Monitoring HIV Infection:
CD4 T Cell Counts and New Viral Load Technologies

Urvi M Parikh, Ph.D.
University of Pittsburgh
Pittsburgh, PA
USA

VL, CD4 and HIV Infection

- Each day, viral population in a patient:
  - Generates billions of new HIV particles
  - Destroys millions of CD4 T lymphocytes

- The body tries to compensate for the loss by making new CD4 T cells, but AIDS happens when the immune system eventually fails to keep up.

-From Mellors, Scientific American 1998

Measurement of CD4 T Cells and Viral Load in MTN trials is very important

- For monitoring:
  - disease progression in participants that test HIV positive
  - treatment effectiveness in participants that begin antiretroviral therapy

- For determining whether these measures are different in women who had ARV product (tenofovir or Truvada gel or pills) compared to those who did not

We will talk about…

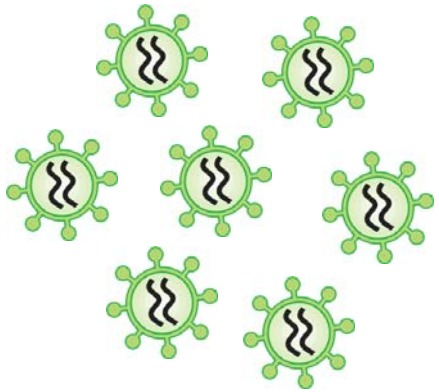
- Viral Load
  - What it is
  - How to measure it
    - Current Roche platform
    - New Abbott platform
- CD4 T Cell Counts
  - What it is
  - How to measure it
- How Viral Load and CD4 are related

What is VIRAL LOAD (VL)?

- Estimation of the amount of virus in a body fluid
  - Generally RNA copies/ml in plasma
  - Each HIV particle contains two strands of RNA, so the level of actual virus is half the RNA count

Why do it?

- Monitor severity of infection
- Track viral suppression
- Evaluate treatment efficacy or failure



History of Viral Load

- Multicenter AIDS Cohort Study (MACS)
  (Mellors 1996)
  - Measured virus in stored plasma samples collected from ~1,600 untreated HIV-infected men
  - Prognosis depended on level of virus
    - VL>30,000 c/ml: 70% died in 6 yrs (avg 4.4 yr)
    - VL <500 c/ml: <1% died in 6 yrs (avg >10 yr)

Monitoring VL is important

- Viral load influences the rate of disease progression
- Lowering viral levels as much as possible for as long as possible with therapy is essential to prolonging life.

Monitoring VL is important

- **Standard Assay: <400 copies/ml**
  - Goal of treatment is to maintain viral load to undetectable in standard assay
- **Ultrasensitive Assay: <50 copies/ml**
  - Offers better protection against developing drug resistance

Major Steps in Viral Load

1. **Extraction**
   - Virus is present in plasma or serum
   - Must isolate RNA from virus
2. **Amplification**
   - Viral nucleic acids are in insufficient quantities to be detected
   - PCR can only amplify DNA - need to convert RNA to cDNA (reverse transcription)
3. **Detection/Quantification**
   - Measure how much DNA has been amplified
   - Calculate viral load

FDA-Approved Assays for VL

- FDA (USA Food and Drug Administration) means results can be reported to patients
- Assays used for viral load at MTN sites include:
  - Roche Amplicor HIV-1 Monitor (sites)
    - Standard vs ultrasensitive
    - Microwell plate (MWP) vs COBAS
  - Abbott M2000 (MTN Virology CORE)

How everyone does VL

Assay	Sites
Standard amplicor HIV-1 monitor vs.1.5	Lilongwe, Blantyre, Uganda
Ultrasensitive amplicor HIV-1 monitor vs.1.5	Harare
Ultrasensitive amplicor HIV-1 COBAS v1.5	Durban
Abbott M2000	Zambia
Abbott M2000	Virology CORE

QUESTION 3

- What is the viral load linear range of the Amplicor HIV-1 Monitor **Standard** Assay?

A. **50 – 75,000 RNA copies/ml**

B. **400 – 750,000 RNA copies/ml**

C. **0 – 1,000,000,000,000 RNA copies/ml**

D. **500 – 500,000 RNA copies/ml**

QUESTION 4

- Which one **IS NOT** a valid result for the Amplicor HIV-1 Monitor **Ultrasensitive** assay?

