

Institute of Clinical Pathology
and Medical Research

Antiviral resistance testing in a clinical laboratory

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Antiviral drug resistance testing

Virus	Resistance testing available	Clinical relevance of resistance testing
HIV	++++	++++
Herpes simplex	++	+
Cytomegalovirus	+++	++
Varicella zoster	+	(+)
Hepatitis B	++	++
Hepatitis C	(+)	(+)
Influenza	+	++
others	-	-

Drug Resistance Testing

Resistance tests use genotyping and/or phenotypic assays to assess susceptibility to drugs

- **Genotypic testing** detects the presence of mutations in a patient's virus population by identifying codon changes that differ from a standard 'wild type' genetic sequence
- **Phenotypic testing** provides information about the relative amount of antiviral drug required to suppress the patient's virus, compared to laboratory or clinical ('wild type') strains

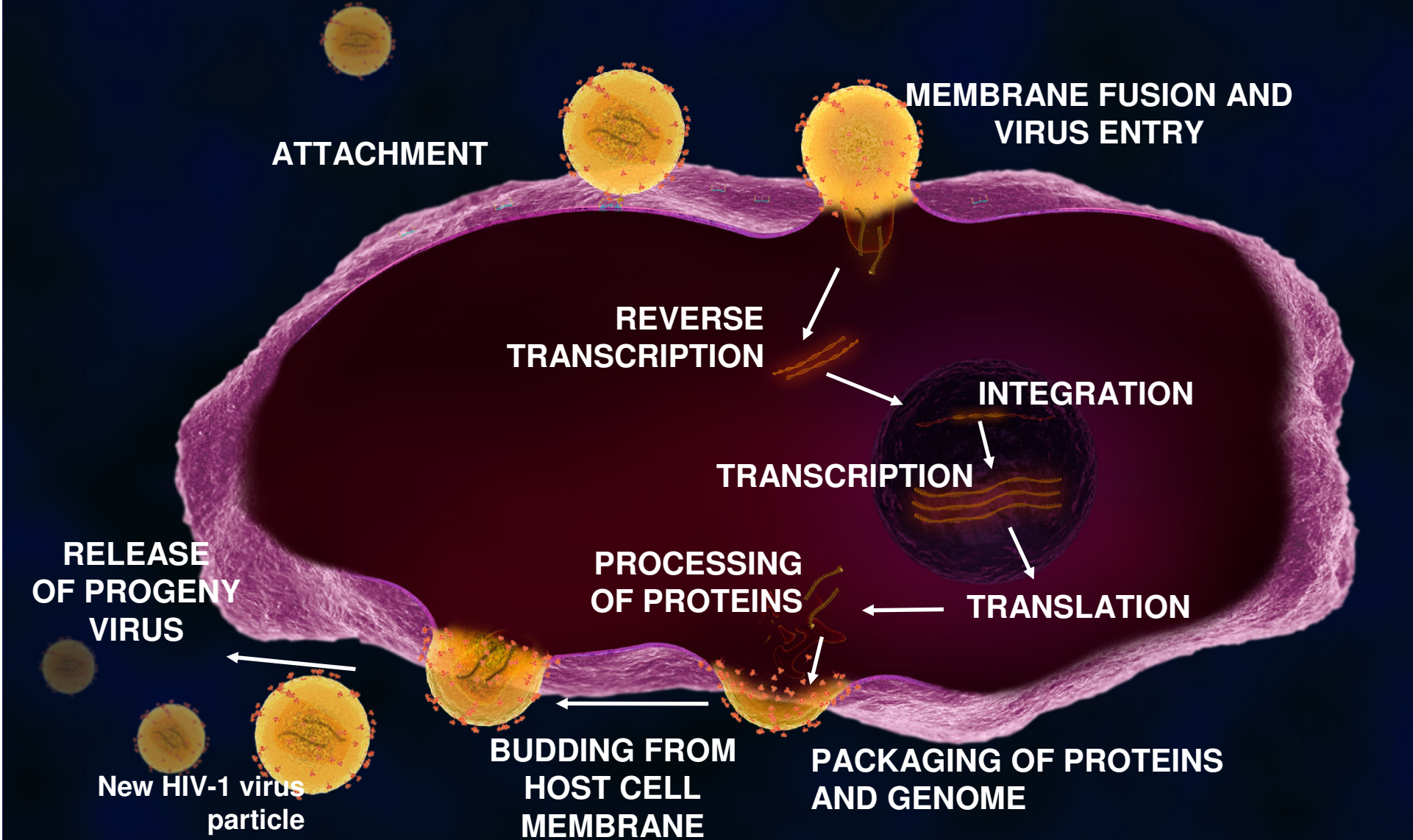
Genotyping methods

- Sequencing
 - drug target
 - (other relevant areas)
- Detecting specific mutations
 - PCR, line probe, RCA etc etc
 - (quantifying specific mutations)

What does 'genotyping' mean?

- drug resistance testing
- establishing linkage between individuals (forensic, medico-legal)
- molecular epidemiology eg. HIV, HCV, HBV, influenza etc
- outbreak investigations eg. measles
- typing viral strains eg. enteroviruses, adenoviruses, influenza

HIV life cycle



Molecular assays in HIV

- Diagnosis
 - Individual (and blood supply)
 - RNA (plasma viral load) and DNA NAT
- Patient management
 - Monitoring and prognosis of *infection*
 - plasma viral load
 - Monitoring and guiding *treatment*
 - plasma viral load
 - **antiretroviral drug resistance testing**
 - HLA-B*5701 PCR (abacavir sensitivity)

Antiretroviral Drugs

- nucleoside reverse transcriptase inhibitors (NRTI)
