NATIONAL HEALTH LABORATORY SERVICE

GeneXpert MTB/RIF

Progress Report

June/July 2012

NATIONAL HEALTH LABORATORY SERVICE

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1. Background to Project

This project was initiated at the request of the Honorable Minister of Health, Dr Aaron Motsoaledi, in early 2011, following the World Health Organization's strong recommendation published in December 2010 that "the new automated DNA test for TB be used as the initial diagnostic test in individuals suspected of MDR-TB or HIV/TB". In essence this comprises the majority of TB suspects in South Africa. A pilot study was proposed by the TB Cluster within the National Department of Health (NDoH) while a project feasibility study was being performed with due diligence.

The pilot study was initiated in microscopy centres in high focus TB areas. The NDoH requested that at least 1 instrument be placed in each province, preferably in high burden districts. Selections were made by the TB cluster, with twenty-five microscopy centres being selected and a total of 30 instruments placed.

The NDoH funded 9 GX16 and 14 GX4 instruments for the project. FIND (The Foundation for Innovative New Diagnostics) donated 6 GX4 analysers and the Infinity or GX48 was supported by PEPFAR Right to Care funds. All instruments were placed by World TB day March 24th 2011. This placement represented about 10% of national coverage. The basis for the calculations was an assumption that 2 smears at diagnosis would be replaced by 1 Xpert® MTB/RIF assay. All instruments were interfaced to the NHLS Laboratory Information System (LIS) allowing for troubleshooting and data collection.

The remainder of the roll-out is being performed in a phased manner by the National Priority Programmes of the NHLS and the NDoH, the progress of which is described in point 4 below.

2. Assays performed to date

In summary, a total of 472,734 specimens have been processed to date (10 July 2012). The total % of *Mycobacterium tuberculosis* complex (MTBC) detected in this cohort was 15.65% (73,960). The percentage positivity has remained on average between 16-17% monthly country-wide. To date Kwa-Zulu Natal (KZN) has performed the greatest number of tests which is probably as a result of the throughput of the GX48 analyzer (Refer to table 1). Average Rifampicin resistance detection rates have remained around 7% since project inception (Refer to table 2).

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Table 1: GeneXpert MTB Results by province

Province	MTB Detected	MTB Not Detected	Test Unsuccessful	Grand Total	% MTB Detected
Eastern Cape	9,334	47,579	1,985	58,898	15.85
Free State	8,035	47,870	158	56,063	14.33
Gauteng	6,857	46,326	1,049	54,232	12.64
Kwa-Zulu Natal	24,287	103,732	3,937	131,956	18.41
Limpopo	3,430	28,955	398	32,783	10.46
Mpumalanga	4,340	22,252	1,659	28,251	15.36
North West	4,525	23,726	1,427	29,678	15.25
Northern Cape	4,906	25,709	833	31,448	15.60
Western Cape	8,246	41,037	142	49,425	16.68
Grand Total	**73,960**	**387,186**	**11,588**	**472,734**	**15.65**

Table 2: Provincial GeneXpert RIF Results in MTB detected cases

Province	Inconclusive	Resistant	Sensitive	No Result	Grand Total	% RIF Resistant
Eastern Cape	122	687	8,409	116	9,334	7.36
Free State	105	452	7,453	25	8,035	5.63
Gauteng	70	457	6,320	10	6,857	6.66
Kwa-Zulu Natal	354	1,914	21,632	387	24,287	7.88
Limpopo	54	254	3,093	29	3,430	7.41
Mpumalanga	64	363	3,835	78	4,340	8.36
North West	62	375	4,079	9	4,525	8.29
Northern Cape	60	304	4,514	28	4,906	6.20
Western Cape	90	387	7,765	4	8,246	4.69
Grand Total	**981**	**5,193**	**67,100**	**686**	**73,960**	**7.02**

Rifampicin concordance is good for both LPA and culture. There is Rifampicin mono-resistance significant geographical variation. The national average is 12% for DST and 18% for LPA. This could be attributed to a number of factors such as geographical variation, laboratory variation, and interpretation of LPA, reliability of gold standard or even strain variation.

Testing and clinical algorithms show variation across provinces, requiring standardisation.