A. 65 copies/ml

B. 278 copies/ml

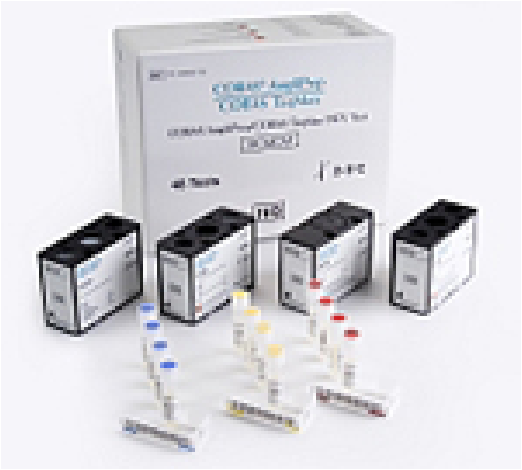
C. 85,400 copies/ml

D. 250,000 copies/ml

The viral load linear range for the Roche ultrasensitive assay is 50 – 100,000 copies/ml

Amplicor HIV-1 Monitor

- Linear Range
  - Standard: 400-750,000 copies/ml
  - Ultrasensitive: 50-100,000 copies/ml
- Manual or Automated
  - Manual → MWP
  - Automated → COBAS
- PCR-based assay using ELISA technology for detection



Roche COBAS Amplicor

Automated version of of the Roche MWP Amplicor

How Amplicor Monitor Works

Extraction

- High molarity guanidinium lyses viral particles in plasma
  - Standard method: Direct lysis from plasma
  - Ultrasensitive method: Concentration of HIV-1 viral particles by high speed centrifugation of plasma
- A quantification standard (RNA) is spiked into the lysis for inclusion as a control
- RNA is precipitated out of solution using alcohol

How Amplicor Monitor Works

Amplification

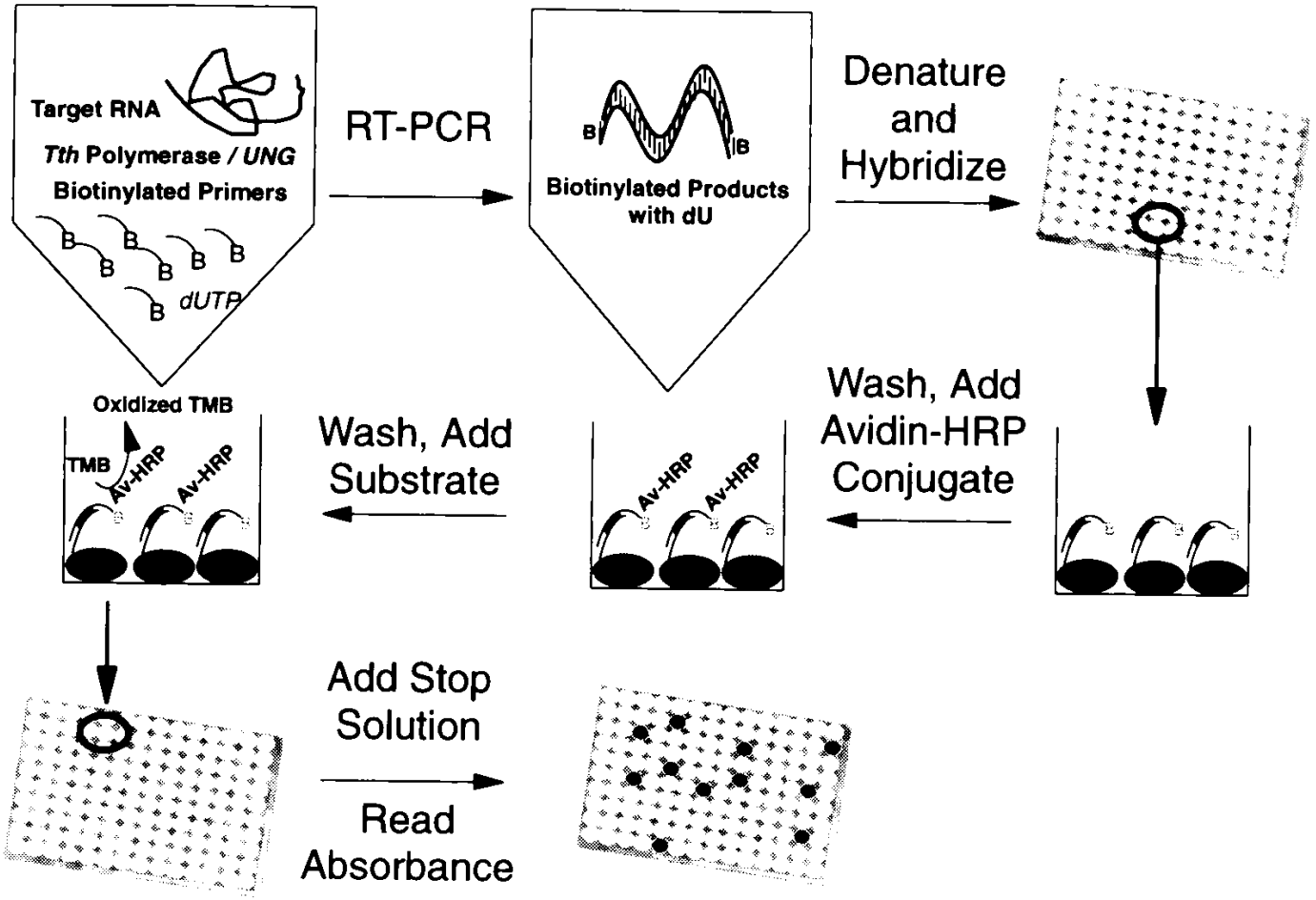
- RNA is reverse transcribed to DNA
- rTh enzyme catalyzes reverse transcription and DNA polymerization in a one-tube reaction using biotinylated primers
- Targets a conserved region of the *gag* gene (structural proteins)
- Amplification is proportional to starting amount of RNA

