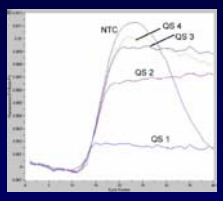
Melting Curve – product analysis





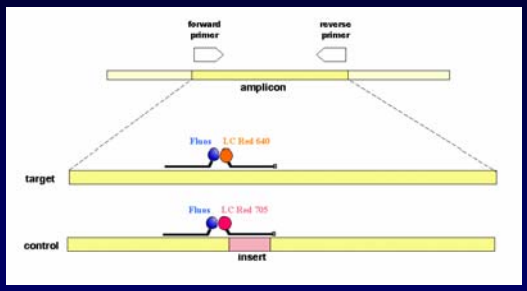
Herpes simplex type differentiation





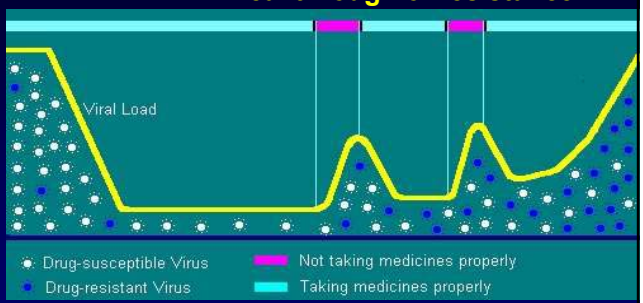
IC detection

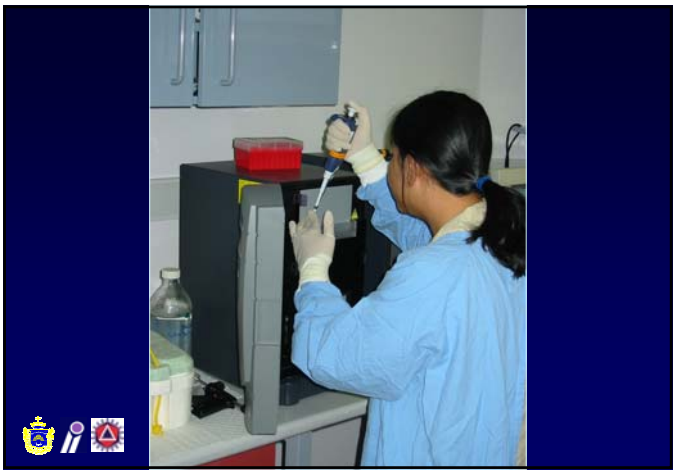
Internal control – 'inhibitor' control

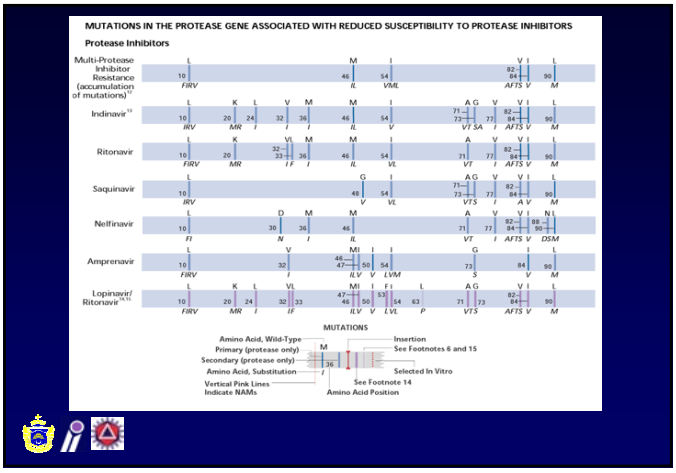


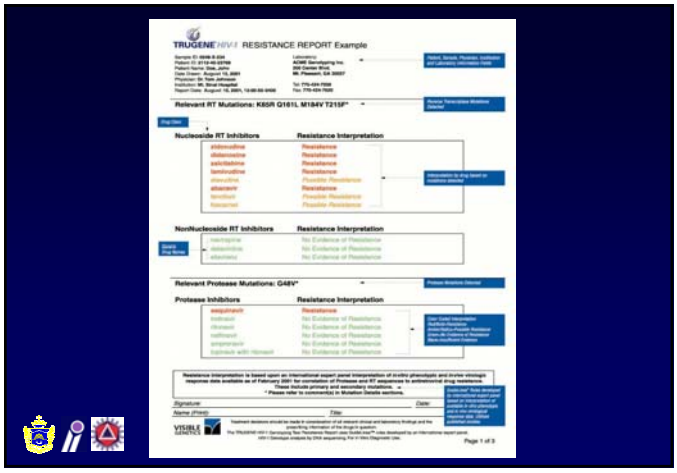
HIV drug resistance testing

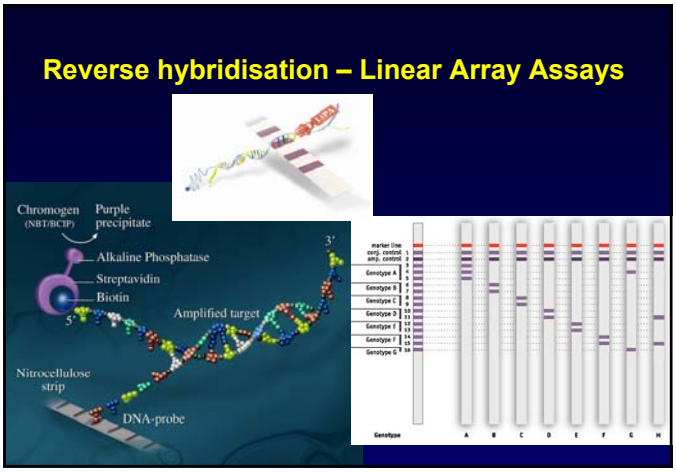
Breakthrough of resistance






MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH REDUCED SUSCEPTIBILITY TO PROTEASE INHIBITORS
Protease Inhibitors
Multi-Protease Inhibitor Resistance (accumulation of mutations)
Indinavir
Ritonavir
Saquinavir
Nelfinavir
Amprenavir
Lopinavir/Ritonavir
MUTATIONS
Amino Acid, Wild-Type
Primary (protease only)
Secondary (protease only)
Amino Acid, Substitution
Vertical Pink Lines Indicate NAMs
Insertion
See Footnotes 6 and 15
Selected In Vitro
See Footnote 14
Amino Acid Position


TRUGENE HIV-1 RESISTANCE REPORT Example
Relevant RT Mutations: K65R G161L M184V T215F*
Nucleoside RT Inhibitors
Resistance Interpretation
NonNucleoside RT Inhibitors
Resistance Interpretation
Relevant Protease Mutations: G48V*
Protease Inhibitors
Resistance Interpretation

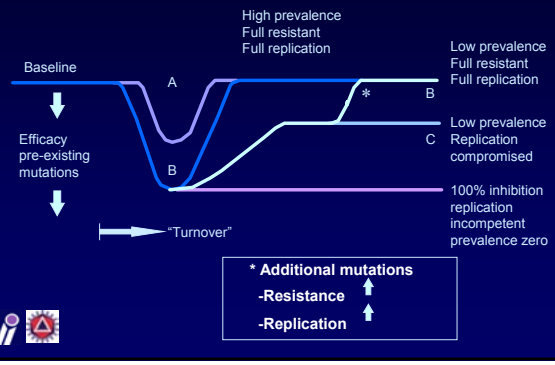

Reverse hybridisation – Linear Array Assays
Chromogen (NBT/BCIP)
