Principles of HIV drug resistance testing and interpretation

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Right to Care

Learning outcomes

Upon completion of this module, you should be able to:

- Summarize the mechanisms of HIV drug resistance development

- Explain the basic nomenclature of HIV drug resistance mutations

- Describe the key principles of genotypic resistance testing and resistance interpretation algorithms

- Recognize the limitations of genotypic resistance testing

What does the viral load tell us?

- **Where does the virus live?**
- **In the Blood?**
- **No**
- **In the liver**
- **Yes**
- **Spleen?**
- **Yes**
- **Lymph nodes?**
- **Yes**
- **So what does it mean if we find it in the blood?**

Is the problem coming from this womans lounge?

So what does the viral load tell us?

- **It tells us that the HIV virus is making…….**

MUTATIONS

- HIV virus produces 10 Billion new viruses per day
- It also makes mistakes when it reproduces itself
- One mistake occurs for every cycle of replication
- The reverse transcriptase enzyme has no prrooof reading mechanism and cant correct the mistakes
- These mistakes are called mutations
- Millions of mutations are formed daily

MUTATIONS
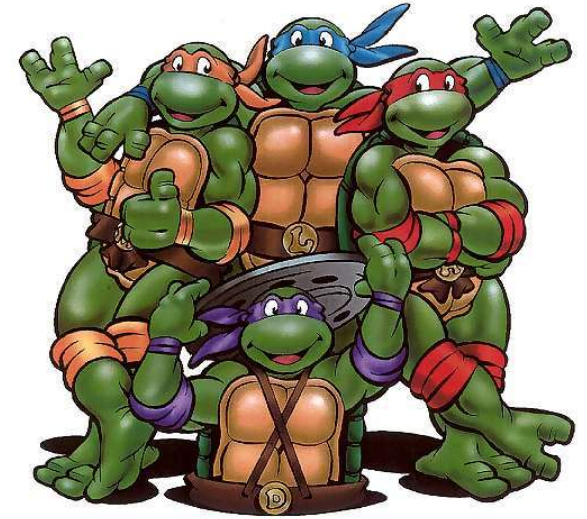
- **If there is no antiretroviral drug around, the mutations quickly disappear**
- **If a mutation causes resistance to a drug, then that quasi species will continue to multiply while the other quasi species are suppressed by that ARV drug.**
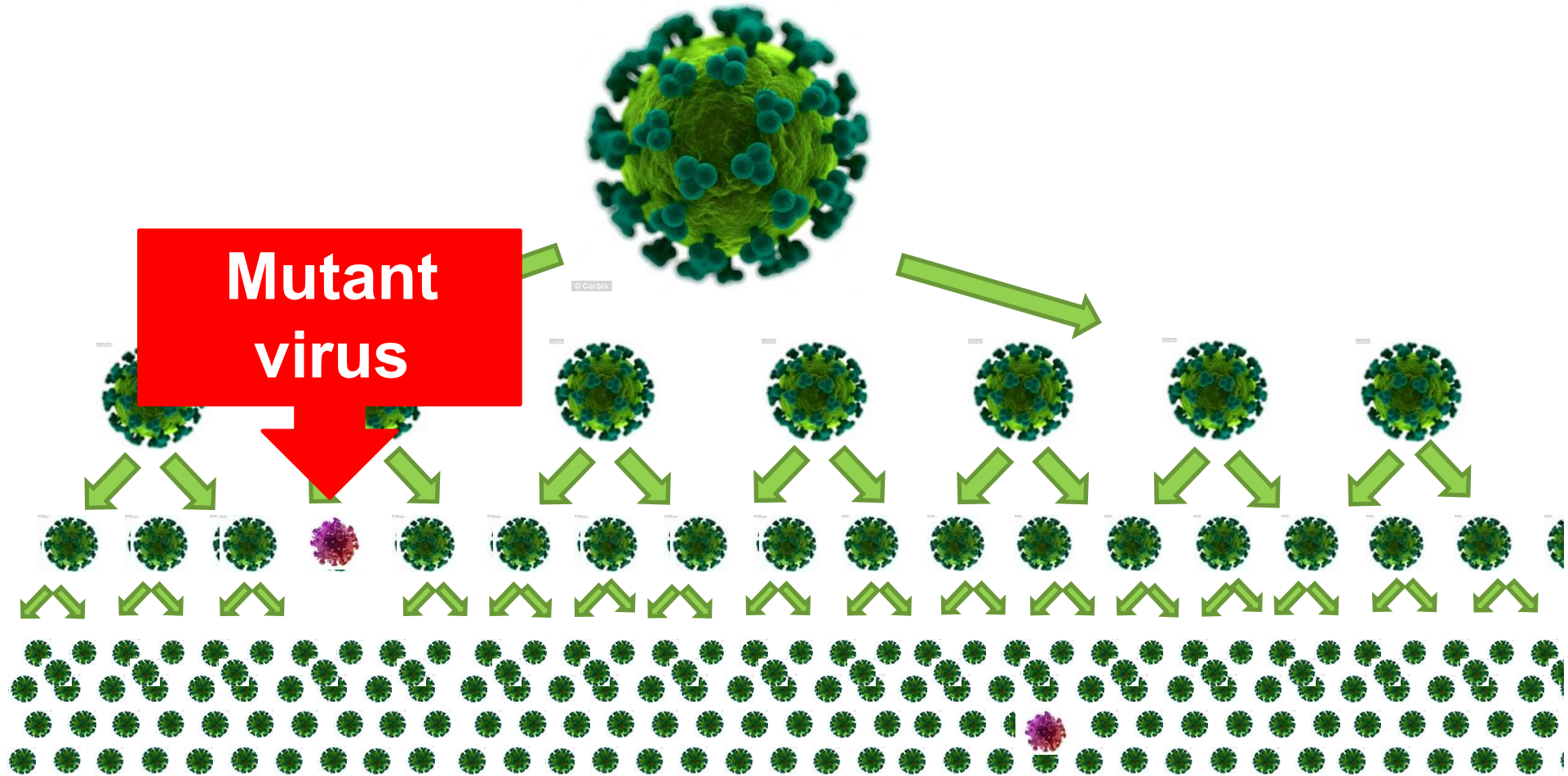- **This will lead to outgrowth of a resistant HIV strain**

