

Tricks in interpreting CD4 and VL results

Suzanne Crowe

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CD4 & HIV RNA are independent predictors of HIV progression

- CD4 tells you the **distance** you have traveled
 - damage to immune system: loss of CD4 T cells



- HIV RNA tells you how **quickly** you are traveling
 - how fast HIV is destroying CD4 T cells



CD4 Count Variation

- LG, 43 yp female, HIV infection diagnosed 4 years ago
- Blood drawn from her a few hours apart at two different labs
- Most if this variation is due to laboratory variation in the WCC and lymphocyte count, rather than in the flow cytometric result

Date	CD4%	Absolute CD4
5 Mar 2002	11	148
5 Mar 2002	13	321

- In this patient, just using the absolute CD4 suggests progressive HIV between June 1998 and August 2000
- But the %CD4 is unchanged
- So the absolute CD4 just reflects physiologic variation
- Cell count should be repeated if unsure
- Watch for trends in CD4 counts as well as clinical symptoms
- Never act just on one CD4 count unless it really fits with symptoms.

Date	%CD4	Absolute CD4
15 Apr 97	18	330
15 Jun 98	19	406
14 Dec 98	20	312
8 Aug 00	19	271
26 Sep 00	19	325
12 Dec 00	18	303

- The variation in Absolute CD4 counts may be more obvious with higher CD4 counts
- Probably due to lab variation in the WCC and lymphocyte count, rather than in the %CD4.

Date	%CD4	Absolute CD4
14 Sept 99	37	788
15 Mar 00	38	1101
16 May 00	38	874
17 Oct 00	38	993
12 Dec 00	37	1219

What can alter the CD4 count?

- **Circadian rhythm**
 - CD4 cells are higher in evening/night
- **Activity and rest**
 - **Rest** decreases the CD4 count
 - From a mean of 1060 cells/ul to 660 cells/ul following 30 minutes rest
 - **Exercise** increases the CD4 count and is synergistic with heat
- **Cigarette smoking**
 - decreases the CD4 count

Campbell PJ et al. Int J STD AIDS 1997 8:423

Kendall A et al. J Appl Physiol 1990 69:251

Wewars MD et al. Am J Respir Crit Care Med 1998 158:1543

What is definition of virologic failure?

- Failure to achieve undetectable VL at 6 months after starting ARV regimen
 - Undetectable VL generally <50 copies/ml
 - Some assays <400 copies/ml
- Significant correlation between HIV RNA >10,000 copies/ml and increased morbidity and mortality
 - therapeutic switching should occur prior to this
- WHO recommend 10,000 copies/ml be considered the definition of virologic failure in RCC

How much variation can be expected with VL results?

- What do you think?
- Is there evidence that the VL is increasing in this patient?
- Would you change clinical management based on these results?

Date	VL Copies/ml	VL Log
01/01/08	31,300	4.50
05/03/08	40,000	4.60
30/04/08	59,300	4.75

How much variation can be expected with VL results?

- Generally accepted there is up to a 0.3 log variation between same samples run in two assays for VL when using nucleic acid techniques
- Recent comparison of COBAS Amplicor Monitor, Bayer versant, Abbott Real time, Cobas Ampliprep Taqman with HIV group M strains up to 0.4 log difference (*Braun et al*)
- So there is no real difference in these results although there is certainly a trend towards higher viral load that appears worrying
- Clinically relevant difference between two results is 0.5 log

Zazzi M et al J Clin Microbiol 1999 37: 333
Murphy DG et al J Clin Microbiol 1999 37:812
Best SJ et al J Clin Microbiol 2000 38:4015
Braun P et al Clin Chem Lab Med 2007 45:93

Practical point

- Should try and always use the same lab and same machine for VL assessments

- Mr AG, banker, 28 years, diagnosed with HIV 18 months ago with weight loss and pulmonary TB
- At diagnosis: CD4 144 cells/ul; VL >100,000 copies/ml
- TB treated, standard therapy
- repeat tests at end of TB treatment
 - CD4 210 cells/ul; VL 62,000 copies/ml
- ARVs started
 - ZDV/3TC/NVP
- After 1 month (January 2008):
 - CD4 250 cells/ul; VL <50 copies/ml
- 3 months later (April 2008)
 - CD4 290 cells/ul; VL <50 copies/ml
- 3 months later: (July 2008)
 - CD4 295 cells/ul; VL 108 copies/ml
- Are you concerned?
- What Questions do you ask and what action do you take?

