



**CAPRISA**

CENTRE FOR THE AIDS PROGRAMME OF RESEARCH IN SOUTH AFRICA

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# **GeneXpert HIV-1 Quantitative assay**

**MTN REGIONAL MEETING 25-29 SEPTEMBER 2016**

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CAPRISA

# INTRODUCTION

- Cepheid HIV-1 Quant Assay is an automated single cartridge-based quantitative nucleic acid amplification test that is designed to detect Human Immunodeficiency Virus Type 1 (HIV-1) total nucleic acids, using human plasma.
- The GeneXpert Dx System consist of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results.

# Types of HIV-1 RNA PCR POC CARTRIDGES

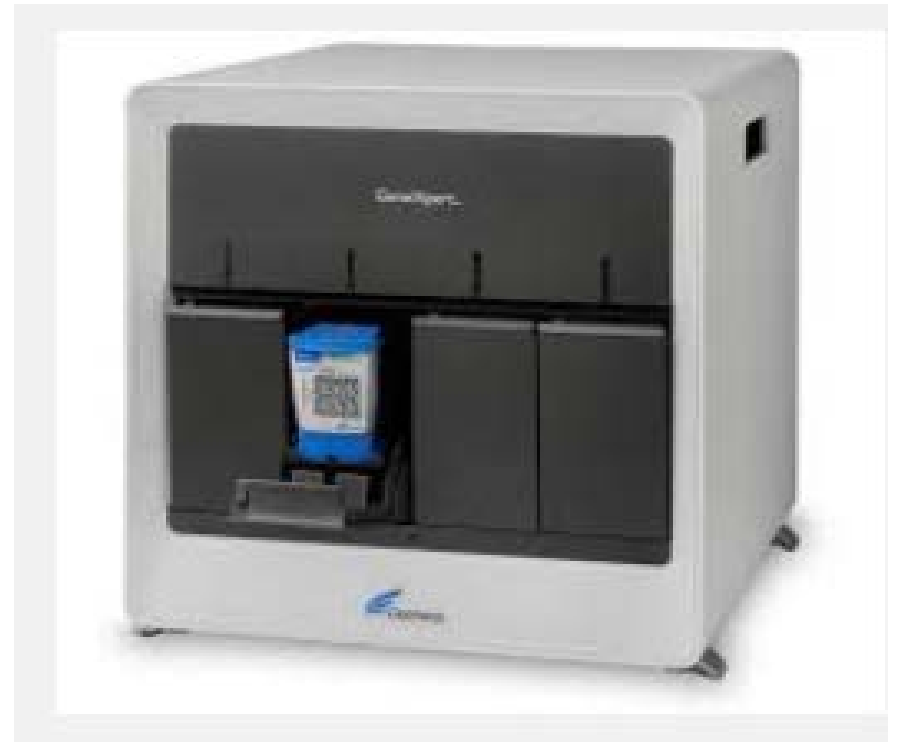


# TYPES OF GENEXPERT INSTRUMENTS

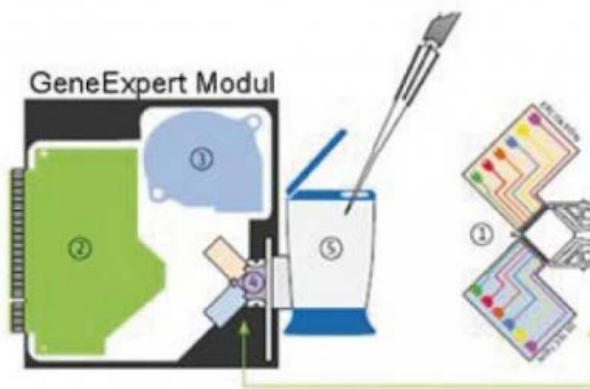


# GENEXPERT INSTRUMENT

- Loading of instrument



## Mode of operation



2 = circuit  
(transfers optical information  
to the computer)  
3 = ventilation  
4 = heating plate  
5 = GeneXpert cartridge

1 = optical block (6 channels)  
(Excitation/emission)

Channel	Excitation	Emission	Calibrated reporter dyes
1	375-405	420-480	CF 1
2	450-495	520-535	FAM
3	500-550	565-590	AltaFluor® 532
4	555-590	606-650	Texas Red®
5	630-650	665-685	AltaFluor® 647
6	630-650	>700	CF 6

*Fig. 3: GeneXpert Gx-IV mode of operation.*

# CARTRIDGE



241 x 528 - ...

- The GeneXpert Dx System integrates and automates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time reverse transcriptase PCR and results are obtained in 90 minutes.
- This system requires the use of single-use disposable GeneXpert cartridge that holds the RT-PCR reagents and hosts the RT-PCR processes. Cross-contamination between samples is minimized due to the cartridges being self-contained.