Purple precipitate
Alkaline Phosphatase
Streptavidin
Biotin
Amplified target
Nitrocellulose strip
DNA-probe
Genotype A
Genotype B
Genotype C
Genotype D
Genotype E
Genotype F
Genotype G
Genotype

Line probe hybridisation – HCV genotype

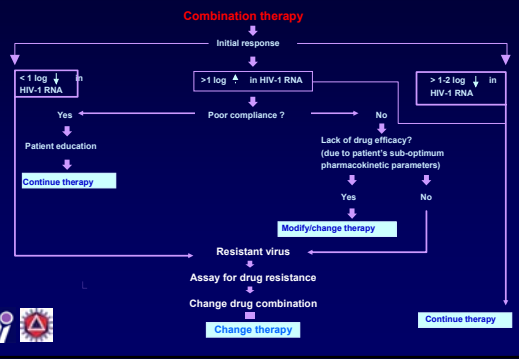
Genotype	Subtype	Prevalence	Geography	Response to therapy
1	1a	approx55% isolates	USA, Europe	Difficult
	1b		USA, Europe, Japan (73%) ; South Korea; China; Taiwan	Most difficult a-IFN
2	2a		Nth USA, Italian and Japanese immigrants	Favourable (2-3 x higher than 1)
	2b		Nth USA, Italian and Japanese immigrants	
	2c		Italy	
3	3a	approx 35% isolates	IVDU USA, Europe, Indian subcontinent; Australian; Thailand; Myanmar; Nepal	Favourable (2-3 x higher than 1)
	3b			
4			Nth Africa; Middle East; Egypt	Difficult, resemble 1
5			South Africa	Difficult, resemble 1
6			Hong Kong Vietnam; Thailand; Indonesia	Difficult, resemble 1
7		(variant of 6)	Vietnam; (Thailand; Indonesia)	
8		(variant of 6)	Vietnam; (Thailand; Indonesia)	
9		(variant of 6)	Vietnam; (Thailand; Indonesia)	
10	10a	(variant of 6)	Indonesia	
11		(variant of 6)	Indonesia	

Individualised management of HIV infection

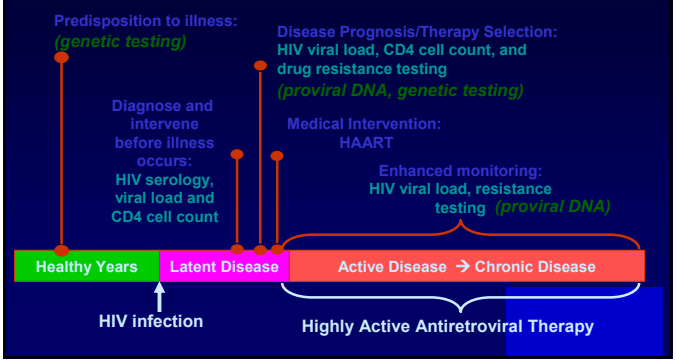
Kinetics of HIV-therapy



Algorithm for assessment of antiviral therapy



Personalized Medicine
HIV infection



Individualised management of HCV infection