We need 2 things for resistance to develop

- **1)Viral multiplication (replication)**
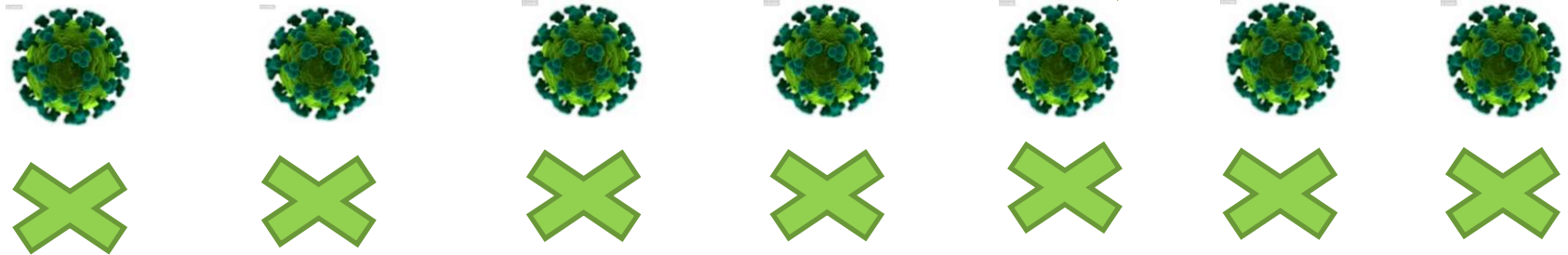
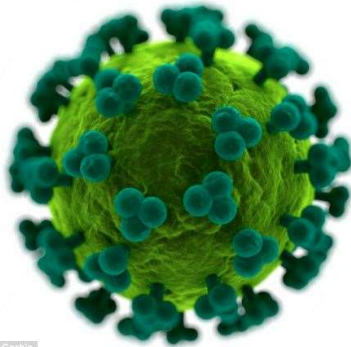- **2)Low levels of ARVs in the body**

NO ARVS

Mutant virus

NO RESISTANCE

Animation sequence courtesy of Dr Julia Turner
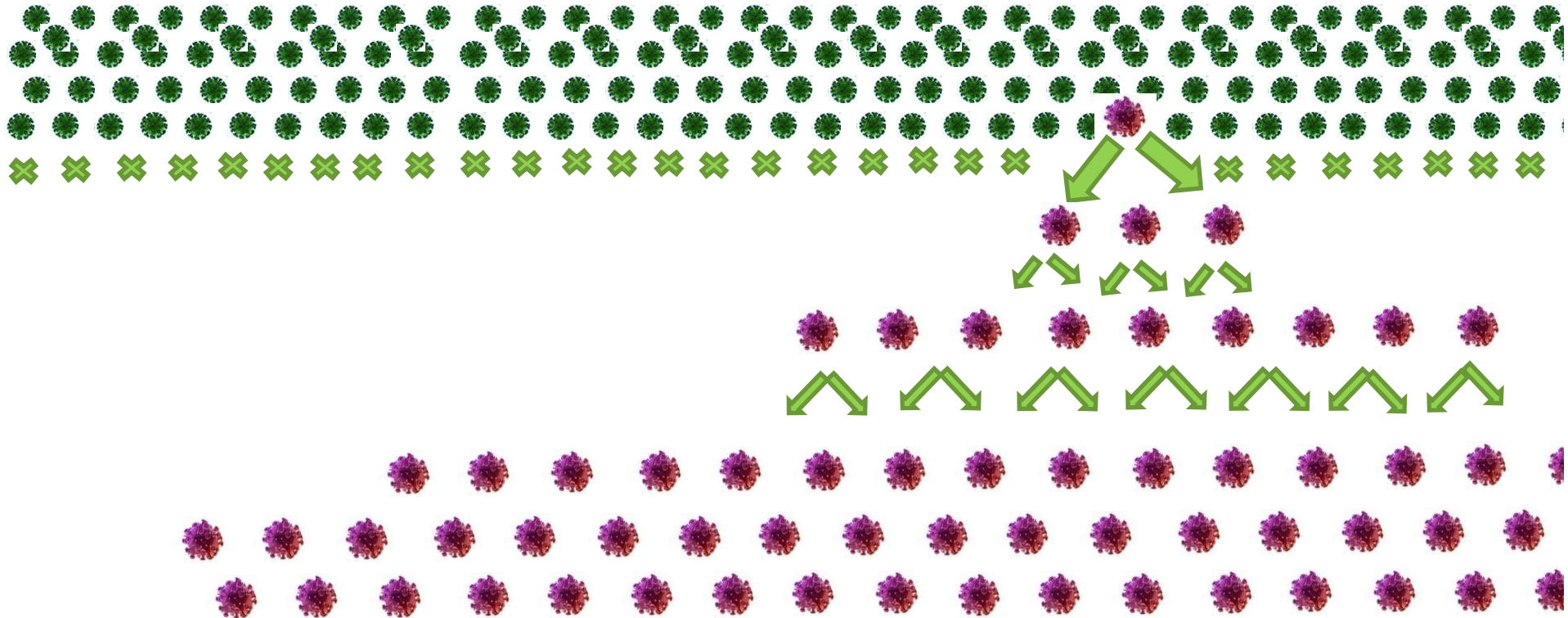
SUFFICIENT LEVELS OF ARVS

NO REPLICATION
NO RESISTANCE

Animation sequence courtesy of Dr Julia Turner

LOW LEVELS OF ARVS PRESENT

REPLICATION + LOW LEVELS OF ARVs=RESISTANCE

Animation sequence courtesy of Dr Julia Turner

Poll 2

- **You do a resistance test on a female patient taking AZT/3TC/LPV/r. It comes back susceptible to all the ARVs.**

- 1. What does this mean? (Multiple Choice)
- A)There is no resistance so she can continue on the same regimen
- B)She has been non-adherent
- C)This is Wild Type virus

- 2. Could there still be viral resistance? (Single Choice)
- D)Yes
- E)No
- F)Not sure

