GeneXpert VL Validation; Experience at UNC Project

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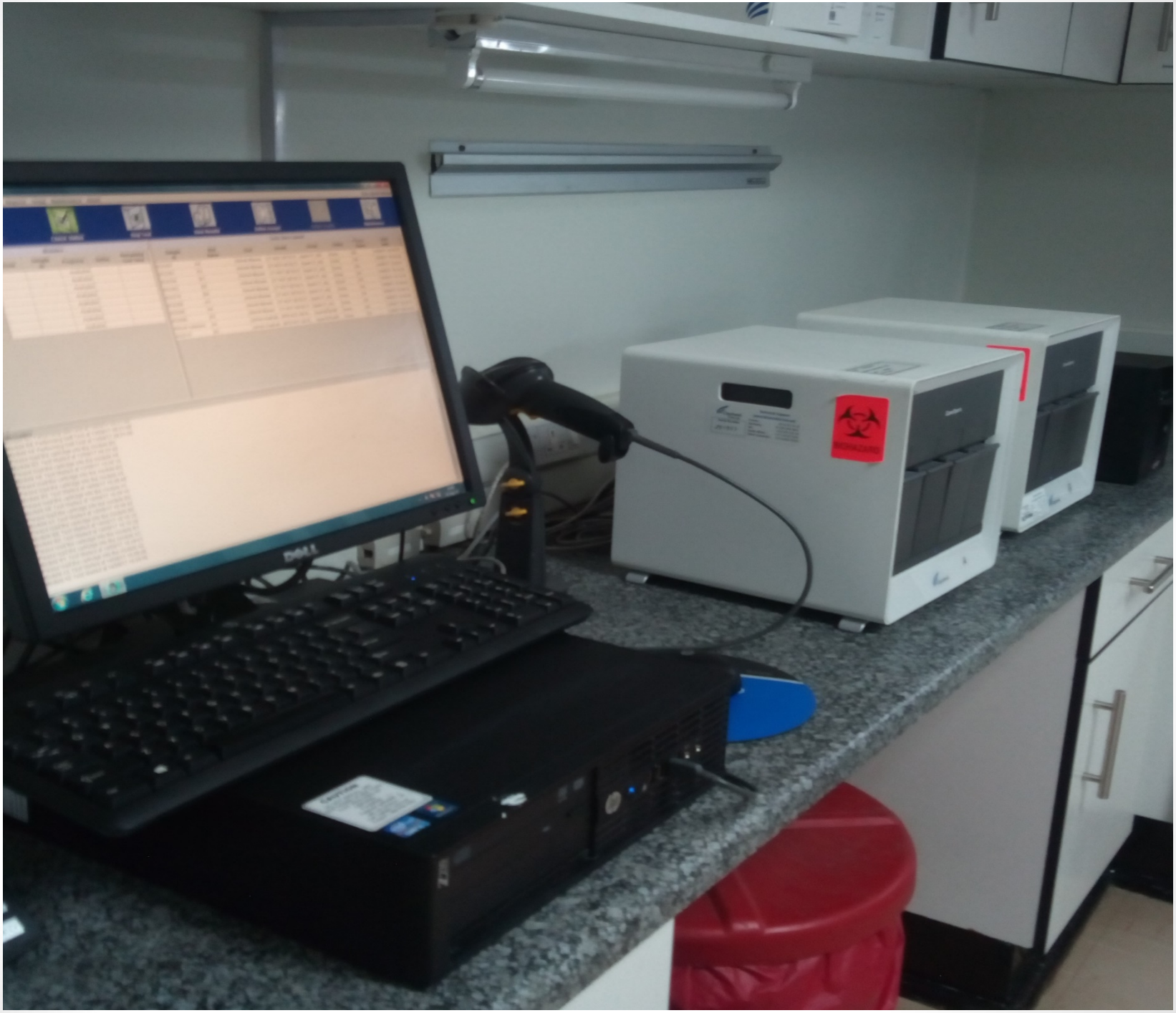
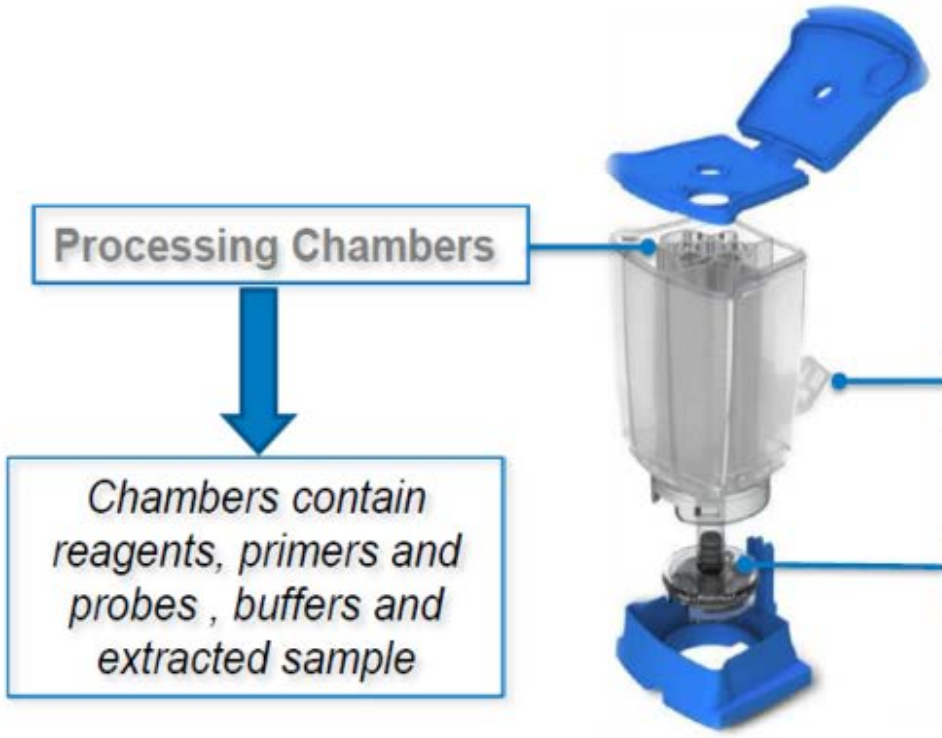
MTN Regional Meeting, Sept. 2017