How Amplicor Monitor Works

Detection/Quantification

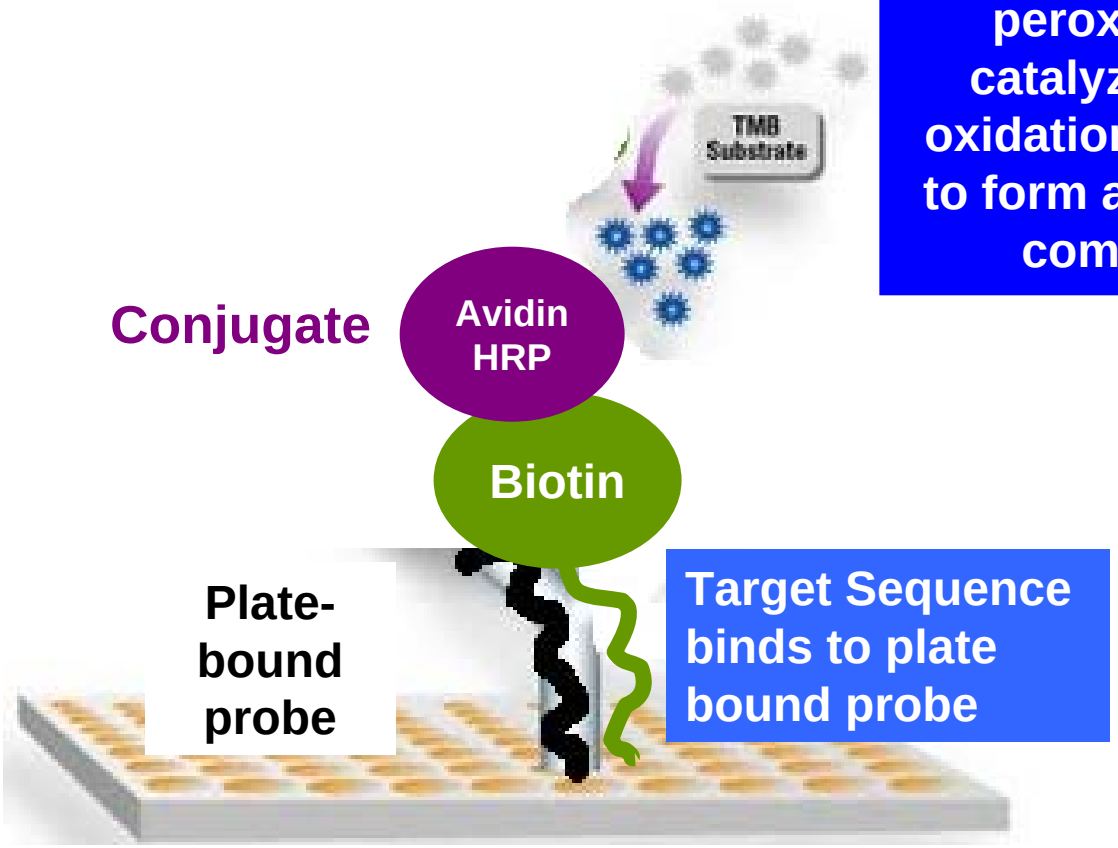
- Amplicon is denatured to make single-stranded DNA
- Biotinylated amplicons are captured by plate-bound probes, either target DNA or control DNA
- Avidin/HRP conjugate provides a colorimetric signal
- OD is proportional to amount of amplicon

Roche Amplicor Assays



Harrigan 1995

Capture ELISA



Bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex

QUESTION 6

- What is the major difference between the Roche Amplicor Monitor **Standard** and **Ultrasensitive** Assays?

The ultrasensitive…

A. includes a high speed centrifugation step to concentrate virus

B. uses a different, more sensitive detection method

C. requires additional rounds of PCR to amplify DNA

D. has a different colored denaturation reagent

QUESTION 7

- After you process your samples and read your plate in the Amplicor Monitor MWP assay, you notice that one of the sample's raw data (O.D.) values are all at background level. What do you do?