- The HIV-1 Quant Assay includes reagents for the detection of HIV-1 RNA in specimens as well as 2 controls used for quantitation of HIV-1 RNA.
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# PROCEDURE

- Blood collected in EDTA tube, spin down for 10 minutes at  $\leq 1300$  rpm.
- Inspect the test cartridge for damage, if damaged do not use.
- Label the HIV-1 Quant cartridge with the sample ID, taking care not to write on the lid or the 2D barcode on the front of the cartridge.
- Open the lid of the cartridge.
- Transfer 1000uL of the plasma, using the 1 mL transfer pipette provided.
- Close the cartridge lid firmly.

- Starting test
- Before starting test ensure all modules status are functioning and have passed self-test after logging onto the GeneXpert Dx system.
- In the GeneXpert Dx System window, click **Create Test**. The Scan Sample ID dialog box appears.

- In the **Sample ID box**, scan or type the sample ID. The scan Cartridge Barcode dialog box will then appear.
- Scan the barcode on the HIV-1 Qual cartridge. The Create Test window appears displaying the barcode information of the cartridge automatically.
- Click **Start Test**. Enter your user name and password.
- A green flashing light will indicate will module is going to perform the test. Open the instrument module door with blinking light and load the cartridge.
- Close the module door until you hear the click and the green light stops flashing, but remains green. Indicating that the test has started.

- Once the test is complete after 1 hour 30 minutes, the green light on the door turns off and the system will release the door lock, opening the module door. You can remove the used cartridge and discard the cartridge appropriately.
- The printer will automatically print the result once the test is complete.
- LOD Whole blood- 200-300 cp/ml
- DBS(70ul) - 500 cp/ml

**1. Transfer of 1ml plasma into HIV-1 viral load cartridge**



**2. Scan, insert cartridge and start test**



**3. Time to result  
90 minutes for  
HIV-1 viral load,  
GeneXpert®**

# Validation

Template #2: one laboratory only interested in validating one 4-module instrument over 5 days.

GeneXpert Instrument #1				
Module				
	1	2	3	4
DAY 1	VQA 150,000	VQA 150,000	VQA 1,500,000	VQA 1,500,000
	VQA 25	VQA 50	VQA 25	VQA 50
	VQA 150,000	VQA 150,000	VQA 1,500,000	VQA 1,500,000
	VQA 50	VQA 25	VQA 50	VQA 25
DAY 2	VQA 1,500	VQA 1,500	VQA 15,000	VQA 15,000
	DONOR 1	DONOR 2	DONOR 3	DONOR 4
	VQA 15,000	VQA 15,000	VQA 1,500	VQA 1,500
	DONOR 5	DONOR 6	DONOR 7	DONOR 8
DAY 3	DONOR 9	DONOR 10	DONOR 11	DONOR 12
	SN DONOR 1	SN DONOR 2	SN DONOR 3	SN DONOR 4
	DONOR 13	DONOR 14	DONOR 15	DONOR 16
DAY 4	SN DONOR 5	SN DONOR 6	SN DONOR 7	SN DONOR 8
	DONOR 17	DONOR 18	DONOR 19	DONOR 20
	DONOR 21	DONOR 22	DONOR 23	DONOR 24
	SN DONOR 9	DONOR 25	DONOR 26	DONOR 27
DAY 5	SN DONOR 10	DONOR 28	DONOR 29	DONOR 30
	DONOR 31	DONOR 32	DONOR 33	DONOR 34
	DONOR 35	DONOR 36	DONOR 37	DONOR 38



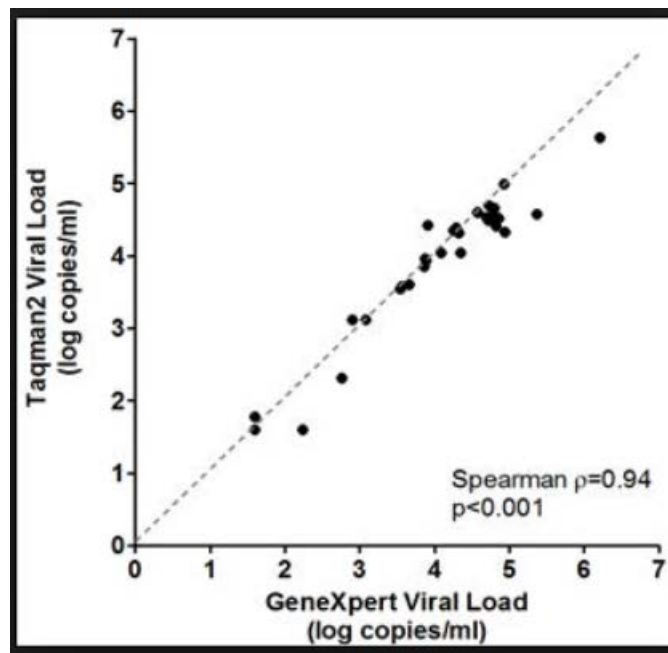
## Diagnostic Accuracy of the Point-of-care Xpert® HIV-1 Viral Load Assay in a South African HIV clinic

