Assessing Real-Time RT-PCR as an alternative to bDNA for both Viral Load quantitation and RNA screening

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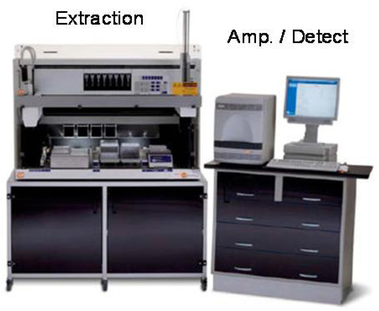
Introduction

- In the last year, the number of HIV viral load test methods has doubled. Two new tests have gained FDA approval in 2007. Each utilizes real-time, reverse transcription-PCR.
- Real-time, RT-PCR appears to possess several advantages over previous technologies from the laboratory point of view.
- Herein, we review some of the key observations we have made regarding Real-Time, RT-PCR in the process of switching from bDNA as the routine viral load test method in our laboratory
- RT-PCR appears to be less laborious, faster, and more sensitive than bDNA;
- *What might be the implications of these differences ?*

Overview of RT-PCR system (Abbott m2000):

- Automated sample extraction (RNA purification);
- Specimen barcode reader
- Real-Time RT-PCR on a separate device, (can be spatially separated)



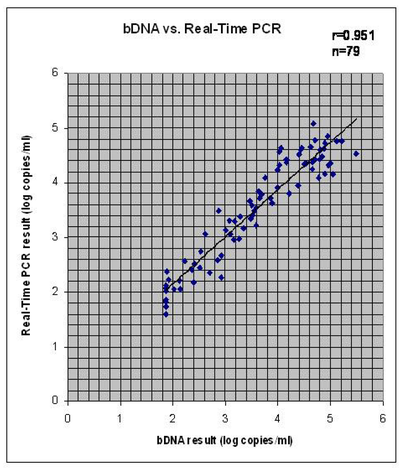
To Perform 1 Full Run:

bDNA (with 340 instrument): 15 controls, 81 patient samples:

- 384 pipetting steps
- 96 supernatant removal steps
- Results in 1.3 days

- **Real-Time PCR:** 9 controls, 87 patient samples:
- 4 pipetting steps
- Results in 6 hours

bDNA and Real-Time PCR compared



- Very high correlation between bDNA and RT-PCR
- 91% of specimens fell within 4x of each other
- 69% within 3x
- 59% within 2x
- 52/239 specimens were RNA+ by Real-Time PCR but below-detectable limit on bDNA
- 4/239 were RNA+ by bDNA, but "not detected" by Real-Time PCR

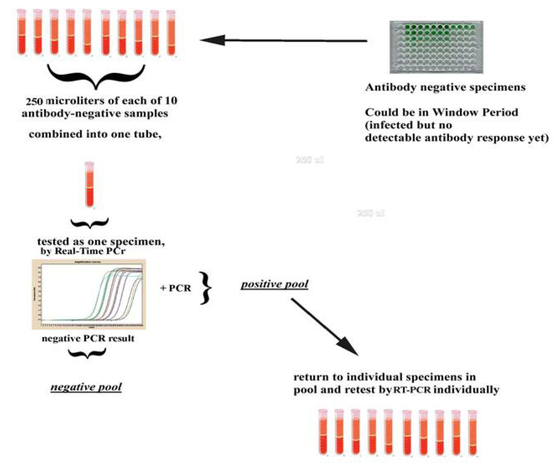
Setting the bar higher (lower ?)?

- 118 HIV+ patient viral load specimens were analyzed by **bDNA** and found to be "<75 copies" by bDNA (undetectable)
- 52 of 118 (44%) found to contain RNA by **Real-Time RT-PCR**
  - 18 of 118 were RNA+ by RT-PCR and gave a numerical value for copy number
  - 34 found to be RNA+ but less than 40 copies
  - 66 were "Target Not Detected" by RT-PCR

Any Implications ?

- Will the results motivate changes in therapy to aim lower ?
- Is there any psychological impact (for patients) in no longer being "below detectable levels" ?

Using Real-Time PCR for RNA screening:



RNA-based pooling for detecting acute (early) HIV Infection:

- In first 2 months of using Real-Time PCR for pooling, experience has been similar to that from bDNA (75 pools-of-10, *4 RNA+ pools found and confirmed, thus far*)

- One pool of 10 Ab-negative specimens was found to be "RNA detected <40 copies" by Real-Time PCR.
- Running all 10 neatly found specimen to contain 355 copies of RNA per ml
- Specimen likely would not have been detected by bDNA in a pool of 10

Conclusions

- Real-Time RT-PCR is more sensitive than bDNA; the method often detects HIV RNA in specimens that are considered below the detectable limit for bDNA
- Results from RT-PCR correlate very well with those obtained from bDNA
- RT-PCR is a sensitive alternative to bDNA for RNA-based screening for HIV infection