A. **Report the VL as 0 copies/ml**

B. **Declare the assay a failure, throw away the plate, yell at your fellow lab tech, then go home.**

C. **Re-run only the specimen of interest if the QS also failed**

D. **Re-run the entire plate regardless of the QS values**

E. **Email Ted**

QUESTION 8

Roche Standard Viral Load Results

	1	2	3	4	5	6
A	0.045	3.498	0.043	3.629	0.621	0.050
B	0.048	3.694	0.043	3.434	0.156	0.055
C	0.045	3.116	0.042	2.232	0.066	0.042
D	0.046	1.960	0.042	0.771	0.046	0.063
E	0.047	0.677	0.043	0.254	0.043	0.042
F	0.046	0.183	0.042	0.093	0.043	0.043
G	2.572	2.839	1.722	2.409	2.627	2.492
H	0.814	0.872	0.493	0.768	0.622	0.883

Which samples will be <400 copies/ml? List by column number.

QUESTION 9

Roche Standard Viral Load Results

3	4	5	6
0.043	3.629	2.471	3.657
0.043	0.047	0.672	3.657
0.042	2.232	0.200	2.678
0.042	0.771	0.081	1.011
0.043	3.434	0.051	0.300
0.042	0.093	0.050	0.093
1.722	2.409	1.774	2.484
0.493	0.768	0.358	0.654

Dilutions are out of sequence

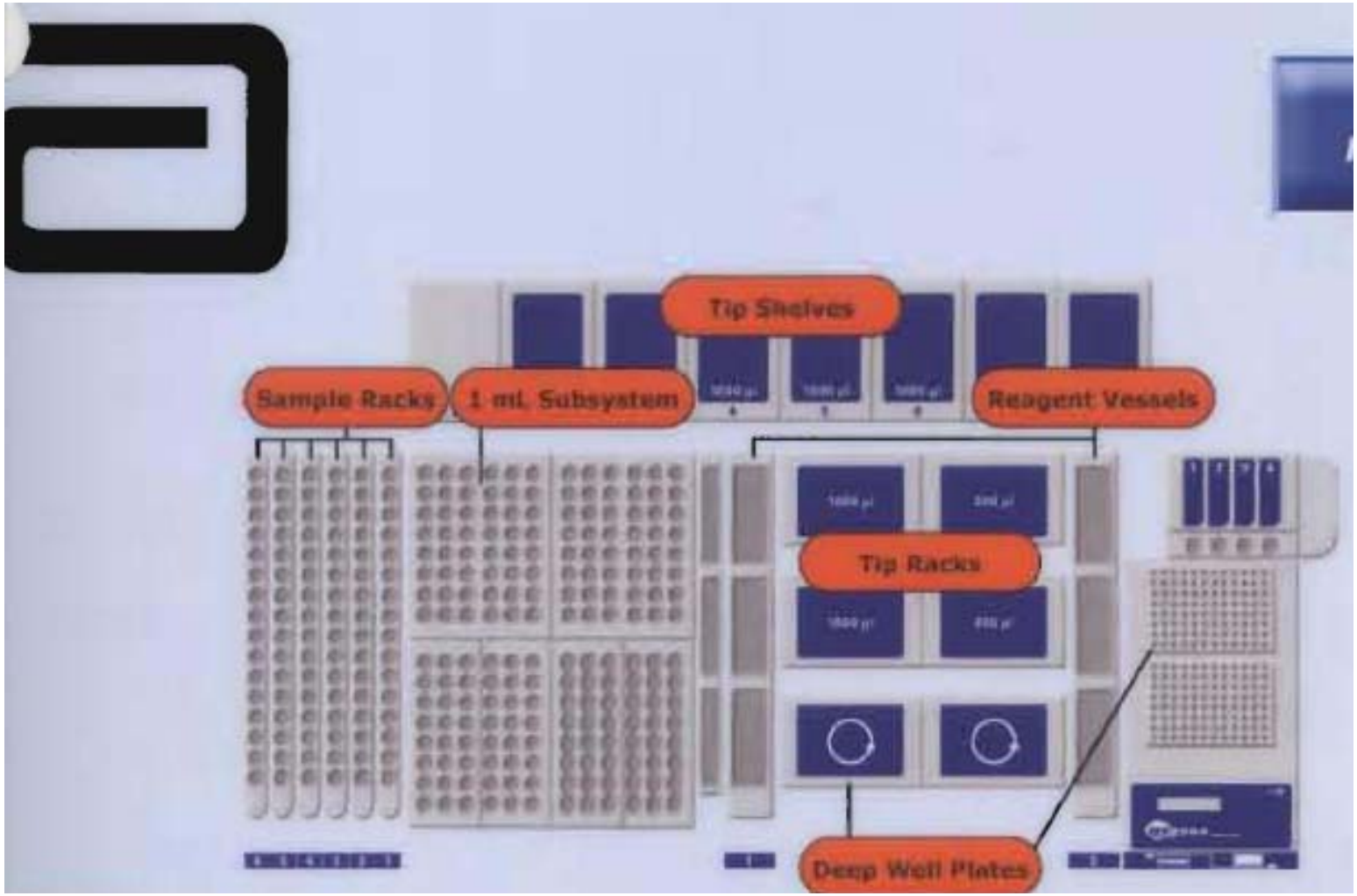
Which column of data is invalid? Why?