Cape Town, RSA.


GeneXpert Infinity-48


DELL

GeneXpert Cartridge



Processing Chambers

Chambers contain reagents, primers and probes , buffers and extracted sample

Amplification tube

Allows you to perform a rapid thermal cycling and optical excitation/detection

Valve Body

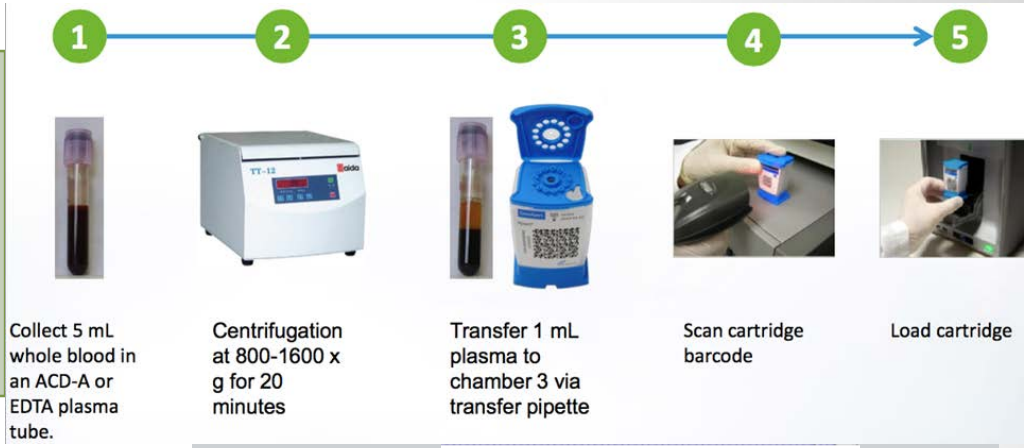
By turning, it directs the fluids into the different chambers and the PCR tube.

This is a self-contained system

Principles of GeneXpert Design

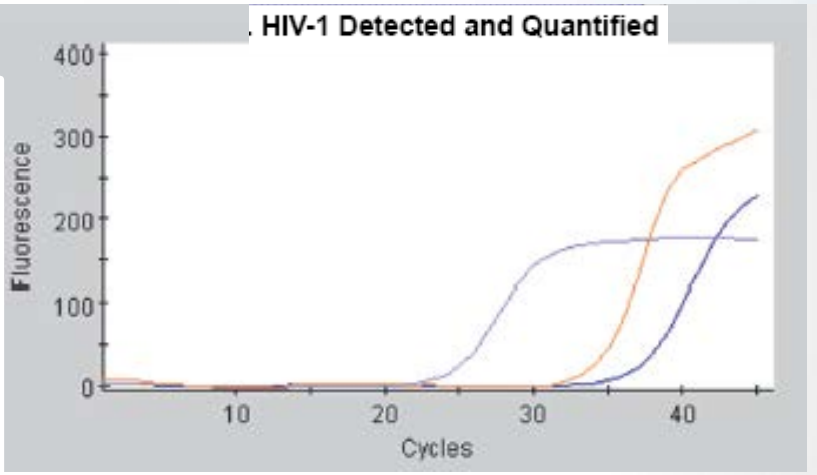
- Real-time PCR (amplification & detection at the same time)
- No wet interface between instrument and cartridge to eliminate carry-over
- Total internal control of reagents system – No separate external positive or negative controls required**
- Software instructions to individual module motherboards to coordinate valve movement and integral hydraulic drives
- Smart fluidics - Flow of liquids directed by micro valves – Allow using micro quantities of reaction components
- Automated data analysis and results interpretation