zidovudine, lamivudine, stavudine, didanosine, abacavir, emtricitabine
- nucleotide RTI (NtRTI)
tenofovir
- nonnucleoside reverse transcriptase inhibitors (NNRTI)
nevirapine, efavirenz
- protease inhibitors (PI)
indinavir, saquinavir, (ritonavir), nelfinavir, fosamprenavir, lopinavir/ritonavir, atazanavir, tipranavir, darunavir
- fusion inhibitors
enfuvirtide

Antiretroviral Drugs

- combinations
 - nucleoside reverse transcriptase inhibitors (NRTI)
eg. AZT/3TC, AZT/3TC/ABV, ABV/3TC, TFV/FTC etc
 - NRTI + NNRTI eg. TFV/FTC/EFV
- new drug classes
 - integrase inhibitors (raltegravir)
 - CCR5 antagonists (maraviroc)

Treatment failure

- **Virologic failure:** incomplete or no plasma HIV RNA response to treatment (repeated measures of >400 copies at week 24 or >50 copies at week 48)
- **Immunologic failure:** no CD4+ T cell rise (25-50 cells/ μ l) over 12 months, or decrease below baseline.
- **Clinical progression:** occurrence or recurrence of HIV-related events, excluding immune reconstitution, after 3 months treatment

Assessing treatment failure

- Patient's adherence to treatment
- Adverse effects of drugs
- Pharmacokinetic issues (including therapeutic drug monitoring)
- Perform drug resistance testing (whilst on treatment) and review treatment history to select at least three new agents

Resistance

Resistance is the ability of HIV to replicate in the presence of drug

it reduces treatment options

it does not mean clinical progression

Resistance

- Need sustained drug pressure
- Phenotypic resistance levels vary widely
- **Major** (at drug contact residues) and **minor** (restore viral fitness) mutations
- Signature mutations for many (but not all) drugs
- Mutations are designated by the wild-type amino acid, then position in the sequence, then the mutant amino acid eg. **M184V** is methionine at position 184 in reverse transcriptase replaced by valine