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- 2 months later: (July 2008)
 - CD4 295 cells/ul; VL 108 copies/ml
- 5 weeks later: (August 2008)
 - CD4 310 cells/ul; VL <50

Blips

- “Blips” are defined as intermittent episodes of detectable low-level viremia in patients on HAART which spontaneously return to undetectable without a change in therapy
- Data suggest that blips represent normal biological variation around a mean of <50 copies/ml.
 - Generally last short time (2.5 days)
 - Usually low magnitude (mean 79 copies/ml)
- No association between blip and virological failure
 - Viruses obtained pre-blip and during blip genetically indistinct
 - No resistance mutations

- **Causes of blips:**
 - Random event
 - Assay variability
 - Method of blood collection and processing may cause some blips
 - Laboratory error (eg contamination within assay)
- **Other possible causes of short term viremia**
 - intercurrent infection or vaccination
- **Must be differentiated from**
 - Imperfect adherence
 - Early virologic failure

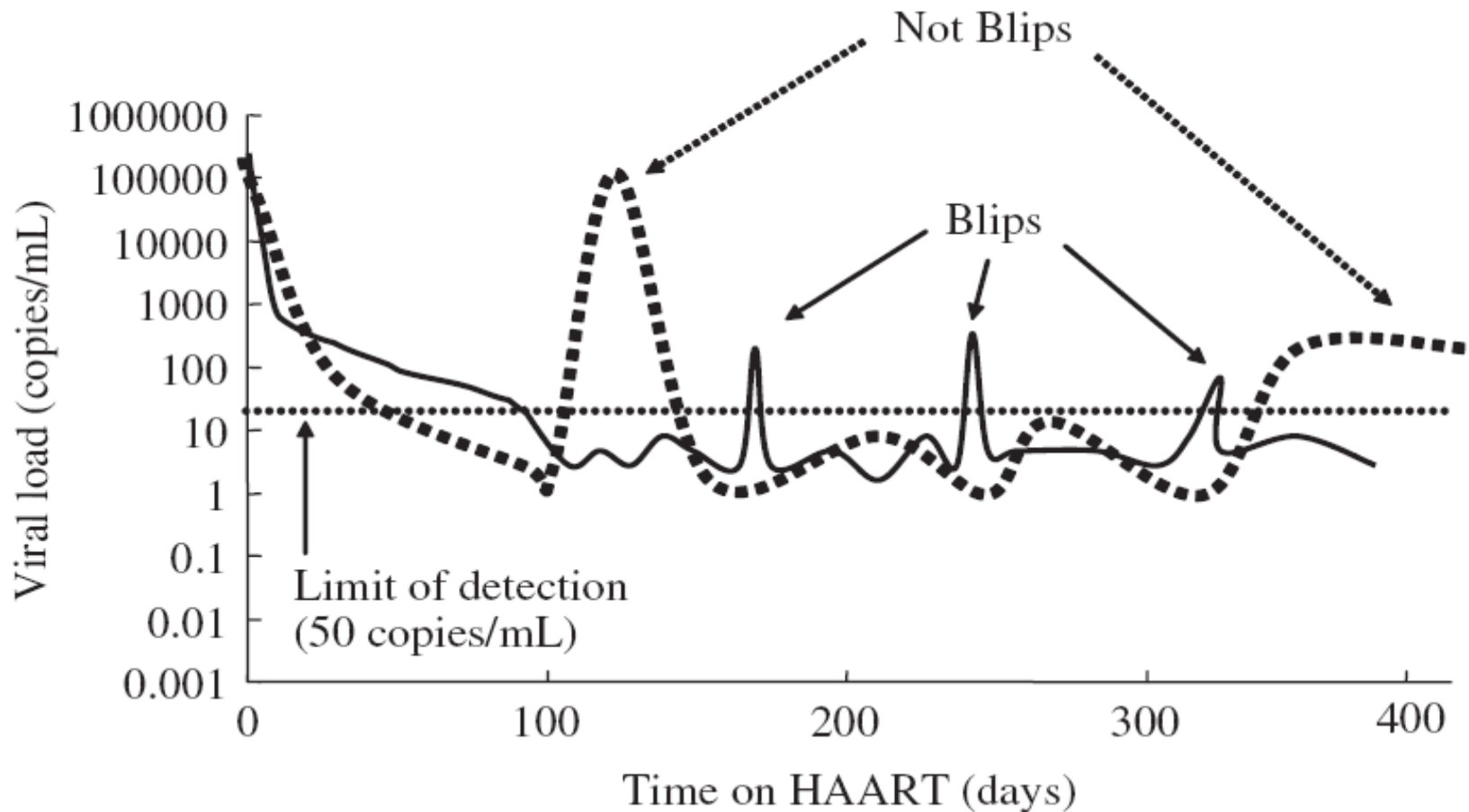


Figure 1. Blips are defined as intermittent episodes of detectable low-level HIV-1 viraemia >50 copies/mL which are preceded and followed by viraemia in the undetectable range without any change in therapy. Episodes of persistently detectable or high-level viraemia are not considered blips.

Lee PK, Kieffer TL, Siliciano RF, Nettles RE. HIV-1 viral load blips are of limited clinical significance. J Antimicrob Chemother. 2006;57:803-5.

