Performance of Xpert® HIV-1 Quant compared to Roche CAP/CTM v2 and Abbott RealTi*m*e HIV-1 on a prequalification plasma validation panel

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HIV Viral load testing

2013 WHO recommends

- VL as the preferred monitoring approach to "diagnose and confirm" ART failure.
  - Using the reduced threshold of viral load failure of 1000copies/ml
  - Based on two consecutive VL values
  - Using plasma specimens
  - Within 6 months after initiating ART, then annually.
  - With adherence support between measurements.

- VL is a currently a laboratory based test and quality testing services rely on good specimen transport logistics.

SA NHLS NPP HIV VL Laboratory footprint to perform >2million VL/an.

Relevant services will need expansion

1. Centralised high-throughput systems (constrained by specimen transport logistics and integrity)
   - Whole blood 6hrs @ 15°C - 37°C
   - Plasma (centrifugation) 24hrs @ 37°C to 5yrs @ -70°C (storage)
2. Decentralized lower-throughput testing platforms.
   - Extend service through sample integrity = DBS (1-2 weeks 37°C to 1 yr @-70°C (storage).
   - Increases access to testing and reduce TAT = (POC).

HIV viral load labs

17 laboratories

8 sites with Abbott m2000 system

9 sites with Roche CAP/CTM

Current instrument capacity (8 hour shift)

6888 samples/day

~ 1000 HIV VL / 8hrs

Multi-testing in a national program

- NHLS currently has 207 Gx testing sites for use in diagnosing pulmonary tuberculosis (Xpert MTB/RIF).
- We investigated the performance of the Xpert® HIV-1 Quant assay for VL.

NATIONAL HEALTH LABORATORY SERVICE
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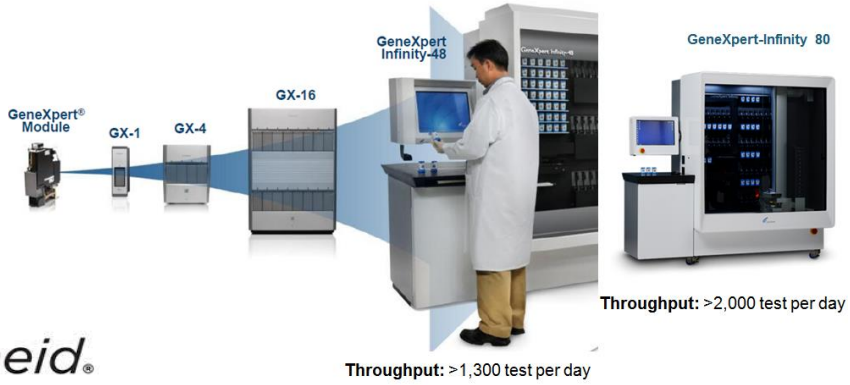
Xpert® HIV-1 Quant assay for VL

- 14 IVD tests on the market
  - Healthcare Associated Infections – 7 tests (eg MRSA and C.Difficile)
  - Critical Infectious Diseases 3 tests (eg. MTB/RIF and Flu)
  - Sexual Health – 3 tests (eg. Chlamydia, gonorrhoeae)
  - Oncology/Genetics – 1 test (Thrombosis)
- All testing done within a closed cartridge
  - Sample preparation
  - Amplification
  - detection



Cepheid

GeneXpert HIV-1 Quant protocol

Step 2: Centrifugation at 800-1600 x g for 20 minutes

Step 3:
1.Transfer 1 mL plasma to chamber 3

Step 1:
Collect 5 ml whole blood in an ACD-A or EDTA plasma tube.

