

# Rapid Molecular Diagnostic Test of HIV-1 Purified RNA from Plasma



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# HIV Diagnostics

- Rapid HIV diagnostics are critical factors in the overall management of HIV epidemics in developing countries.
- UNAIDS 90 90 90: testing is the first 90



diagnosed



on treatment



virally suppressed

- Currently available diagnostic testing methods
  - Antibodies
  - Antigens
  - CD4

# Nucleic acid-based tests (NAT)

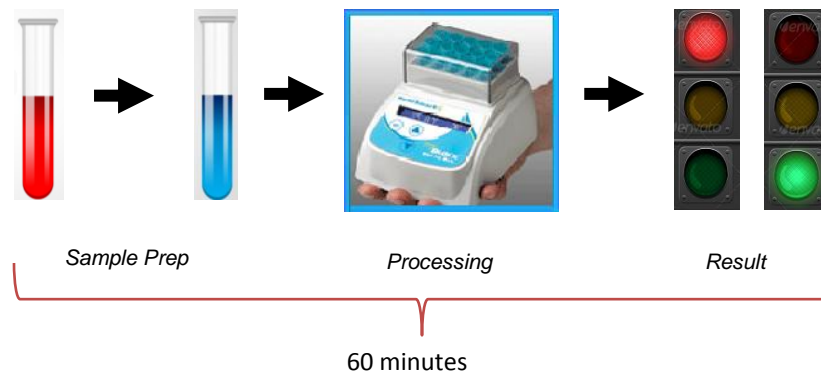
- NAT: testing RNA or DNA
- Only NAT tests can be used for detection of early HIV infection
- Only NAT tests can determine viral load-key part of AIDS treatment
  - Antiretroviral Therapy and Highly Active Antiretroviral Therapy
- PCR testing performed in developed countries
  - Expensive equipment and skilled technician
- Essentially unavailable in developing countries

# DASL Rapid™ HIV-1 RNA Assay

(under development)

## Design Objectives:

- High sensitivity (>90% detection rate)
- Low-cost: \$1-5 per test
  - a simple heater, no other equipment needed
- Rapid: <60 min from sample to results
- Ease of use: visual detection-traffic lights-like results



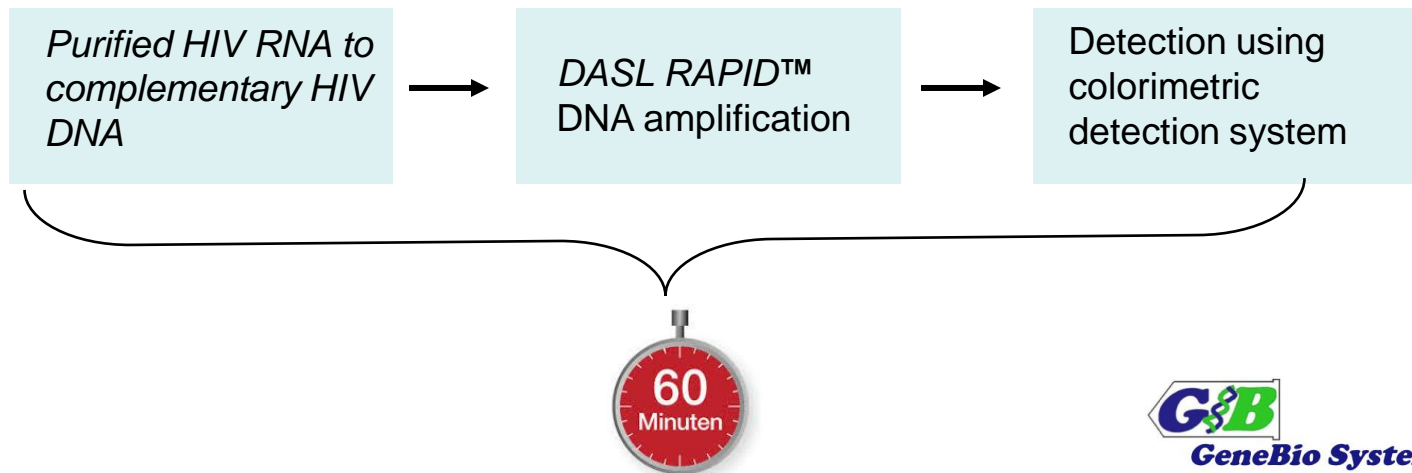
# Assay Development Platform

- *DNA Amplification via Scissors-Like structures – DASL RAPID™*
  - Isothermal DNA amplification
  - Developed by GeneBio Systems, a molecular diagnostic company
  - Has been used to develop other assays in food safety and disease diagnosis
  - Can detect RNA, after it is converted to DNA
    - Reverse transcription