- Patient MN on ART, claims compliant, no recent infection or vaccination, tested every second month and was undetectable for >6 months.
- Following test the VL was detectable.
 - Is patient failing therapy?
- VL was retested two weeks later and was undetectable.
 - Rise in VL was a blip

Date	VL Copies/ml	VL Log
09/10/07	<50	<1.70
18/11/07	<50	<1.70
12/12/07	<50	<1.70
15/01/08	200	2.30

30/01/08	<50	<1.70
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- VL assay 15/01/08: negative control was not negative, but assay results were all still reported
- When same sample was repeated it was <50
 - Rise in VL in this case was found to be due to low level contamination in assay

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12/12/07	<50	<1.70
15/01/08	200	2.30

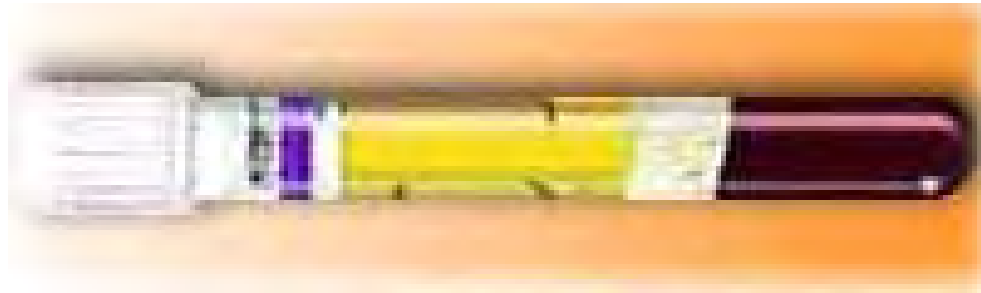
15/01/08	<50	<1.70
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RT-PCR... main problems

- Expensive, technically sophisticated
- Requires separate areas or separate labs to perform different aspects of PCR tests
 - Contamination common... even in the best labs
- Need to repeat whole assay if controls are not within range or if negative control is positive
 - This means throwing out results for whole kit
 - Many resource-constrained labs do not repeat assay when problems occur because of the cost → results may be unreliable if lab not following quality regulations