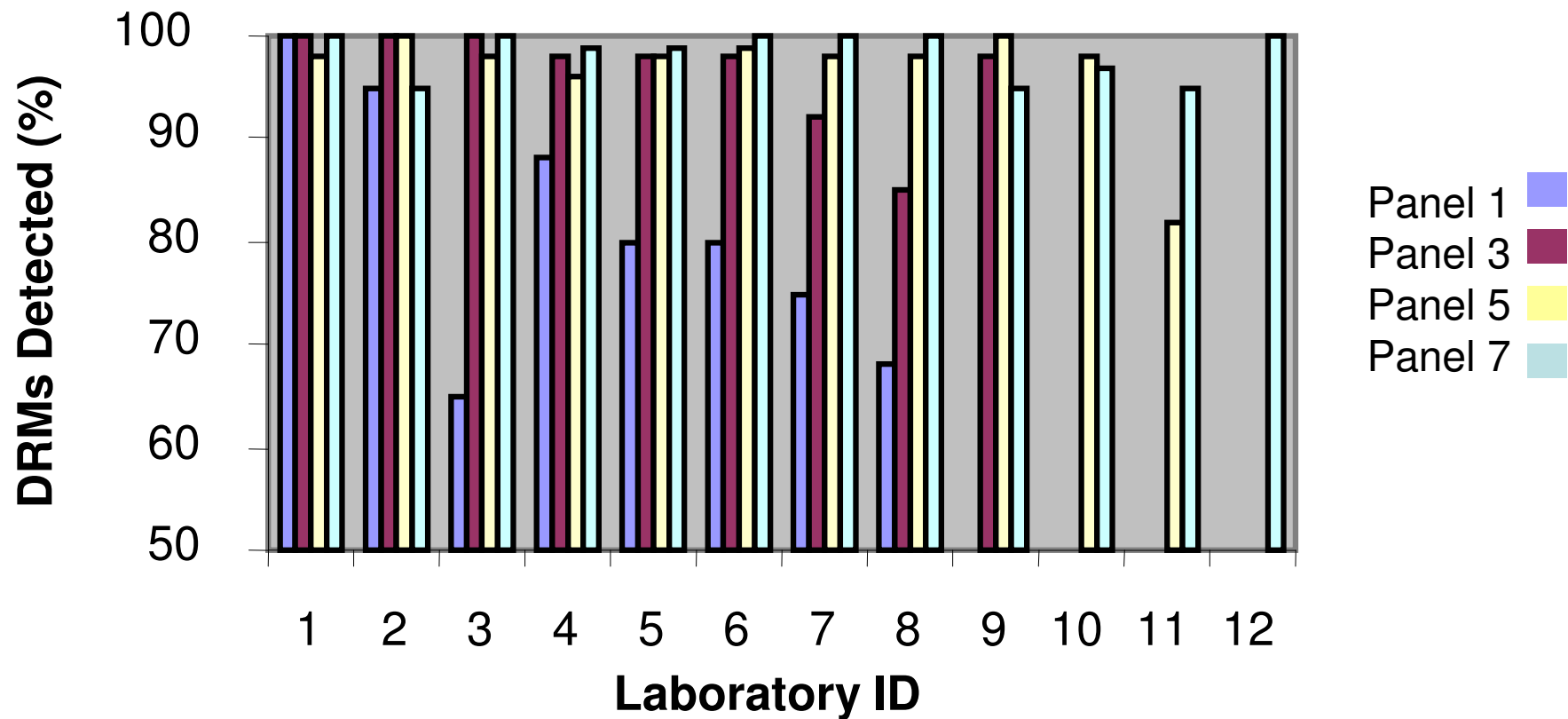
HIV drug resistance testing

- Used in treatment failure and treatment-naïve individuals
- Genotyping done in 8 laboratories in Australia
- 'In-house' primers or commercial systems to generate sequences that span protease and reverse transcriptase
- Sequences compared to databases (eg, Stanford HIV Database, CREST, company) and mutations identified
- National QA program