Class	Drug	* STAN ▪ v6.2.0 29/05/2012
NRTI	Zidovudine	S
	Didanosine	S
	Stavudine	S
	Lamivudine	S
	Emtricitabine	S
	Abacavir	S
	Tenofovir	S
NNRTI	Nevirapine	S
	Efavirenz	S
	Etravirine	S
	Rilpivirine	S
PI/Boosted PI	Indinavir/r	S
	Saquinavir/r	S
	Nelfinavir	S
	Fosamprenavir/r	S
	Lopinavir/r	S
	Atazanavir/r	S
	Tipranavir/r	S
	Darunavir/r	S

The battle of the HIV Viruses

Resistant virus

Wild-type virus

Slide courtesy of Dr Anna Maria Geretti
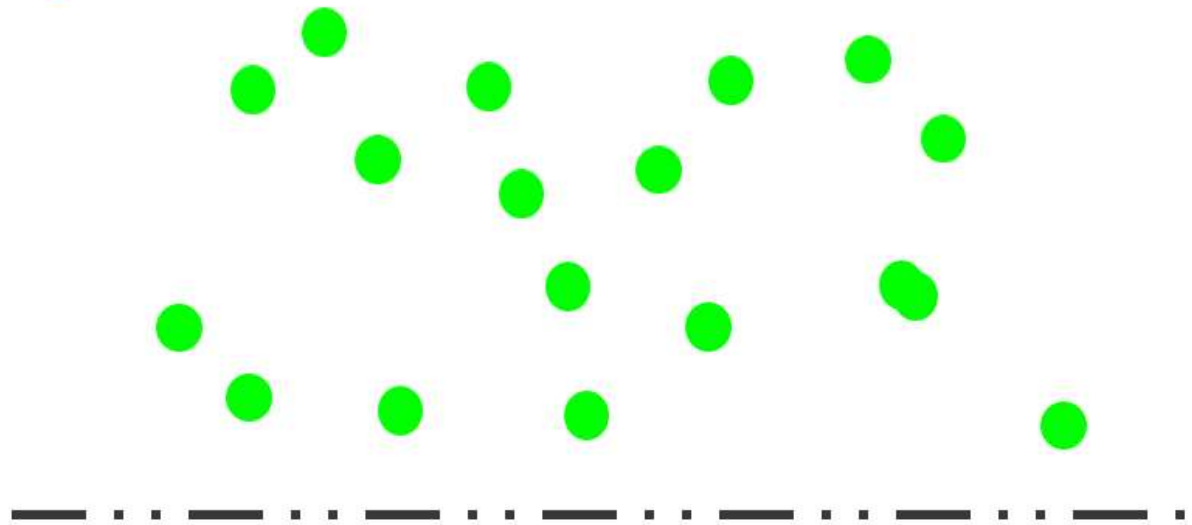
20-30%

How to do resistance testing

- Therefore make certain that patient is adherent before doing resistance testing
- Maintain patient on current antiretroviral therapy - resistance testing must be performed whilst the patient is taking the antiretroviral therapy regimen they are failing
- Ask patient to take ARVs regularly for 1 month and then do resistance testing

Poll 3

- **High Viral Loads**

Question?

If you see 2 patients with the following viral loads, which one do you think is more likely to have resistance?

- VL: 2 000
- VL: 200 000

Resistance mutations generally weaken the HIV virus and reduce its replicability

MUTATION NOTATION

M184V

MUTATIONS

M184V
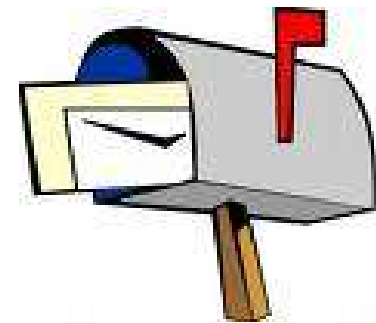
What should be there
in wild type
(Methionine)

MUTATIONS

M184V

↑
What should be there
in wild type
(Methionine)

↑
**What is there
instead (Valine)**

Genetic barrier to resistance

- Some drugs have a low genetic barrier to resistance

- Some drugs have a high genetic barrier to resistance
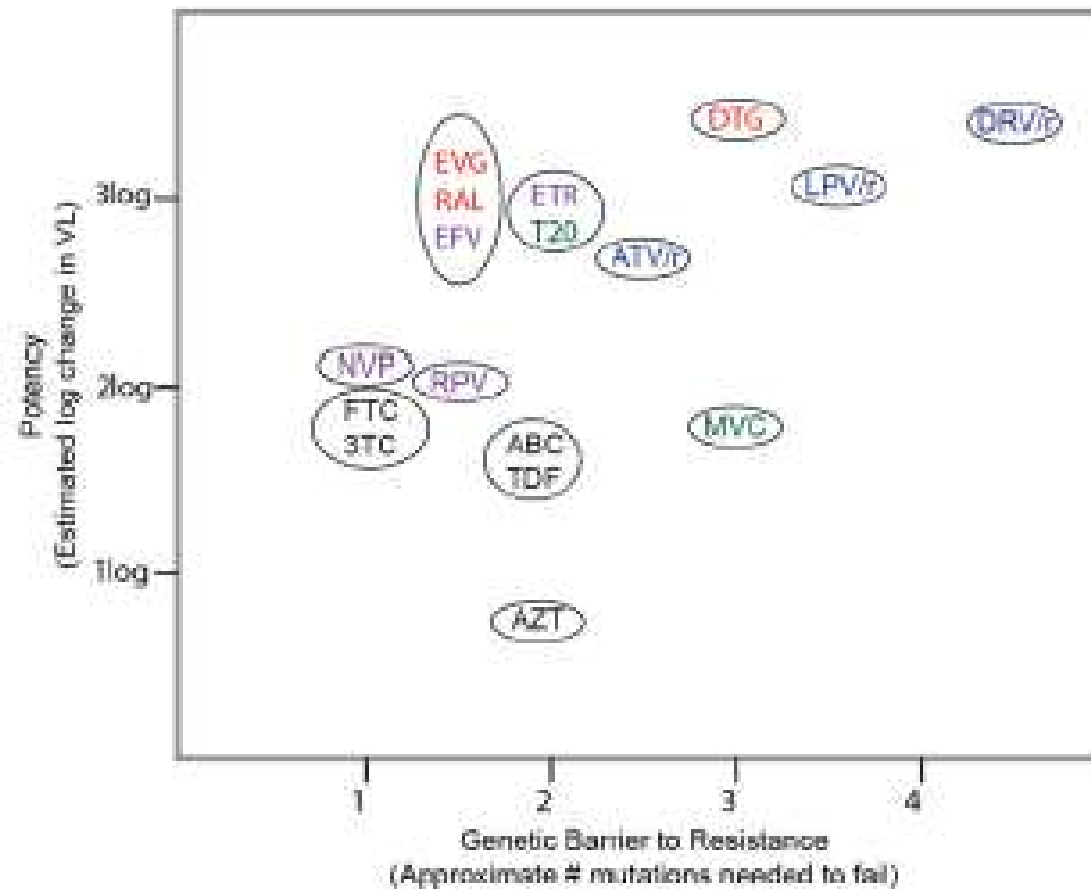
Fig. 1. ARV potency versus genetic barrier to resistance. Abbreviations: ARV; antiretroviral; VL: viral load; ABC: abacavir; ATV/r: boosted atazanavir; DRV/r: boosted darunavir; DTG: dolutegravir; EFV: efavirenz; FTC: emtricitabine; EVG: elvitegravir; T20: enfuvirtide; ETR: etravirine; 3TC: lamivudine; LPV/r: boosted lopinavir; MVC: maraviroc; NVP: nevirapine; RAL: raltegravir; RPV: rilpivirine; and TDF: tenofovir. ARVs in black font are nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), those in purple font are non-NRTIs (NNRTIs), those in blue font are protease inhibitors (PIs), those in red font are integrase strand transfer inhibitors (INSTIs), and those in green font are entry inhibitors. ARVs appearing together in the same ellipse should be considered to have roughly equivalent potencies and genetic barriers to resistance.