Blood collection can alter VL results

- The type of collection tube can give different HIV viral load results
 - Plasma preparation tubes (PPT) can elevate HIV VL results



- In most assays EDTA anticoagulant is recommended

http://www.hopkins-hivguide.org/images/publications/nl_05_nov.pdf

García-Bujalance S, et al. J Microbiol Methods. 2007;69:384-6

Intercurrent infections can increase VL

- Mean VL increase during illness 7.8 fold
- Associated with immune activation
 - ↑ plasma levels of sTNFR-I, -II and sIL-2R
- Reduction in VL (1.5 fold) during 15 day convalescence

Can HIV subtype affect VL results?

- HIV-1 sequence variations can impact on the performance of nucleic acid based VL assays by affecting the primer or probe binding sites
- This may lead to underestimation or failure to detect some HIV-1 subtypes
- Non-nucleic acid based assays eg Cavidia ExaVir or p24 based assays are not affected

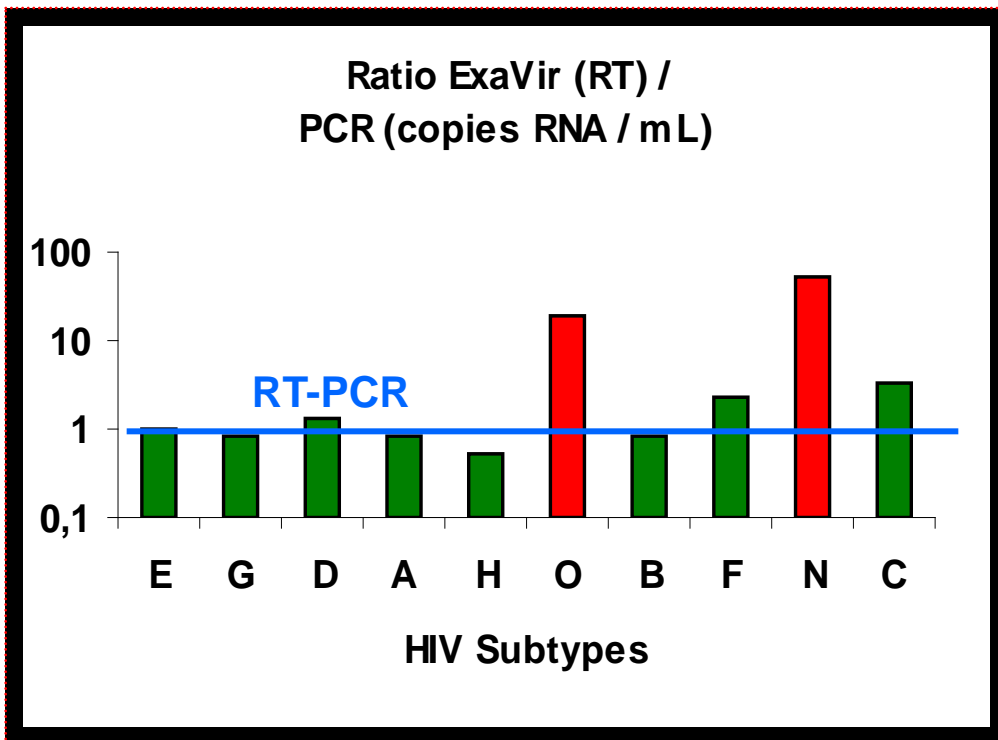
Subtype detection

Table 1 Viral load results in copies/mL $\times 10^3$ (undiluted and 10-fold dilutions) of different HIV-1 subtypes measured with all systems.