Australian laboratories that perform antiretroviral drug testing

SA	Institute of Medical and Veterinary Science In-house PCR sequencing and Stanford HIV Database
WA	Immunology, Royal Perth Hospital In-house PCR sequencing and Stanford HIV Databas
VIC	Clinical Research Laboratory, Burnet Institute ViroSeq HIV-1 genotyping system version 2.0
VIC	Victorian Infectious Diseases Reference Laboratory In-house PCR sequencing and Stanford HIV Database
NSW	CIDMLS, ICPMR, Westmead Hospital In-house PCR sequencing and Stanford HIV Database
NSW	Immunology, Royal Prince Alfred Hospital In-house PCR sequencing and Stanford HIV Database
NSW	Centre for Immunology, St Vincents Hospital
QLD	Immunology, Pathology Queensland Abbott PE Applied Biosystems - ViroSeq HIV genotyping system

External Quality Assurance Scheme (EQAS) Detection of mutations in plasma



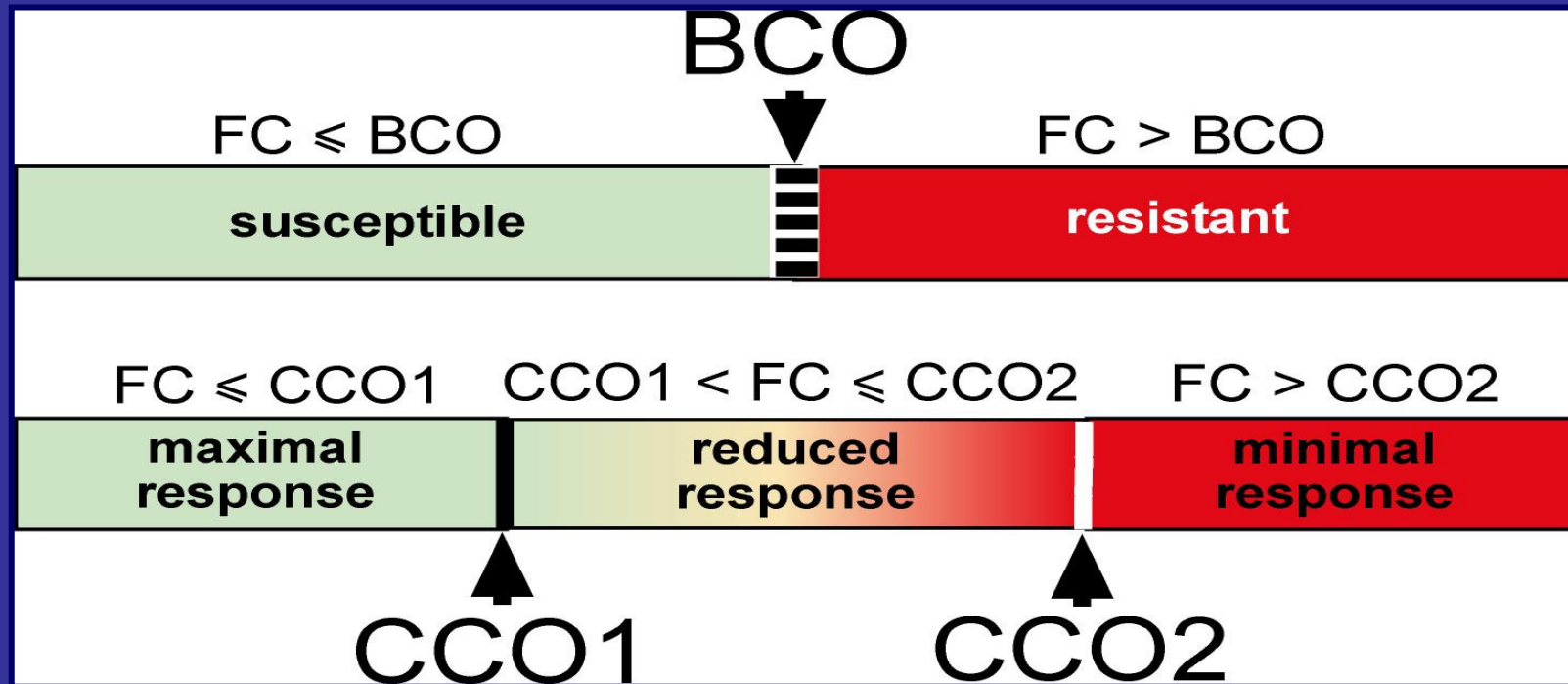
The **genotypic sensitivity score** (GSS) is based on the number of drugs in a study regimen to which the patient has genotypic sensitivity. Score 1 if genotyping shows no resistance mutations to the drug and 0 otherwise.