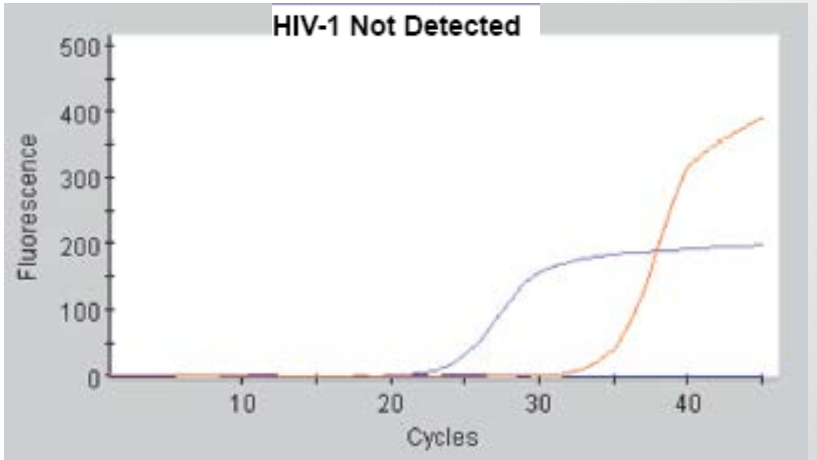
Xpert HIV-1 VL Assay principle



GeneXpert HIV-1 VL

- 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
- TAT <95mins





VQA Validation Template

Template #1: One laboratory interested in parallel testing two 4-module instruments over 5 days.

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

Total: 72 cartridges

Running of the samples

- 24 VQA copy controls were used for the validation with the following concentrations: 25; 50; 1,500; 15,000; 150,000 and 1,500,000
- 38 SP and 10SN Donor samples were used for validation
- The validation was conducted from 17 January 2017 to 27 January 2017
- Validation temporarily stopped on 19 January 2017 but resumed on 25 January 2017
- Two technicians who are competent on running the Xpert did the testing
- Data was submitted to VQA for analysis

Results - Precision

- Total assay SD should not exceed a target of 0.15

			STANDARD DEVIATION		
Lab-Assay	Nominal Concentration (Copies/mL)	CSALR[1]	Intra-Assay	Inter-Assay	Total-Assay[2]
291-Xpert-VL	1,500	0.0164	0.114	-	0.114
291-Xpert-VL	15,000	-0.0235	0.087	-	0.087
291-Xpert-VL	150,000	-0.0169	0.027	-	0.027
291-Xpert-VL	1,500,000	-0.0435	0.102	-	0.102
291-Xpert-VL	Combined	-0.0169	-	-	0.083