Time to result in GeneXpert ~95 min

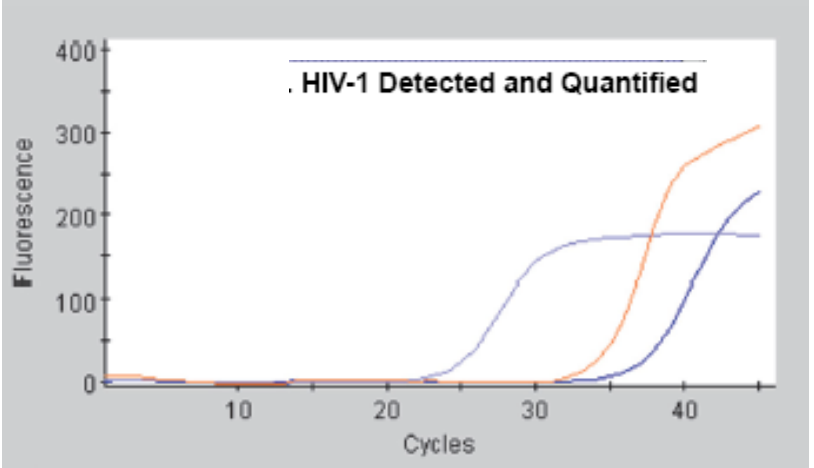
Ch 3

Step 5: Load into GX and close door

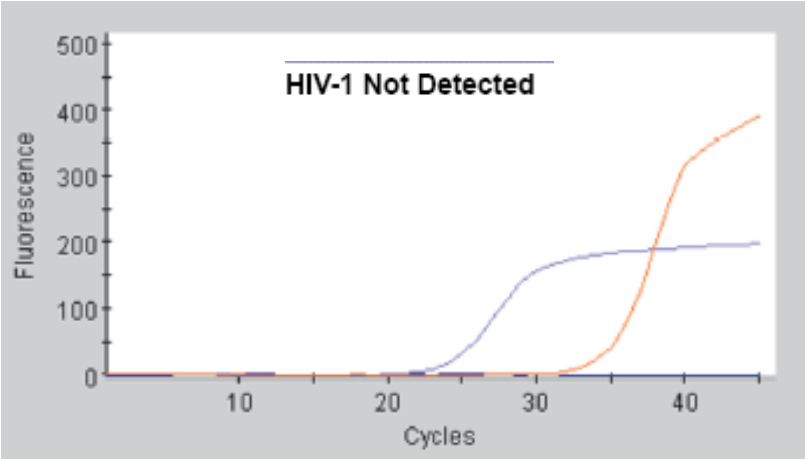
Step 4: Scan cartridge barcode

Xpert® HIV-1 Quant assay principle

GeneXpert HIV-1 Quant
- Fully automated
- Real time molecular cartridge based
- Two internal quantification standards.
- Requiring 1ml plasma



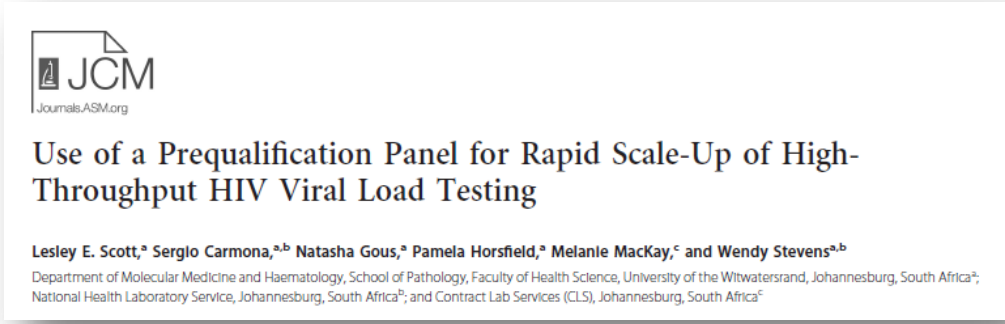
- LODetection ~20cp/ml
- LOQuantification 40cp/ml
- Linear range: 40 – 10million cp/ml
- Targets 3’ end 5’ LTR
- Detects HIV Group M,O,N and recombinants.
- TAT <95mins



Methods

- Collect blood (ACD or EDTA), centrifuge, transfer 1ml plasma into cartridge ….test.
- Training within 1 day (requires computer literacy).

- A 42 member plasma HIV-1 subtype C panel used to validate the CAP/CTMv2 (Roche) and RealTi*me* HIV-1 (Abbott) during implementation of these platforms in NHLS.
- Frozen plasma was shipped to Cepheid, Sweden, where testing was performed
- Analysed in Johannesburg. CAP/CTMv2 and RealTi*me* HIV-1 were reference methods.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Use of a Prequalification Panel for Rapid Scale-Up of High-Throughput HIV Viral Load Testing