# Detection of Purified HIV RNA by Prototype Kit Assay



- 24 reactions/ kit box
- lyophilized Key reagents
- packaged with protective porch



# HIV Synthetic DNA Detection

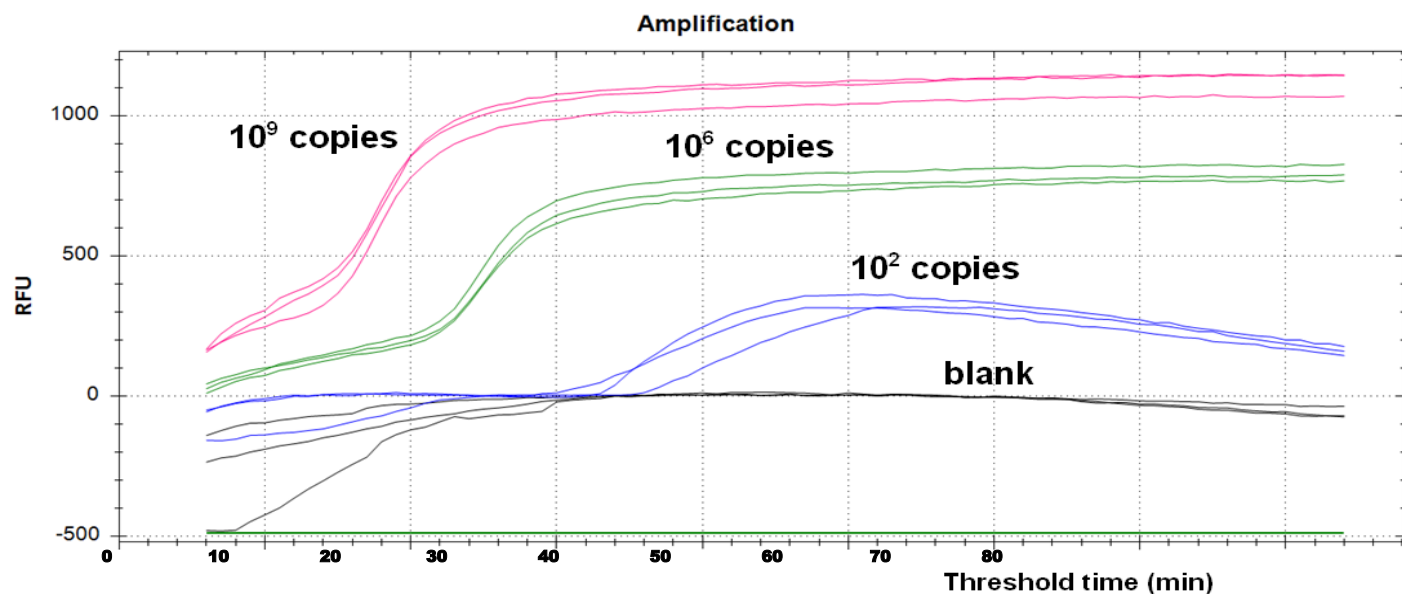
- *DASL RAPID*<sup>™</sup> DNA amplification
- Visual colorimetric detection system