Genetic barrier to resistance

- Lamivudine – single mutation (M184V) confers high-level resistance to lamivudine **(low genetic barrier)**

- Efavirenz – single mutation (e.g. K103N) confers high-level resistance and cross-class resistance **(low genetic barrier)**

- Lopinavir/ritonavir – requires several mutations to confer resistance **(high genetic barrier)**

- Dolutegravir-**high genetic barrier**

HIV drug resistance testing

1. genotype

Genotype tests look to see how the structure of a sample of your HIV may have changed.

your hiv

2. phenotype

Phenotype tests see whether HIV drugs still work to control your type of HIV.

your hiv   drug to test
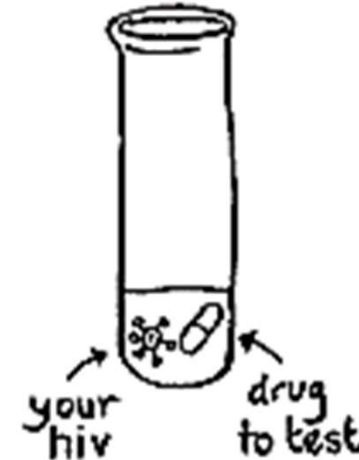
Genotyping	Phenotyping
Sequencing of the HIV-1 gene to detect resistance-associated amino acid substitutions (i.e. mutations)	Measures the ability of a virus to replicate in cell culture in serial dilutions of ARV drugs, thereby directly quantifying the concentration of drug required to inhibit viral replication

How is genotypic drug resistance testing done?
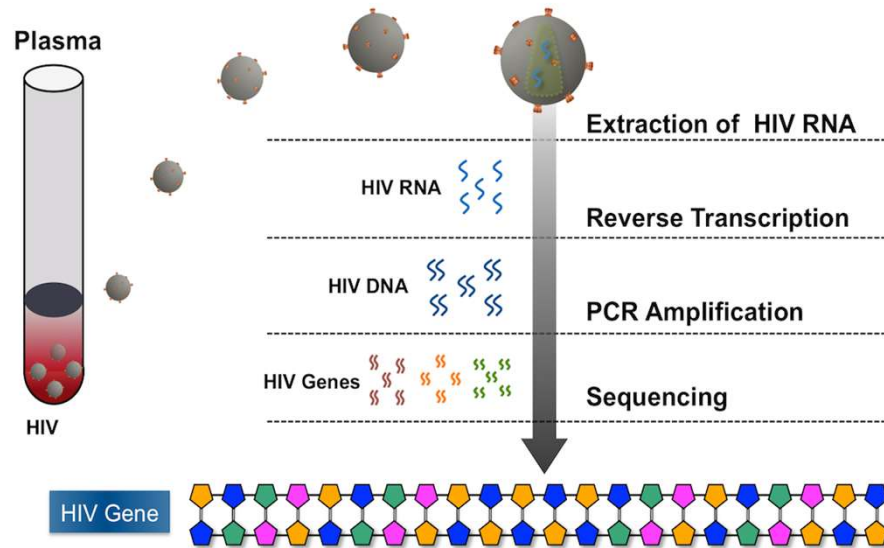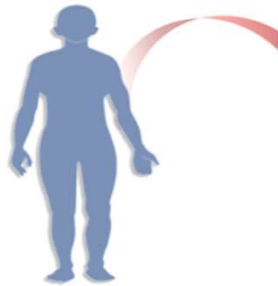
Sanger sequencing