Abbott M2000

- Linear Range
  - 50 – 10,000,000 RNA copies/ml
- Automated Only
- PCR-based assay using real-time PCR technology for detection

Abbott M2000

Inside of M2000



How M2000 Works

Extraction

- Internal Control added to lysis
- Magnetic particle technology capture nucleic acids
- Particles are washed to remove unbound sample
- Bound nucleic acids are eluted

QUESTION 10

- From what is the Abbott Internal Control (IC) target sequence derived?

A. Potato

B. Pumpkin

C. Banana

D. Bee Hive Wax

How M2000 Works

Amplification

- Also uses rTh polymerase for one step RT-PCR; primers have fluorescent probe
- Targets a region in *pol* integrase

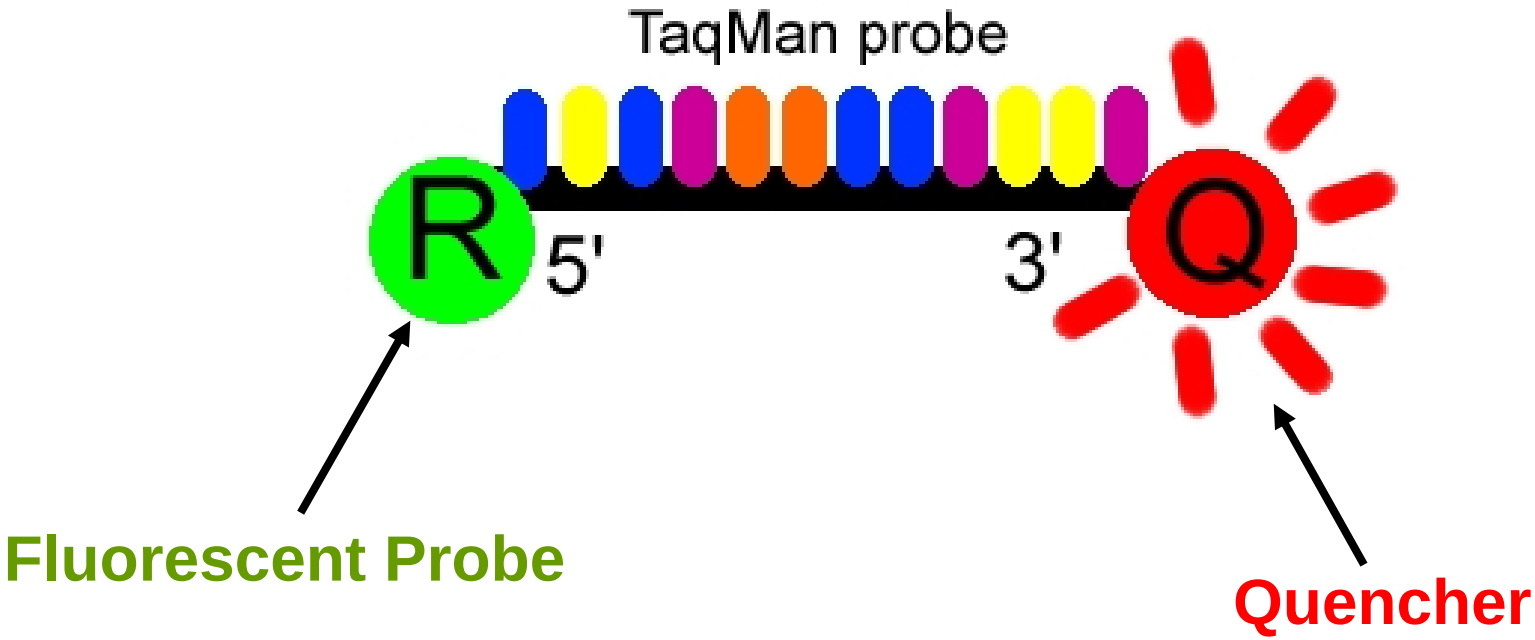
From Qiagen website

How M2000 Works

Detection/Quantification

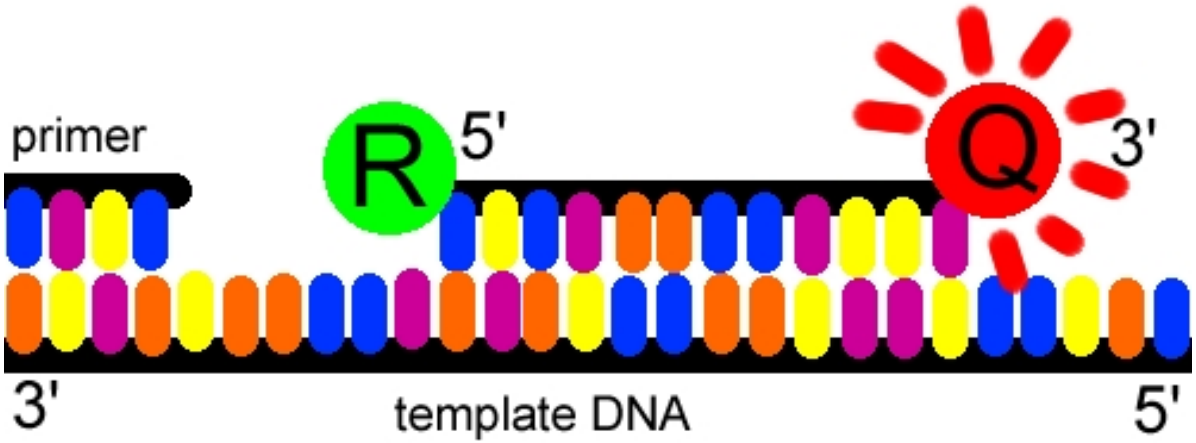
- Amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent labeled oligonucleotide probes
- Probes do not generate signal unless they are specifically bound to the amplified product
- Amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample

Real-time PCR



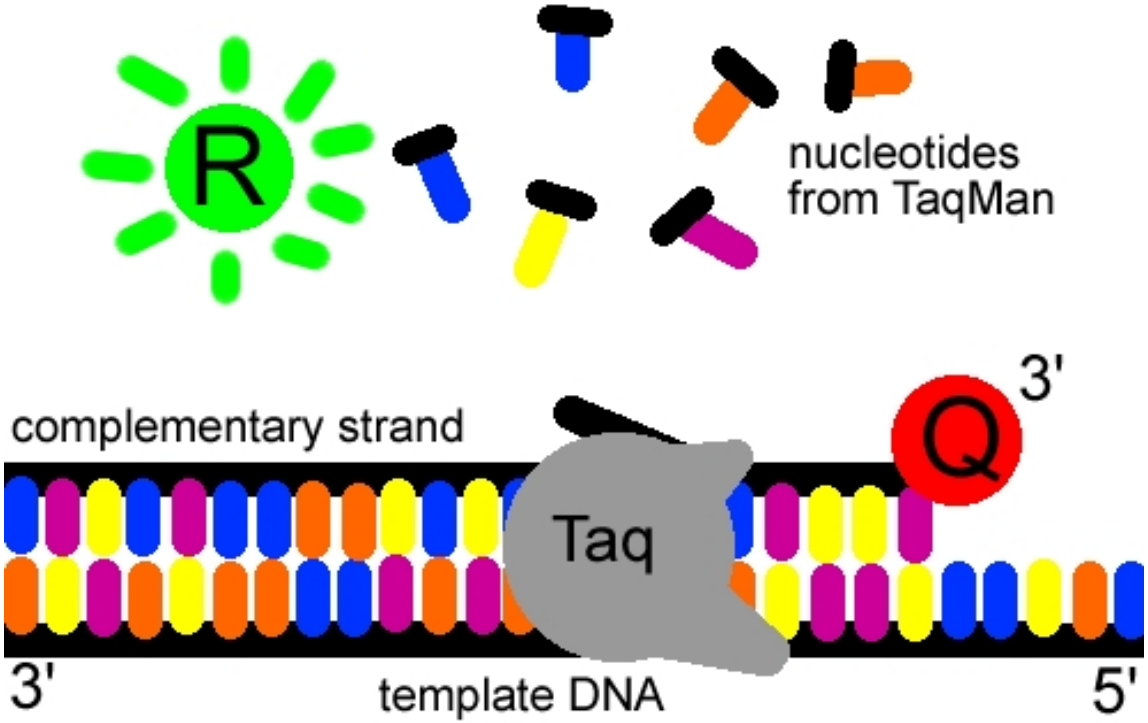
In absence of target sequence, fluorescent probe is quenched.