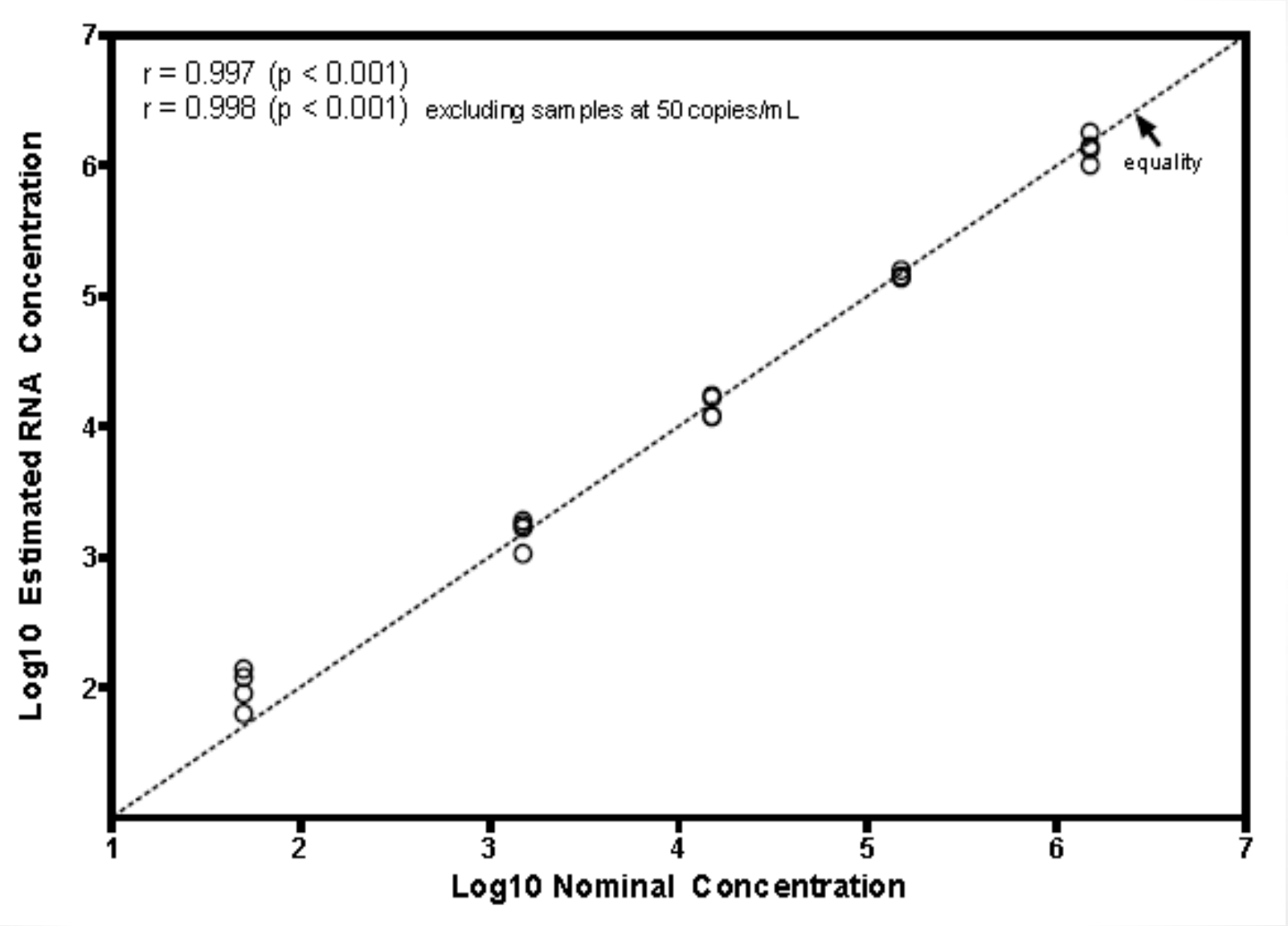
Results - Accuracy and Linearity

- All the parameters fell in the acceptable threshold

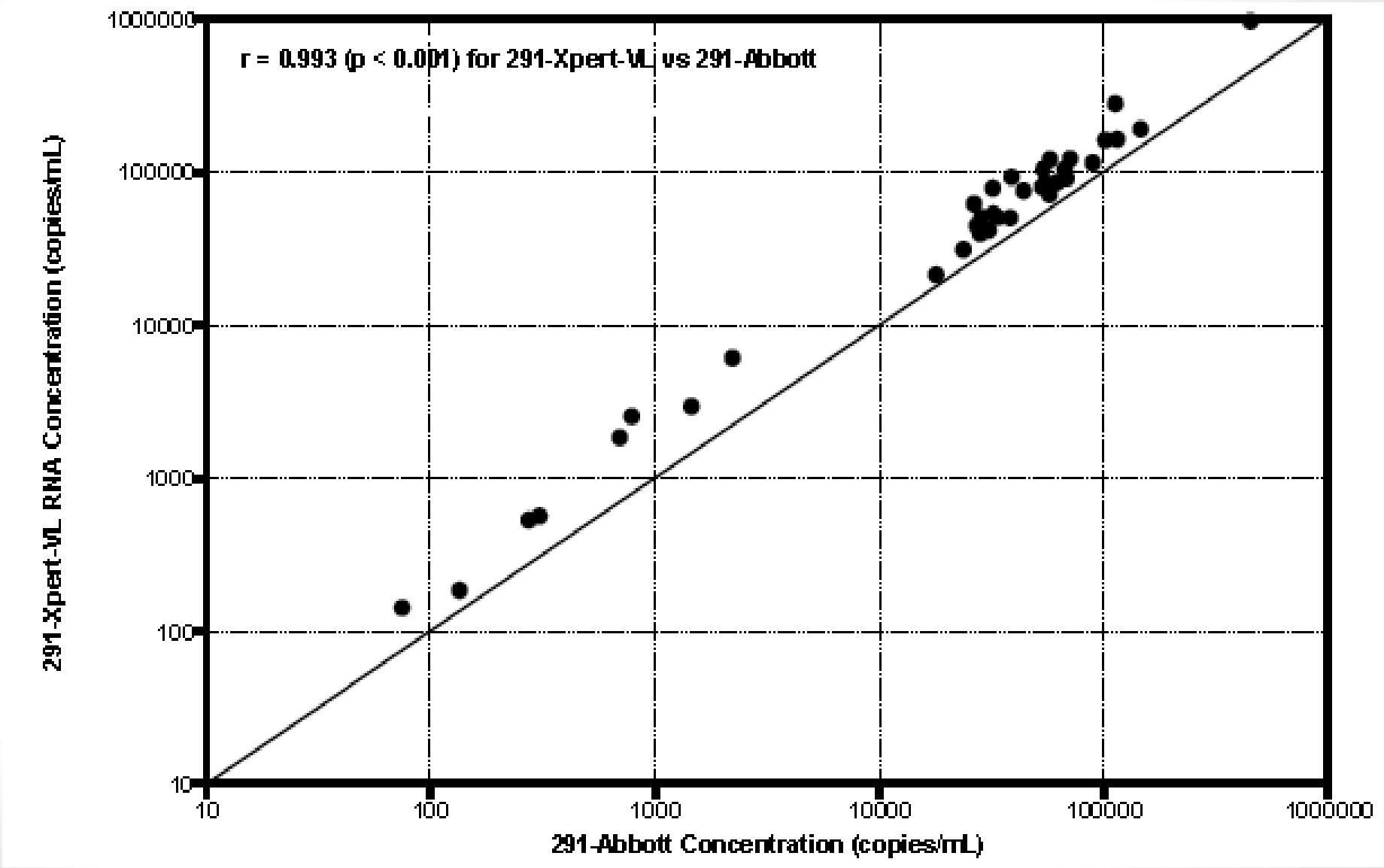
	LINEARITY PARAMETERS[1]		
Lab-Assay	**Slope[2]**	**SD(resid)**	**SEM**
Acceptance Threshold	0.056	0.096	0.091
291-Xpert-VL	-0.017	0.013	0.025

[1]Slope=the slope of the regression line fit to the CSALR
SD(resid)=the residual standard deviation of the regression line fit to the CSALR
SEM=standard error of the CSALR
[2]Threshold values are presented as absolute values

Log10 Estimated RNA Concentration vs Log10 Nominal Concentration



Comparison of Xpert and Abbott m2000



Sensitivity

	NOMINAL CONCENTRATION (Copies/mL)	
Lab-Assay	25 N = 4	50 N = 4
291-Xpert-VL	100%	100%

Lab-Assay	% > 50 copies/mL N = 4
291-Xpert-VL	100%

Analysis of Carryover

	HIV SERO-NEGATIVE DONORS		1,500,000 COPIES/ML VQA CONTROLS	
Lab-Assay	# Tested	# Negative Results	# Tested	# Detectable Results
291-Xpert-VL	10	10	4	4
291-Abbott	10	10	-	-

[1]Results indicate that carryover was not detected

Summary of results

- No problems noted on precision, linearity, carryover or sensitivity using VQA copy controls
- No discordant results on clinical samples
- All results within ±0.7 log10 HIV RNA Window
- Xpert results consistently higher than Abbott (Avg. log difference =0.234, about 1.7 fold difference.)

Challenges encountered

- Invalid runs due to inadequate sample volume
- Use of multiple kits because the initial kit expired during the validation period
- The instrument had to be used for other assays as well during the validation period like MTB, CT/NG and HPV
- Data was not being reviewed in real time to determine if repeat testing was necessary – a result of >10,000,000cp/ml was reported instead of diluting the sample.

Lessons Learnt

- Sample volume is critical for the assay to minimize cartridge wastage
- Proper planning to minimize use of multiple kit lot numbers
- Constant dialogue with VQA very important

Acknowledgements

- *UNC Project Laboratory staff*
- *MTN Network Laboratory team for the support and initiation of the process*
- *Rush University VQA Center specifically Cheryl Jennings for the provision of the validation plan, VQA copy controls and analysis of results*