Polymerase chain reaction

Output File

>Sequence1

GTAAAACTAAAGCCAGGAATGGATGGCCCAAAGATTAAACAATGGCCATTGAC
AGAAGAGAAAATAAAAGCATTAACAGAAATTTGTAACGAAATGGAGAAGGAAGG
AAAAATTACAAAAATTGGGCCTGAAAATCCATATAACACTCCAATATTTGCCATA
AAAAAGAAGGACAGTACTAAGTGGAGAAATTAGTAGATTTCAGGGAACTCAAT
AAAAGAACTCAAGACTTTTGGGAAGTTCAATTAGGAATACCACATCCAGCAGGG
TTAAAAAGAAAAATCAGTGACAGTACTGGATGTGGGGGATGCATATTTTTCA
GTTCCTTTAGATGAAGACTTCAGGAAATATACTGCATTTACCATACCTAGTATAA
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AAGGATCACCAGCAATATTCCAAAGTAGCATGATAAAAATCTTAGAGCCCTTTA
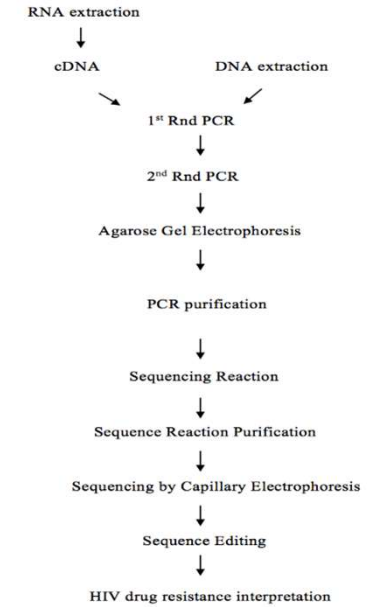GAACAAAAAATCCAGAAATAGTCATCTATCAATATATGGATGATCTGTATGTAG
GATCTGACTTAGAAATAGGGCAACATAGAGCAAAAATAGAGGAGTTAAGAGCA
CATCTATTAAAGTGGGGATTTACCACACCAGACAAGAAACATCAGAAGGAACC
CCCATTTCTTTGGATGGGGTATGAACTCCATCCTGACAAATGGACAGTACAGC
CTATACAGCTGCCAAAAAGGATAGCTGGACTGTCAATGATATACAGAAGTTAG
TGGGAAAACTAAACTGGGCAAGTCAGATTTACCCAGGGATTAAAGTAAGGCAA
CTTTGTAAACTCCTTAGGGGGGCTAAAGCACTAACAGACATAGTACCACTAACT
GAAGAAGCAGAATTAGAATTAGCAGAGAACAAGGAAATACTAAAAGA

https://hivdb.stanford.edu

HIVdb Program

Genotypic Resistance Interpretation Algorithm

HIVdb accepts user-submitted protease, RT, and integrase sequences or mutations and returns inferred levels of resistance to the most commonly used protease, nucleoside, non-nucleoside, and integrase inhibitors. Its purpose is educational and as such it provides extensive comments and a highly transparent scoring system that is hyperlinked to data in the HIV Drug Resistance Database. A detailed description of the program as well as all updates is in the Release Notes. A web service has been created to allow users to access HIVdb programmatically.

Sequences can be entered as plain text if just one sequence is entered. Sequences must be entered using the FASTA format if multiple sequences are entered. Sequences can be pasted in the text box or uploaded using the File Upload option. The upper limit is currently 1000 sequences containing 3000 nucleotides per sequence.

By default the program output will contain an HTML page with a navigation sidebar linking to the results for each sequence. Users selecting the spreadsheet output options, will obtain links to tab-delimited files containing tabular sequence summary data, tabular resistance summary data, and a formatted amino acid sequence alignment. Detailed descriptions of the HTML and spreadsheet output are explained in the Release Notes.

Drug display options

By default, results will be shown for checked ARVs. Use checkboxes for additional ARVs. (select all ARVs, revert to default)

NRTI: ☑ ABC ☑ AZT ☑ FTC ☑ 3TC ☑ TDF ☐ D4T ☐ DDI

INSTI: ☑ DTG ☑ EVG ☑ RAL

NNRTI: ☑ EFV ☑ ETR ☑ NVP ☑ RPV

PI: ☑ ATV/r ☑ DRV/r ☑ LPV/r ☐ FPV/r ☐ IDV/r ☐ NFV ☐ SQV/r ☐ TPV/r

---

Input mutations | **Input sequences**

Header: [   ] (optional)

Upload text file: [Choose File] No file chosen

>Sequence1
CCTCAAATCACTCTTTGGCAACGACCCTTTGTCACAATAAAAGTAGGGGGCCAGACAAGAGAGGCTCTCCTAGACACAGGGGCAGATGATACAGTATTAGAAGAAATAAATTTGCCAGGAAAATGGAAACCAAAATAATAGGAGGAATTGGAGGTTTTGT
CAGAGTAAAACAGTATGATGATGTAACTATAGACATTTGCGGAAAAAAGGTTACAGGTACAGTGGTGGTAGGGCCTACACCTGCCAACATAATTGGAAGAAACATGATGACTCAGCTTGGATGCACACTAAATTTTCCAATTAGCCCCATTAACACTGTACC
AGTAAAACTGAAGCCAGGAATGGATGGCCCAAAGGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATCTGTGAAGAAATGGAGAAGGAAGGAAAAATTACAAAAATTGGGCCTGACAATCCATATAACACTCCAATATTTGCCAT
AAAAAAGAAGGACAGTACTAAGTGGAGAAAAGTAGTAGACTTCAGGGAGCTTAATAAAAGAACTCAAGACTTTTGGGAGGTTCAATTAGGAATACCACACCCAGCAGGGTTAAAAAAGAGCAAATCAGTGACAGTGCTAGATGTGGGAGATGCATATTTTT
CAGTTCCTTTAGATGAAGACTTTAGGAAGTATACTGCATTCACCATACCCAGTGTGAACAATGAGACACCAGGGATTAGATATCAATATAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATTTTAGAACCCT
TTAGAGCAAGAAATCCAGAAATGGTCATCTATCAATATGTGGATGACTTGTATGTAGGATCTGATTTAGAAATAGGGCAACATAGAAAAAAGATAGAAGAGTTAAGAGAACATCTATTAGCGTGGGGACTTACCACACCAGATAAGAAACATCAGAAAGAAC

Output options

🔘 HTML ⚪ Printable HTML ⚪ Spreadsheets (TSV) ⚪ XML

[Reset] [Analyze]

Drug Resistance Interpretation: PR

PI Major Resistance Mutations: M46I, I54V, L76V, V82A, L90M
PI Accessory Resistance Mutations: L10F
Other Mutations: I15V, L19T, K20R, M36I, R41K, K55R, R57K, Q61D, I62V, L63T, E65D, H69K, A71V, I72T, L89M, I93L