The GSS is defined as the sum of the genotypic sensitivities for all drugs in the regimen.

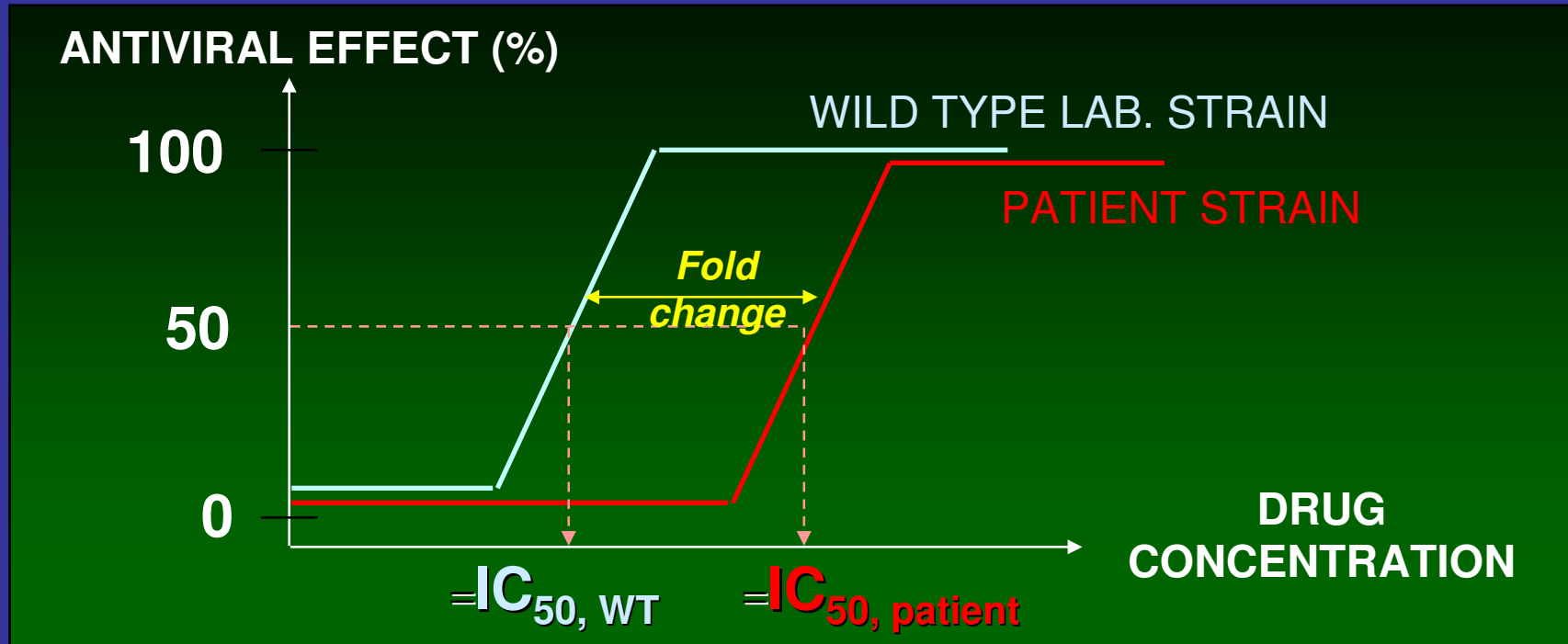
The **phenotypic sensitivity score** (PSS) is based on the number of drugs in study regimen to which the patient has phenotypic sensitivity.

- **Replicative capacity** is the capacity of virus to replicate in the absence of drug (compared to wild type virus)
- **Viral fitness** is the ability of virus to grow in vivo (and depends on the replicative capacity, host factors, drug activity)

Phenotype reports



What is a fold-change?