- Clear difference between negative and positive results

# HIV Synthetic DNA Detection

- *DASL RAPID*<sup>™</sup> DNA amplification
- Fluorescent detection



- Detected high, mid and low levels of DNA
- No detection in the blank

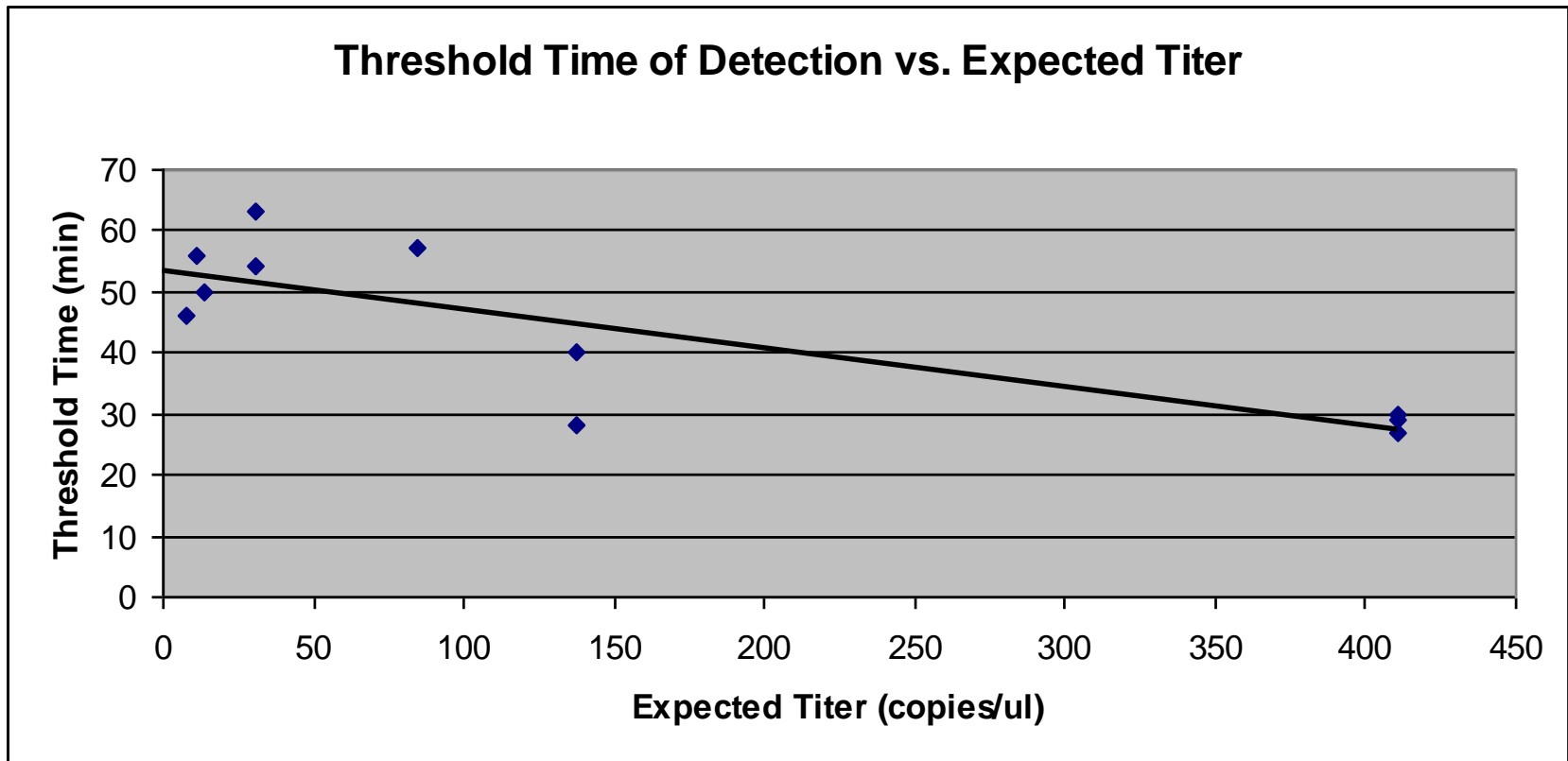


# Purified HIV RNA Detection

| HIV RNA | Titer (copies/ $\mu$ l) | Threshold time (min)* | Detection |
|---------|-------------------------|-----------------------|-----------|
| #1      | 13.6                    | 50                    | Yes       |
| #2      | 0.05                    | N.D.                  | No*       |
| #3      | 5.8                     | N.D.                  | No*       |
| #4      | 410.8                   | 27                    | Yes       |
| #5      | 1.05                    | N.D.                  | No*       |
| #6      | 30.4                    | 54                    | Yes       |
| #7      | 7.94                    | 46                    | Yes       |
| #8      | 137.9                   | 28                    | Yes       |
| #9      | 84.4                    | 57                    | Yes       |
| #10     | 11                      | 56                    | Yes       |