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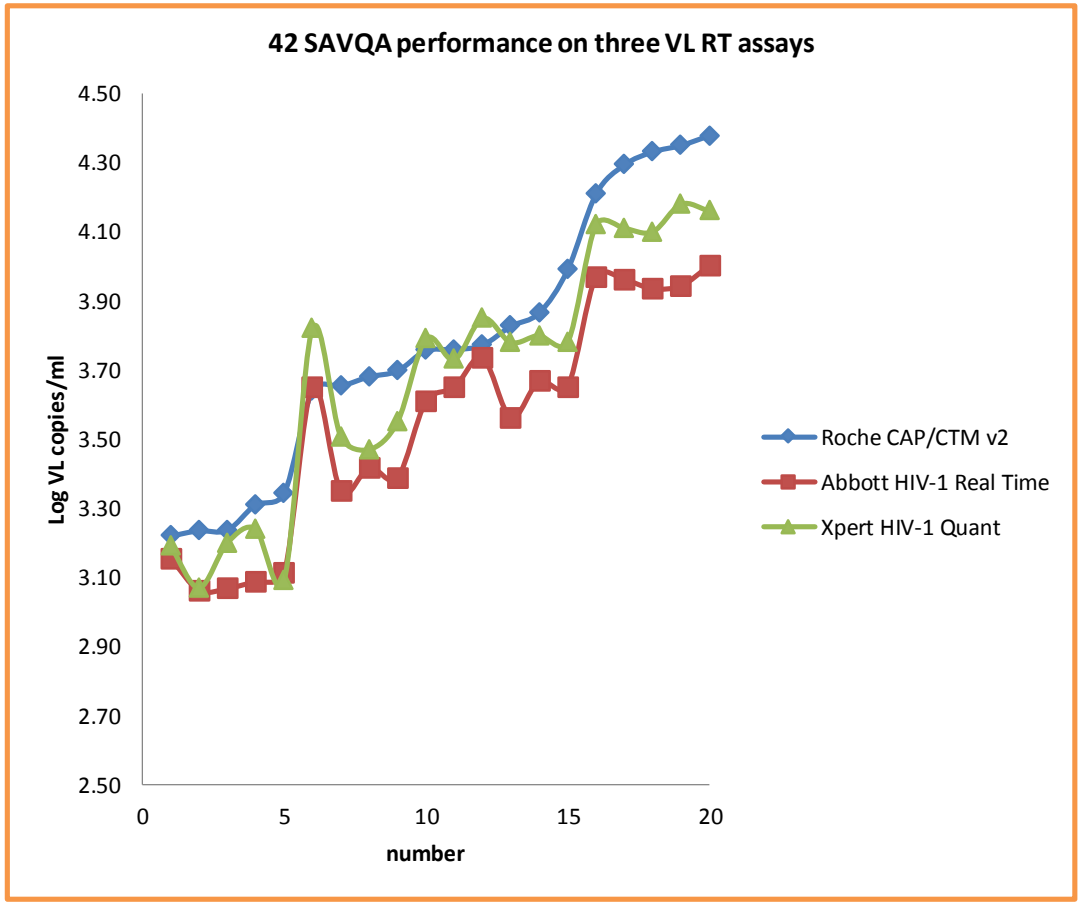
Acceptance criteria:

- <log 0.19 cp/ml SD
- <35%CV copies/ml
- <log 0.3 cp/ml absolute bias
- ≤2.9% similarity CV

Results

Expected	Xpert® HIV-1 Quant observed
All HIV negative specimens	100% correctly identified
Carryover	none
Results not reported	n=1, due to instrument error
Intra-variability (within Precision)	
Standard Deviation	
CAP/CTMv2	Log0.15copies/ml
RealTi*m*e HIV-1	Log0.15copies/ml
Xpert® HIV-1 Quant	Log0.16 copies/ml
%CV	
CAP/CTMv2	36%CV
RealTi*m*e HIV-1	35.9%CV
Xpert® HIV-1 Quant	36.8%CV

Bias	
Xpert® HIV-1 Quant generates lower values than CAP/CTMv2.	log0.18Lopies/ml [SD bias Log0.1copies/ml]
Xpert® HIV-1 Quant generates higher values than RealTi*m*e HIV-1	Log-0.17copies/ml [SD log 0.08copies/ml].
Concordance correlation	
CAP/CTMv2	*Pc* = 0.922
RealTi*m*e HIV-1	*Pc* =0.918
Percentage Similarity CV	
CAP/CTMv2	1.5%CV
RealTi*m*e HIV-1	0.9%CV



Summary

- The GeneXpert HIV-1 VL assay shows good performance on a plasma validation panel compared to existing reference methods.
- Footprint already well established in SA
  - Laboratories already “trained” on technology, but implementation will require:
    - Laboratory integration work flow for HIV and TB Gx cartridge testing.
    - Connectivity (LIS established for TB, Gx Remote Connect under development and manages multi-test suite).
    - Cost evaluation.
- A full clinical validation is needed
- True POC whole blood and DBS needs development and evaluations

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