Subtype		Env/gag/pol	Viral load, copies/mL $\times 10^3$							
ID	Abbr.		Amplicor HIV-1 Monitor (Roche)		Versant HIV-1 RNA 3.0 (Bayer)		CAP/CTM HIV-1 (Roche)		Abbott RealTime HIV-1 (Abbott)	
			Undiluted	1:10 dilution	Undiluted	1:10 dilution	Undiluted	1:10 dilution	Undiluted	1:10 dilution
92UG029	A	A/A/A	135	10.1	154	10.5	472	43.6	537	39.8
92TH026	B	B/B/B	135	19.3	109	13.1	275	31.0	363	20.4
92BR025	C	C/C/C	70	9.7	29.7	4.0	170	17.5	208	10.2
92UG021	D1	D/A/D	64.9	6.2	51.7	6.4	235	16.5	190	13.8
92UG035	D2	D/A/A	387	32.6	168	15.4	1.050	44.0	954	53.7
92UG024	D3	D/D/D	116	19.2	95.9	9.7	338	21.6	323	16.9
92TH022	E1	E/A/crf01AE	120	20.6	86.9	7.9	315	42.5	398	20.8
92TH009	E2	E/A/crf01_AE	157	11.5	62.2	5.7	282	38.5	288	18.6
93BR029	F1	F/B/B	163	30.9	104	11.4	472	18.4	380	21.3
93BR020	F2	F/F/F	163	12	57.5	7.4	175	7.2	309	17.7
ARP173/ RU570	G	G*/G	351	23	62.3	4.7	195	30.8	588	37.1
HIV V1557	H	H*/H	427	29.2	125	10.0	955	68.8	426	26.9
Di 0019	J	–	0.93	0.09	0.30	<0.05	0.349	0.127	1.1	–
MVP5180	O1	O	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3467	263
HIV 1 CA 9	O2	O	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	> 10,000	2884

Taken from: Braun P, Ehret R, Wiesmann F, Zabbai F, Knickmann M, Kühn R, Thamm S, Warnat G, Knechten H. Comparison of four commercial quantitative HIV-1 assays for viral load monitoring in clinical daily routine. *Clin Chem Lab Med.* 2007;45(1):93-9.

Detection of different HIV-1 subtypes



- Different HIV-1 subtypes containing 2000 - 20,000 copies/ml were assayed using Roche RT-PCR and Cavidu ExaVir Load.

-Note the level of detection particularly for subtypes O and N.

1 = equal detection Cavidu & PCR
>1 = better detection using Cavidu
<1 better detection with RT-PCR