- 7 of 10 purified RNA samples detected
- 3 of 10 with titers too low were not detected

# Correlating RNA detection results with expected titers

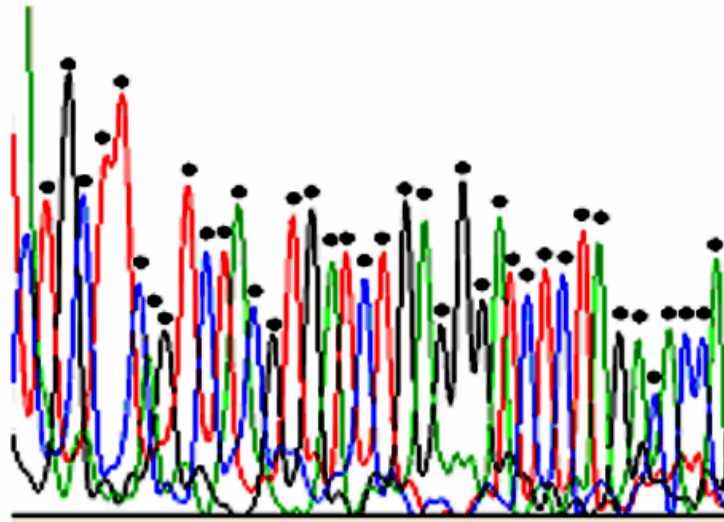


The threshold time results from the Prototype assay correlated with expected titers

# DNA sequencing results

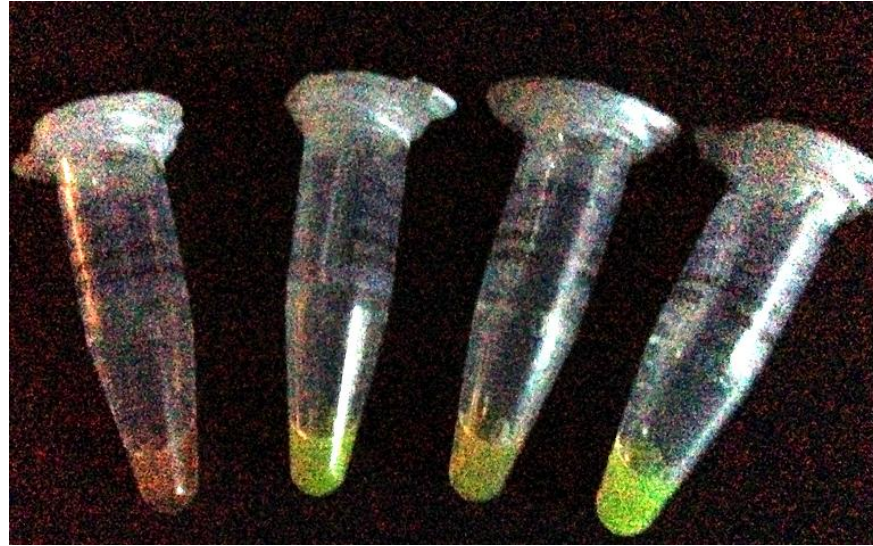
**Observed:**   
**Expected:** CTG CTT CAG TCT ACG TGA TCTG AGG GAT CTC TAG NNA CCA

**Observed sequence matches expected sequence**



DNA sequencing confirmed the correct HIV *ltr* sequence in detected sample

# Detection of Purified HIV RNA



Negative Control    Sample #8    Sample #6    Positive Control

- Two HIV RNAs and positive control showed green
- The negative control had an orange color

# Next steps towards meeting design objectives

- Improve sensitivity of detection From 70% to 90%
- Assay time to 30-60 min
  - Plasma sample to detection results
- Source OEM, and custom made reagents to reduce cost to \$1-5 per test
- Optimize colorimetric detection to maximize positive-negative difference

# Acknowledgement

- Grand Challenge Canada for funding support
- Team at GeneBio Systems, Inc. and CustomBiologics™
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