Real-time PCR



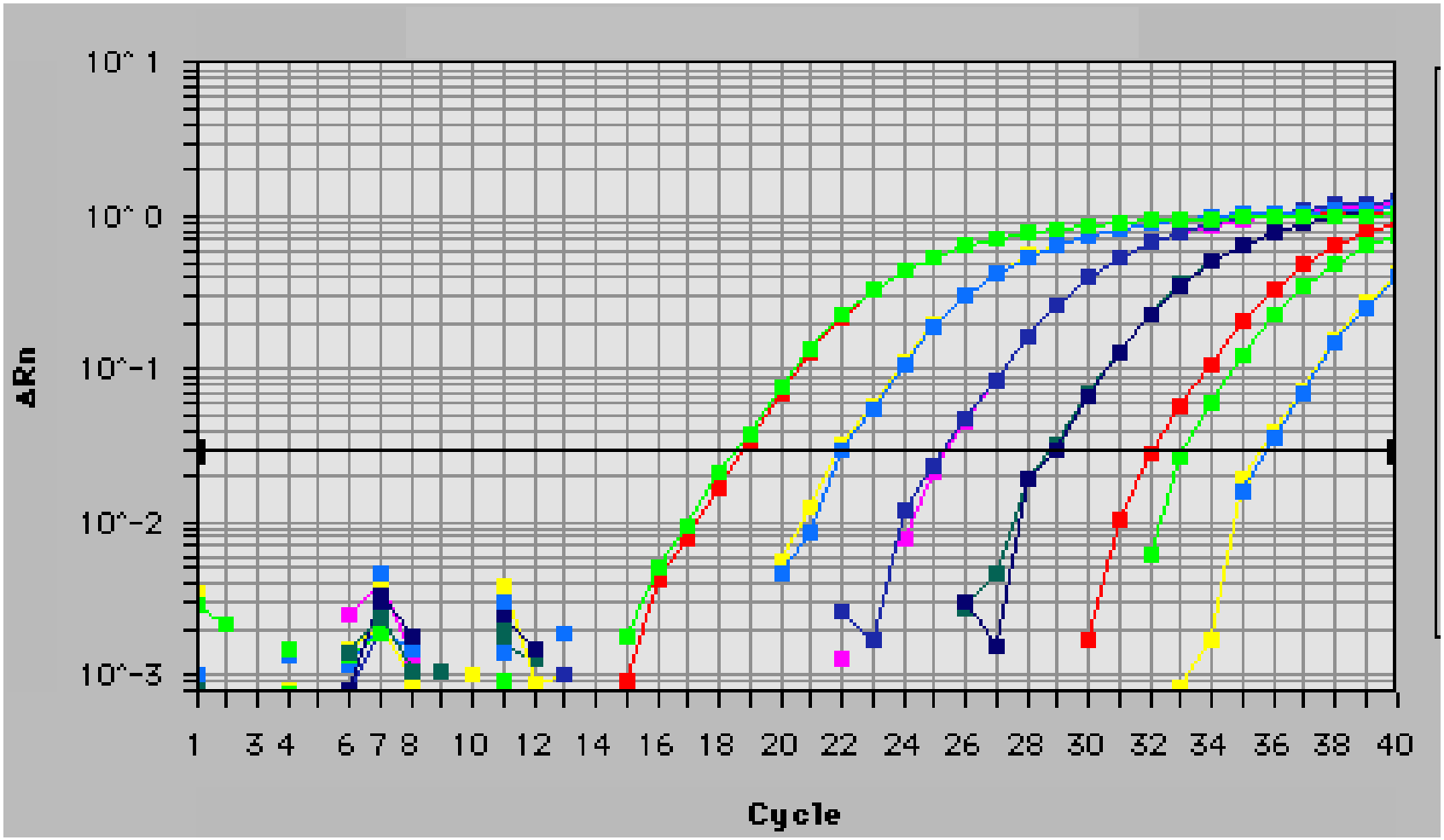
Complementary probe binds to target sequence (HIV-1 DNA)

Real-time PCR



Taq polymerase copies DNA, releasing the fluorescent probe. Fluorescence is detected.

What Real-Time Data Looks Like



QUESTION 11

- The Abbott M2000 Viral Load Assay uses technology most similar to:

A. Roche Monitor MWP Assay

B. Bio-Rad Western Blot

C. Roche Monitor COBAS Assay

D. Roche TaqMan Assay

QUESTION 12

- An error code is given for a specimen that is being processed by an automated method (e.g. Abbott M2000). It states that there is insufficient volume in the vessel to perform an aspirate or dispense operation. What should you do to troubleshoot?