- $IC_{50} = 50\%$ Inhibitory Concentration

$$\frac{IC_{50, patient}}{IC_{50, WT}} = \text{Fold change}$$

Cut-offs are drug-specific reference values used to determine the significance of a given fold-change value

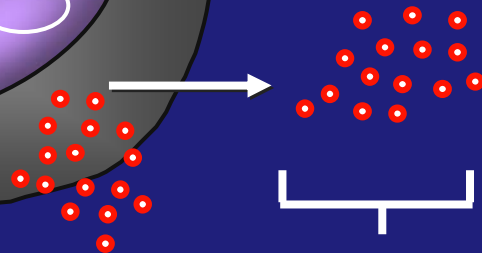
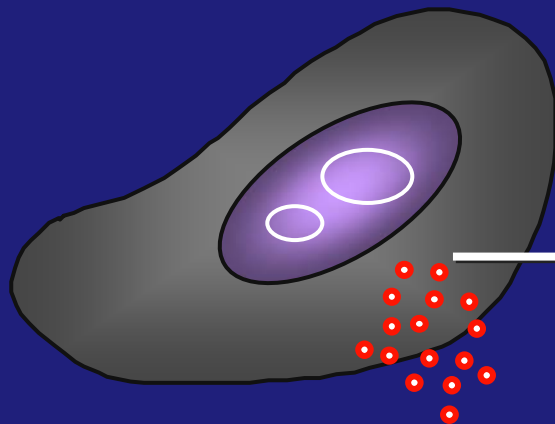
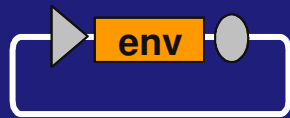
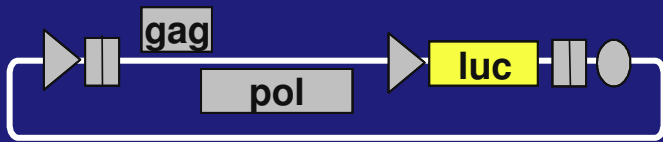
- *Biological cut-offs*

- Define what is resistant and nonresistant based on how viruses respond to a drug *in vitro*.

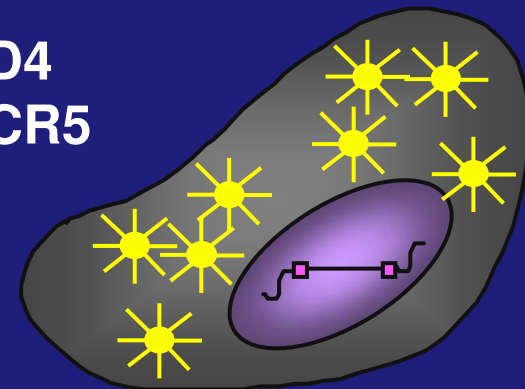
- *Clinical cut-offs*

- Define what is resistant and nonresistant based on how patients respond to a drug *in vivo*.

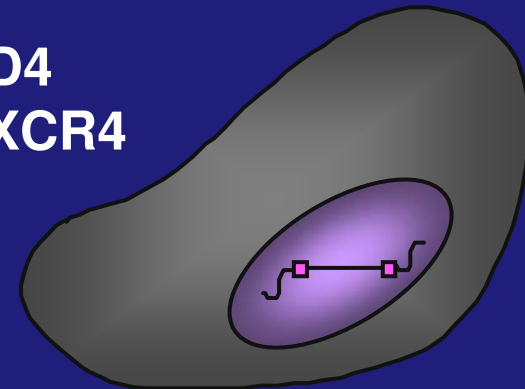
Trofile™: R5-tropic virus



CD4
CCR5



CD4
CXCR4



- R5-tropic
- X4-tropic
- R5/X4-tropic (dual)

Developments in HIV drug resistance testing at the clinical level

- Assessing resistance to new drugs in current classes
- New classes
 - fusion inhibitors (envelope gp41)
 - integrase inhibitors
- Methods to detect 'early' or low-level resistance
- Phenotyping
- Tropism assays (Monogram for CCR5 antagonists)
- Viral 'fitness' assays