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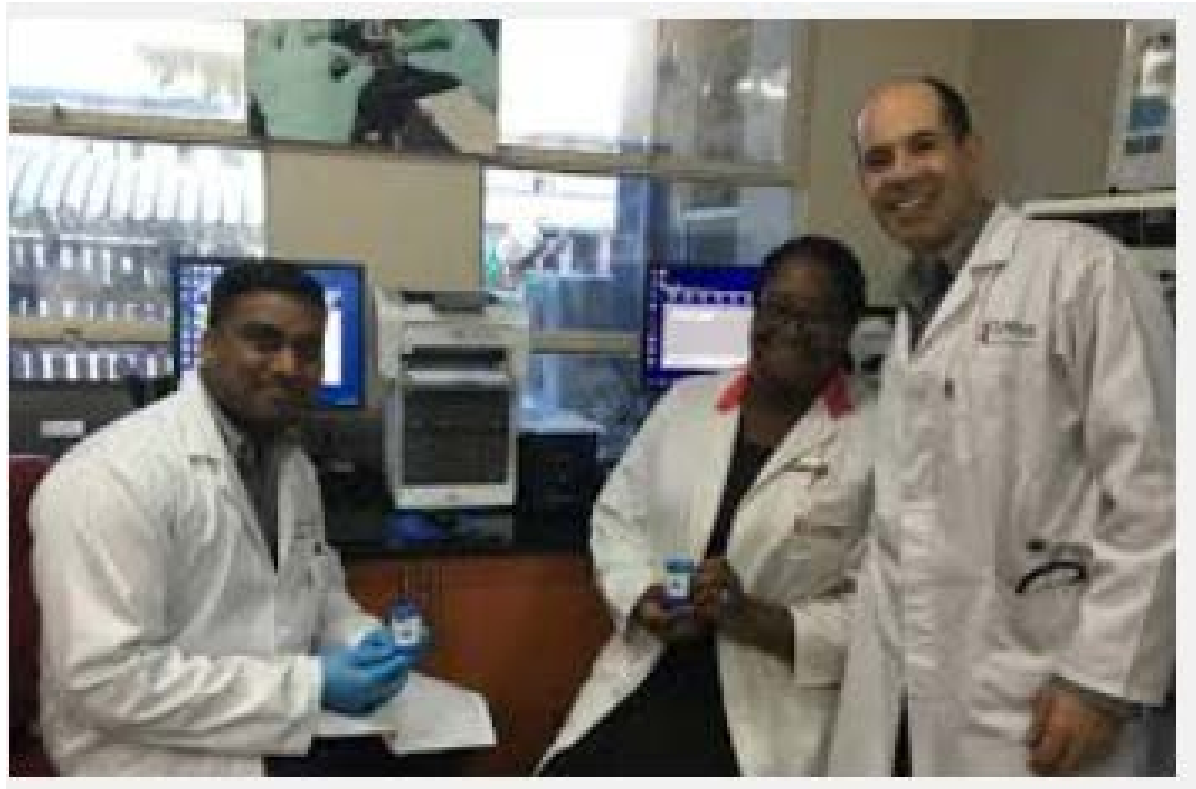


- Min of 1000ul of plasma is required for the assay
- EDTA tube may be stored at 15-35 degrees celsius for up to 6 hours or at 2-8 degrees Celsius for up to 72 hours prior to preparation and testing.
- Specimens are stable frozen and can be used for the assay. Need to ensure sample is completely thawed, spin to remove any clots and then introduce into the GeneXpert cartridge.

- Ensure sample is introduced into the cartridge properly with no air bubbles being introduced as well as that will affect the assay.
- Note that most GeneXpert kits currently have short shelf life, will improve as further tests indicating stability of reagents progress
- Clean and service the GeneXpert according to manufacturers instructions
- Do not use reagents or cartridges post expiry date.
- Do not open a cartridge lid until ready to perform testing.
- Cartridge should be used within 30 minutes after opening from pouch.

- The assay is very easy to perform.
- A report prints out once the test is complete that includes the primary result.
- The beauty of the GeneXpert POC VL assay in addition to the short turn around time is that
  - you can start different patient tests at different times and
  - also you can run different assays like CT/NG or TV or MTB Rif on the different modules for patients at once.

# USERS



# Acknowledgements

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- **Cepheid**



CAPRISA hosts a DST-NRF Centre of Excellence in HIV Prevention



CAPRISA hosts a MRC HIV-TB Pathogens Treatment Research

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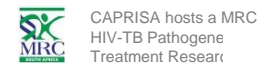
# Example of Acknowledgements

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