Can choose more than one

A. Check that the specimen has the correct volume in the sample tube

B. Yell at the machine, it's obviously not your fault

C. Call Ted on the phone immediately

D. Inspect the sample for clots and/or bubbles

E. Mark the run as invalid and start over

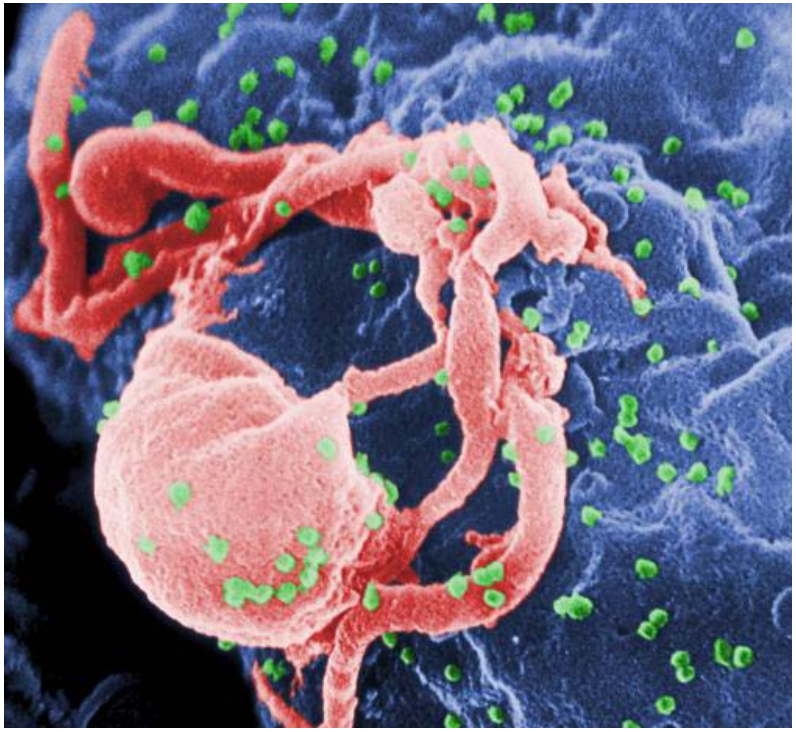
Factors That Can Affect VL

Box 4.5 Factors known to influence viral load measurements

Factor	Effect on viral load measurement
Selected aspects of specimen handling (delay in processing, freeze/thaw, etc)	Variable effects, but handling of specimens should be consistent
Intrinsic variability of assays	0.1-0.2 log standard deviation
Immunisations, including influenza vaccine, pneumococcal vaccine, hepatitis B vaccine, and presumably other vaccines	Viral load may increase for 2 to 4 weeks after immunisation
Tuberculosis, herpesvirus infections, and presumably many other infections	Increased viral load for 2 to 4 weeks after infection
Sex	Women may have lower viral load; Viral load may decrease during ovulation

Adapted from: Johnson SC, Kuritzikes DR. Monitoring therapy with plasma HIV RNA and CD4 counts. HIV Advances in Research and Therapy 1997;7:13-8.

CD4+ T Cells



From CDC.gov

- **What are they?**
  - Part of the immune system
  - Help protect the body against infection
  - Type of cell that HIV infects
- **Why do we count them?**
  - Monitor disease progression

CD4+ T Cell Count

- In 1 ml of blood (1/5 teaspoon or ~1 drop), if a person has:

- **700 – 1000 CD4+ T cells**
  - Healthy immune system in a person not infected with HIV

- **<200 CD4+ T cells**
  - HIV positive person progresses to AIDS
  - The body's immune system is no longer strong enough to prevent illness and infection

QUESTION 15

- In general, when viral load goes up:

A. CD4 T cell count goes down

B. CD4 T cell count stays the same

C. CD4 T cell count goes up

D. There is no relationship between viral load and CD4 T cells

Therapy Initiation Based on CD4

World Health Organization (WHO) Recommendations

CD4 criteria for the initiation of ART in adults and adolescents

CD4 (cells/mm³) [a]	Treatment recommendation [b]
<200	Treat irrespective of clinical stage [c] **[A-III]**
200–350	Consider treatment and initiate before CD4 count drops below 200 cells/mm³ [c d e] **[A-III]**
>350	Do not initiate treatment **[A-III]**

a CD4 cell count should be measured after stabilization of any intercurrent condition.

b CD4 cell count supplements clinical assessment and should therefore be used in combination with clinical staging in decision-making.

c A drop in the CD4 cell count below 200 cells/mm³ is associated with a significant increase in opportunistic infections and death.

d The initiation of ART is recommended for all patients with any WHO clinical stage 4 disease and some WHO clinical stage 3 conditions, notably pulmonary TB (see Section 12.1) and severe bacterial infections.

e The initiation of ART is recommended in all HIV-infected pregnant women with WHO clinical stage 3 disease and CD4 <350 cells/mm³ (see Section 11.2).

Viral Load and CD4+ T Cells