Protease Inhibitors

atazanavir/r (ATV/r)	High-Level Resistance
darunavir/r (DRV/r)	Low-Level Resistance
lopinavir/r (LPV/r)	High-Level Resistance

PR Comments

PI Major

- **M46I/L** are relatively non-polymorphic PI-selected mutations. In combination with other PI-resistance mutations, they are associated with reduced susceptibility to each of the PIs except DRV.
- **I54V** is a non-polymorphic PI-selected mutation that contributes reduced susceptibility to each of the PIs except DRV.
- **L76V** is a non-polymorphic mutation selected by IDV, LPV and DRV. It reduces susceptibility to these PIs and to FPV and NFV. It increases susceptibility to ATV, SQV and TPV. **L76V** is included in the Tibotec DRV genotypic susceptibility score.
- **V82A** is a non-polymorphic mutation selected primarily by IDV and LPV. It reduces susceptibility to these PIs and contributes cross-resistance to each of the remaining PIs except DRV and TPV.
- **L90M** is a non-polymorphic PI-selected mutation that reduces susceptibility to each of the PIs except TPV and DRV.

PI Accessory

- **L10F** is a common non-polymorphic, PI-selected accessory mutation associated with reduced susceptibility to DRV, FPV, IDV, LPV, and NFV.

Other

- **K20R** is a highly polymorphic PI-selected accessory mutation.
- **A71V/T** are polymorphic, PI-selected accessory mutations that increase the replication of viruses with other PI-resistance mutations.

Dosage Considerations

- There is evidence for low-level **DRV** resistance. If **DRV** is administered it should be used twice daily.

Mutation Scoring: PR

PI	ATV/r	DRV/r	LPV/r
M46I	10	0	10
I54V	15	0	15
V82A	15	0	30
L90M	25	0	15
M46I + V82A	10	0	10
M46I + L90M	10	0	0
I54V + V82A	10	0	10
I54V + L90M	10	0	5
V82A + L90M	10	0	5
L10F	0	5	5
L76V	0	20	30
M46I + L76V	0	0	10
Total	115	25	145

Drug Resistance Interpretation: RT

NRTI Resistance Mutations: L74V, M184V

NNRTI Resistance Mutations: K103S

Other Mutations: E6N, V35T, T39E, S48T, E53D, V60I, K122E, I135V, K173A, Q174R, D177E, I178M, T200K, Q207E, R211A, F214L, V245K, A272P, L283I, T286A, E291D, V292I, I293V

Nucleoside Reverse Transcriptase Inhibitors

abacavir (ABC)	High-Level Resistance
zidovudine (AZT)	Susceptible
emtricitabine (FTC)	High-Level Resistance
lamivudine (3TC)	High-Level Resistance
tenofovir (TDF)	Susceptible

Non-nucleoside Reverse Transcriptase Inhibitors

efavirenz (EFV)	Intermediate Resistance
etravirine (ETR)	Susceptible
nevirapine (NVP)	High-Level Resistance
rilpivirine (RPV)	Susceptible

RT Comments

NRTI

- **M184V/I** cause high-level in vitro resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, **M184V/I** are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication.
- **L74V/I** cause high-level resistance to ddI and intermediate resistance to ABC.

NNRTI

- **K103S** is a non-polymorphic mutation that causes high-level resistance to NVP and intermediate resistance to EFV. Because **K103S** is a 2-bp change from the wildtype K and a 1-bp change from K103N, patients with **K103S** may be likely to have once had K103N.

Mutation Scoring: RT

NRTI	ABC	AZT	FTC	3TC	TDF
L74V	30	0	0	0	0
M184V	15	−10	60	60	−10
L74V + M184V	15	0	0	0	0
Total	60	−10	60	60	−10

NNRTI	EFV	ETR	NVP	RPV
K103S	45	0	60	0
Total	45	0	60	0

Input mutations | Input sequences

Reverse Transcriptase

D67N x | K70KR x | L74LV x | L100IL x | K103KN x
M184V x | T215FIST x | K219EK x
Input mutation(s)

Protease

M46I x | I54V x | L76V x | V82A x
Input mutation(s)

Select mutations:

40	41	44	62
--- ▼	--- ▼	--- ▼	--- ▼

65	67	68	69
--- ▼	--- ▼	--- ▼	--- ▼

70	74	75	77
--- ▼	--- ▼	--- ▼	--- ▼

90	98	100	101
--- ▼	--- ▼	--- ▼	--- ▼

103	106	108	115
--- ▼	--- ▼	--- ▼	--- ▼

116	118	138	151
--- ▼	--- ▼	--- ▼	--- ▼

Select mutations:

10	11	13	20
--- ▼	--- ▼	--- ▼	--- ▼

23	24	30	32
--- ▼	--- ▼	--- ▼	--- ▼

33	35	36	43
--- ▼	--- ▼	--- ▼	--- ▼

46	47	48	50
--- ▼	--- ▼	--- ▼	--- ▼

53	54	58	63
--- ▼	--- ▼	--- ▼	--- ▼

71	73	74	76
--- ▼	--- ▼	--- ▼	--- ▼

Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	**M46I, I54V, L76V, V82A**
PI Accessory Resistance Mutations:	None
Other Mutations:	None

Protease Inhibitors

atazanavir/r (ATV/r)	High-Level Resistance
darunavir/r (DRV/r)	Low-Level Resistance
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PR Comments

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  except DRV and TPV.

Drug resistance interpretation algorithms

- At the heart of most genotype interpretation algorithms are simple scoring systems, based on the presence of specific mutations.