How does the VL early after infection help with prognosis

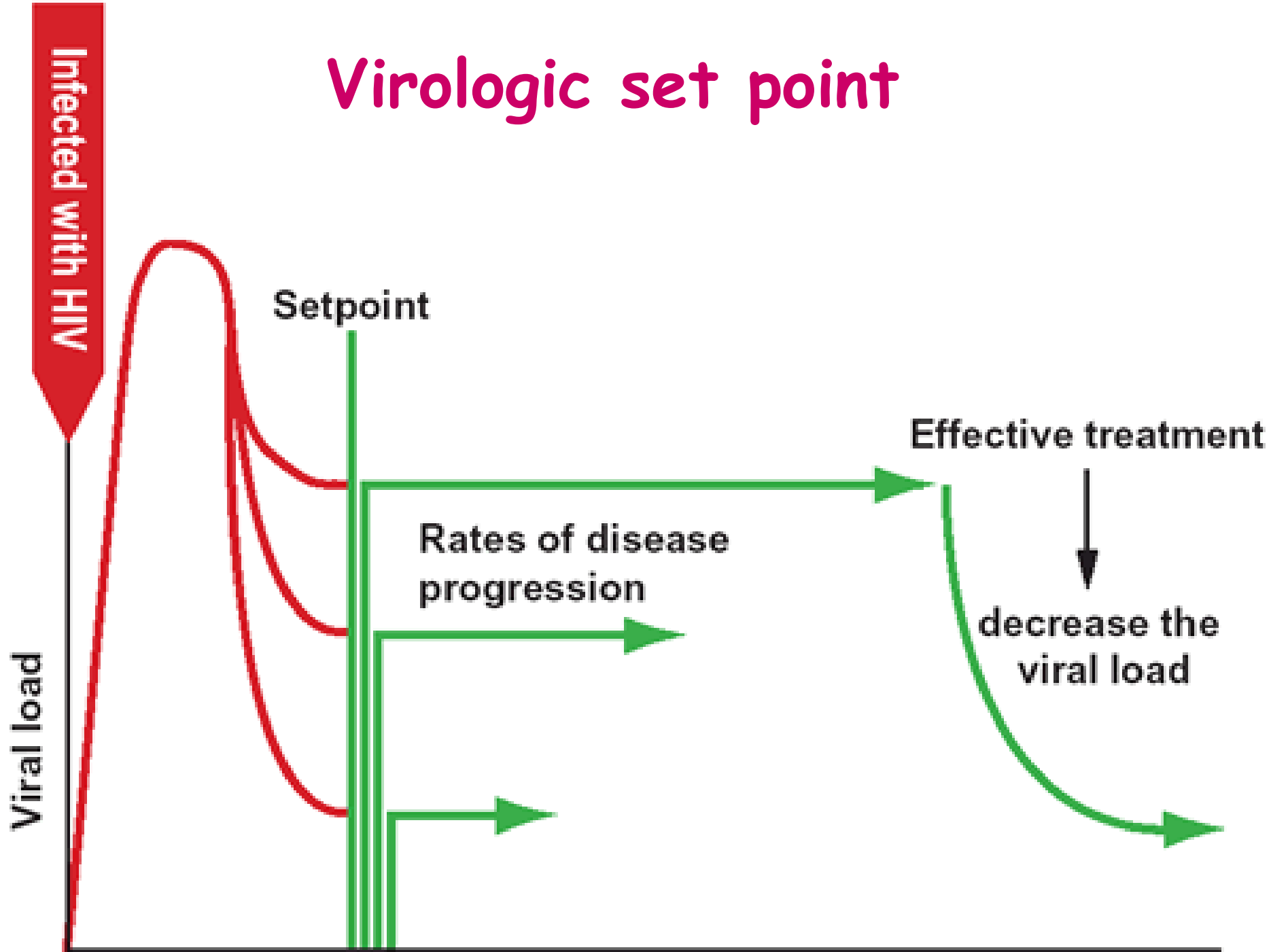
- Within about 6 months after infection the VL stabilizes
 - May be undetectable (very uncommon)
 - May be low (<10,000 copies/ml) (relatively uncommon)
 - May be high (>30,000 copies/ml) (most common)
- Called virologic set point

Study of plasma HIV set point in 168 Kenyan women followed from seroconversion

Plasma set point VL at 4 -24 months after infection	
<4 log ₁₀ HIV RNA copies/ml	45 (27%)
4-5 log ₁₀ HIV RNA copies/ml	68 (39%)
>5 log ₁₀ HIV RNA copies/ml	57 (34%)

Median plasma VL at set point : 46, 770 (log 4.67)

Virologic set point



How to use the virologic set point with an individual patient?

- Use VL set point result with CD4 count
- If VL remains high, need to keep closer watch on CD4
 - May need to start treatment earlier than expected
- If VL becomes undetectable or declines to low level
 - Person likely to have less rapid course
 - Can space clinic visits accordingly

Use of VL result for HIV diagnosis?

- numerous reports of false positive HIV viral load results
 - detectable VL but HIV -ve by serology and culture
- Expect high levels of HIV RNA in acute HIV syndrome
 - False positive results <10,000 copies/ml
- One RNA assay approved by FDA for diagnosis (Aptima qualitative HIV RNA, Gen-Probe)

Rich JD et al, Ann.Int. Med. 1999;130:37.
Daar ES, et al., Ann.Int. Med. 2001;134:25.
de Mendoza C, et al. AIDS 1998;12:2076.
Gallant JE. Hopkins HIV Report 2005;17:1.
Rich JD, et al. Ann.Int. Med.1999;130:37.
Salimnia H, et al. J.Clin.Micro.2005;43:4635