- Each mutation is associated with a penalty score for each drug, so predicted susceptibility to each drug is based on sum of penalty scores from all mutations

- Once the total score is calculated the estimated level of resistance is summarized as follows:

  - Hypersusceptible: total score < 0

  - Susceptible: total score 0 to 9

  - Potential low-level resistance: total score 10 to 14

  - Low-level resistance: total score 15 to 29

  - Intermediate resistance: total score 30 to 59

  - High-level resistance: total score ≥60

Pros and cons of genotypic resistance testing

Pros

- Genotypic testing can be used to detect mutations that are causing resistance on a current regimen

- It can also help to conserve treatment options by showing the ineffective drug within a particular regimen

Cons

- Generally requires minimum viral load of at least 1000 copies/mL to ensure adequate viral amplification

- Cannot detect low-frequency mutations (minority variants) at ≤20%-30% of the viral population

- Will only detect currently circulating strains, with no information on archived mutations

- It is better at determining which drugs won't work than which drugs will work

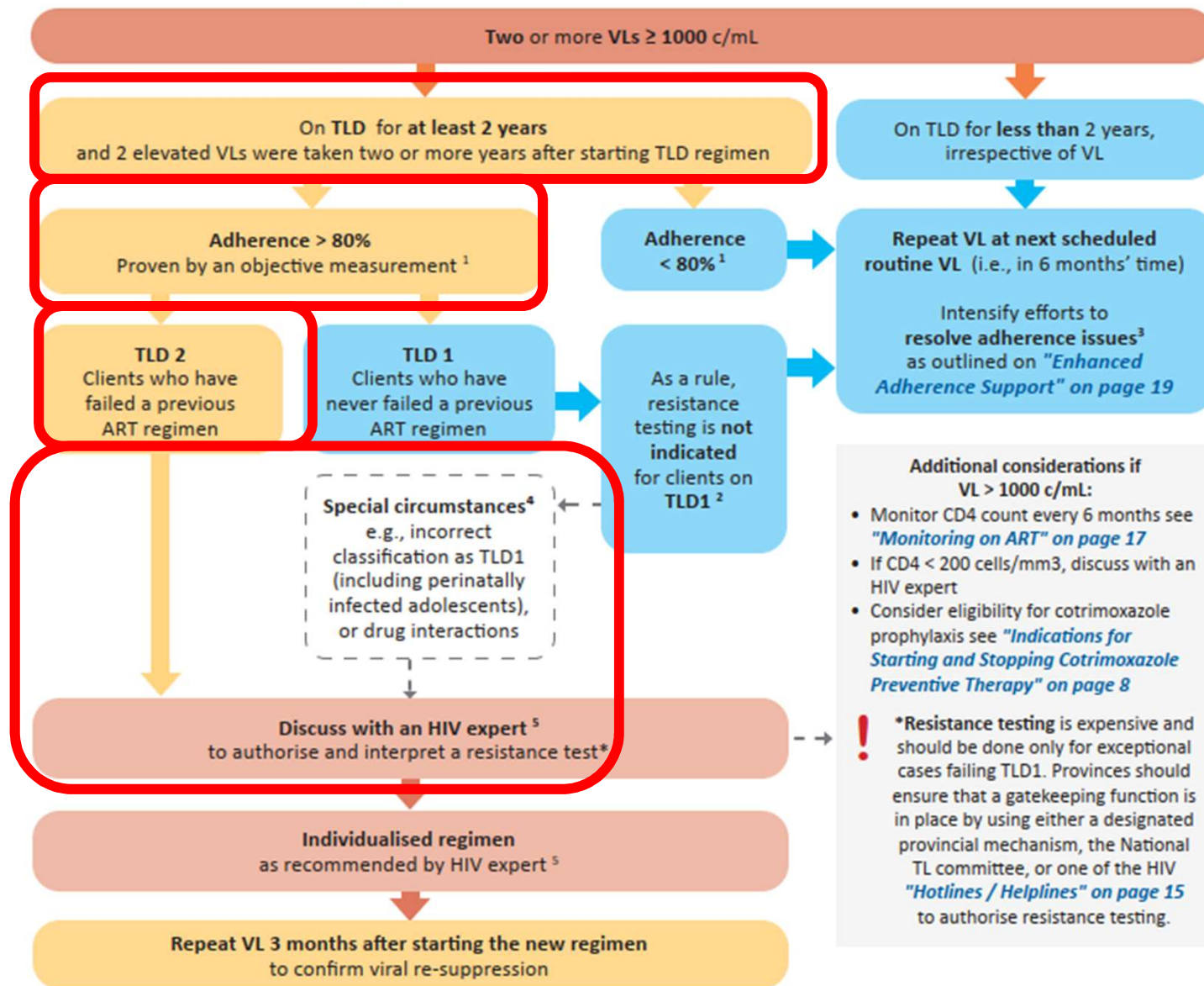# Indications for Resistance testing in 2023 NDoH Guidelines

Switching Existing Clients to DTG-containing Regimens
(Adults, adolescents or children who have never used a DTG-containing regimen in the past)
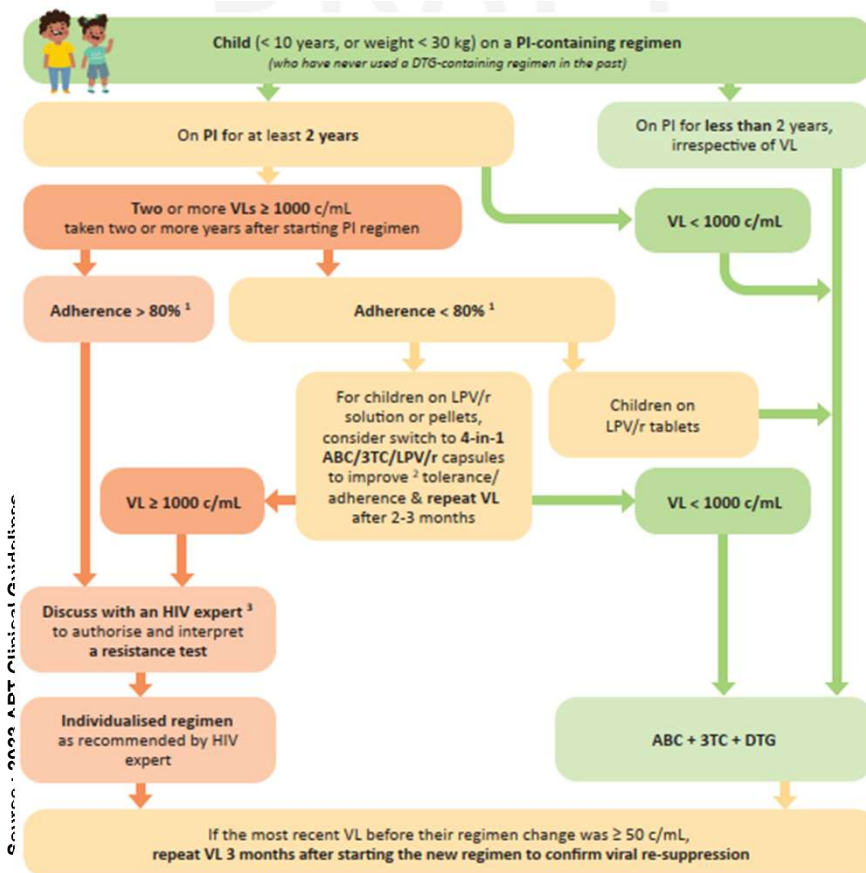
VL-dependent regimen switches Relevant to all clients who have been on PI-based regimens for more than two years: their VL result in the last 12 months will influence the decision of how and when to switch to a DTG-containing regimen			
VL considerations	**Current Regimen**	**Criteria for switch**	**Regimen if change indicated**
VL < 1000 c/mL	**Any LPV/r or ATV/r regimen** for **more than 2 years**	**Switch all to a DTG-containing regimen** If VL in last 12 months was ≥ 50 c/mL, continue to switch same day, but do ABCDE assessment, provide EAC if needed, and repeat the VL after 3 months as per *"The VL non-suppression algorithm" on page 19*	**TLD** provided no renal dysfunction and age ≥ 10 yrs and weight ≥ 30 kg If clients does not qualify for TDF **ABC¹/3TC/DTG**
[2] **Two or more VLs ≥ 1000 c/mL** taken **two or more years** after starting PI regimen	Adult or adolescent on any **LPV/r or ATV/r** regimen and adherence **less than 80%³**	**Switch all to a DTG-containing regimen Do not do a resistance test** These clients are unlikely to have PI resistance mutations. Rather switch to a more tolerable once daily FDC regimen which is likely to support adherence. Manage as per *"The VL non-suppression algorithm" on page 19*	**TLD** provided no renal dysfunction and age ≥ 10 yrs and weight ≥ 30 kg If clients does not qualify for TDF **ABC¹/3TC/DTG**
	Adult or adolescent on any **LPV/r or ATV/r** regimen and adherence **more than 80%³**	Clients who meet the definition of confirmed virological failure and have confirmed adherence more than 80% may need a resistance test. **These clients do not qualify for a same-day switch.** Discuss with an HIV expert⁴ to authorise and interpret a resistance test. Provide individualised regimen as recommended by HIV expert. Repeat VL 3 months after the regimen change to confirm re-suppression, as per the *"Management of Confirmed Virological Failure on TLD" on page 21*	
	Child < 10 years, or weight < 30 kg on any **LPV/r or ATV/r regimen**	These clients do not yet qualify for TLD and may require a resistance test. Refer to algorithm *"Switching children on PI-containing regimens to DTG-containing regimens" on page 16*	

Management of Confirmed Virological Failure on TLD
(also applicable to other DTG-containing regimens)

Two or more VLs ≥ 1000 c/mL

On TLD for at least 2 years
and 2 elevated VLs were taken two or more years after starting TLD regimen

On TLD for less than 2 years,
irrespective of VL

Adherence > 80%
Proven by an objective measurement [1]

Adherence < 80% [1]

Repeat VL at next scheduled routine VL (i.e., in 6 months' time)

Intensify efforts to **resolve adherence issues** [3] as outlined on *"Enhanced Adherence Support" on page 19*

TLD 2
Clients who have failed a previous ART regimen

TLD 1
Clients who have never failed a previous ART regimen

As a rule, resistance testing is **not indicated** for clients on TLD1 [2]

Special circumstances [4] e.g., incorrect classification as TLD1 (including perinatally infected adolescents), or drug interactions

Discuss with an HIV expert [5]
to authorise and interpret a resistance test*

Individualised regimen
as recommended by HIV expert [5]

Repeat VL 3 months after starting the new regimen
to confirm viral re-suppression

Additional considerations if VL > 1000 c/mL:
- Monitor CD4 count every 6 months see *"Monitoring on ART" on page 17*
- If CD4 < 200 cells/mm3, discuss with an HIV expert
- Consider eligibility for cotrimoxazole prophylaxis see *"Indications for Starting and Stopping Cotrimoxazole Preventive Therapy" on page 8*

❗ *Resistance testing is expensive and should be done only for exceptional cases failing TLD1. Provinces should ensure that a gatekeeping function is in place by using either a designated provincial mechanism, the National TL committee, or one of the HIV *"Hotlines / Helplines" on page 15* to authorise resistance testing.

The NDoH recommends all children ≥4 weeks and ≥3 kg be transitioned to a DTG containing regimen

All children should be initiated on a DTG based regimen

refills or attendance of scheduled clinic visits in the previous 6-12 months of <80%, adherence difficulties in young children are often linked to poor tolerability of unpalatable formulations, particularly LPV/r solution. It is important to ask the caregiver about how the child tolerates the medication e.g., does the child refuse to swallow the medicine or spit out or vomit the medicine, and whether the caregiver has been able to overcome this. Considering these limitations, objective measures of good adherence could include one of the following:

   a) Pharmacy refills > 80% in the last 6-12 months (if this is known)
   b) Attendance of > 80% of scheduled clinic visits in the last 6-12 months (if this is known)
   c) Detection of current antiretroviral drug/s in the client's blood or urine, if available

2. If a switch to the 4-in1 capsules does not improve adherence, or is not available, continue to switch to ABC + 3TC + DTG as for non-adherent children on LPV/r tablets
3. The following would qualify as HIV experts: the HIV Helplines, a paediatric infectious disease specialist or the paediatric Third line ART committee

References & additional resources

Clavel D, Hance AJ. HIV drug resistance. N Engl J Med 2004; 350:1023-1035. https://doi.org/10.1056/NEJMra025195

International Antiviral Society-USA. Drug Resistance Mutations in HIV-1. https://www.iasusa.org/2018/01/30/drug-resistance-mutations-in-hiv-1/

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