Table 3: Rif Concordance by LPA or DST

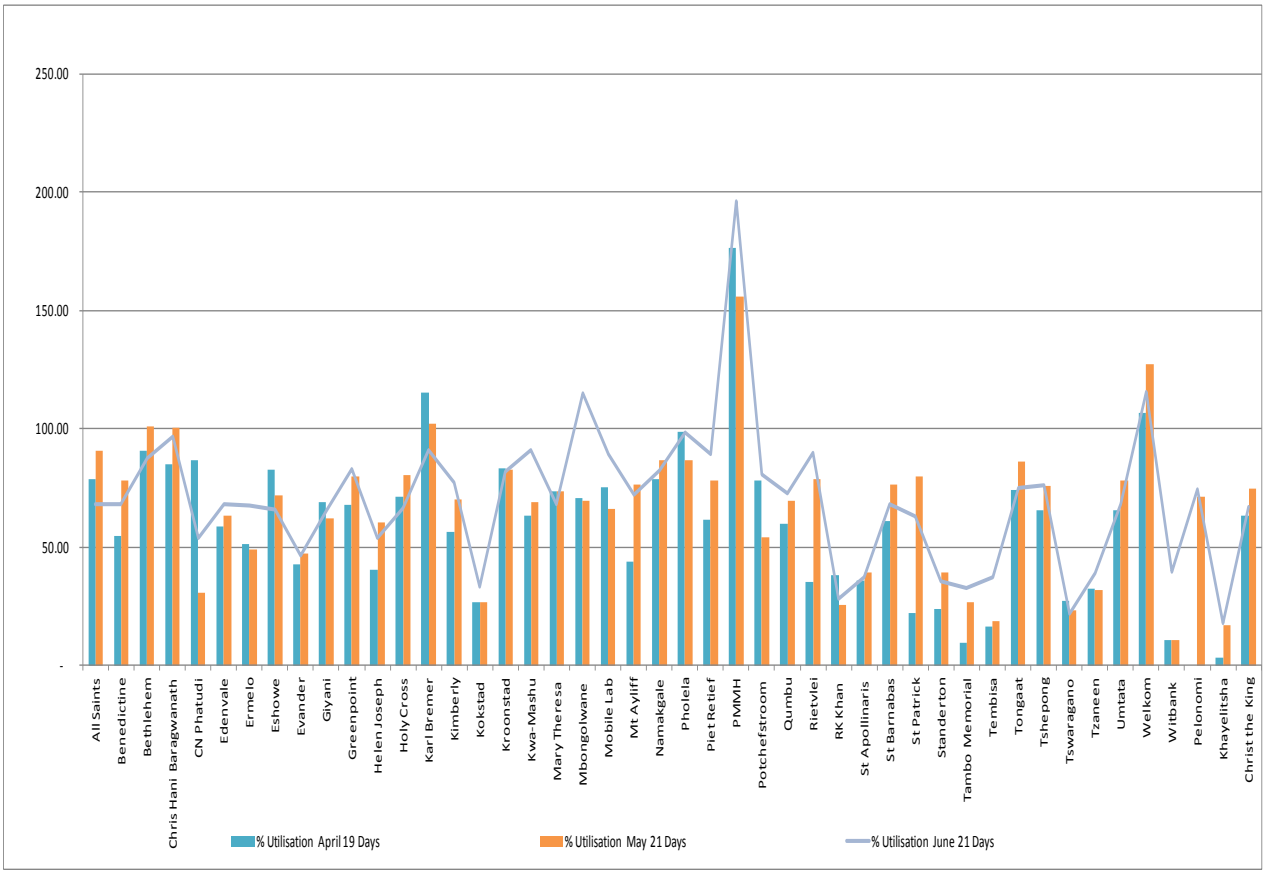
Province	LPA	DST
Eastern Cape	93.3%	12.5%
Free State	83.3%	75.0%
Gauteng	92.3%	88.2%
Kwazulu-Natal	82.2%	93.3%
Limpopo	80.0%	94.4%
Mpumalanga	81.0%	97.2%
North West	100.0%	50.0%
Northern Cape	76.2%	66.7%
Western Cape	95.9%	100.0%
National	**87.2%**	**89.7%**

Errors have ranged consistently below 3%. Details of invalid results, which likely represent sample issues remains below 1%. These are being monitored regularly and corrective action implemented where necessary.

Table 4: Number of Unsuccessful Tests and Reasons

Province	Error	Invalid	No Result	GXP Result	Grand Total	% Error Total
Eastern Cape	1,797	148	40	56,913	58,898	3.05
Free State	137	15	6	55,905	56,063	0.24
Gauteng	914	104	31	53,183	54,232	1.69
Kwa-Zulu Natal	2,946	838	153	128,019	131,956	2.23
Limpopo	322	65	11	32,385	32,783	0.98
Mpumalanga	1,532	115	12	26,592	28,251	5.42
North West	1,299	116	12	28,251	29,678	4.38
Northern Cape	587	225	21	30,615	31,448	1.87
Western Cape	115	17	10	49,283	49,425	0.23
Grand Total	**9,649**	**1,643**	**296**	**461,146**	**472,734**	**2.04**

3. Utilization rates of instruments within the field



Instrument utilization remains variable over the months, but has increased significantly across all testing facilities, with the exception of 5. Utilization is dependent on requests from various health care facilities that refer samples to the laboratories. Other factors affecting utilization could be attributed to clinical training, staff turnover, implementation of fee for service, number of public holidays, as well as decentralization of stock ordering.

4. Further project phases as defined in the NTCM model

Phase I has been completed and has been reported on in the section above.

Phase IIa involves full capacitation of existing labs: Completed October.

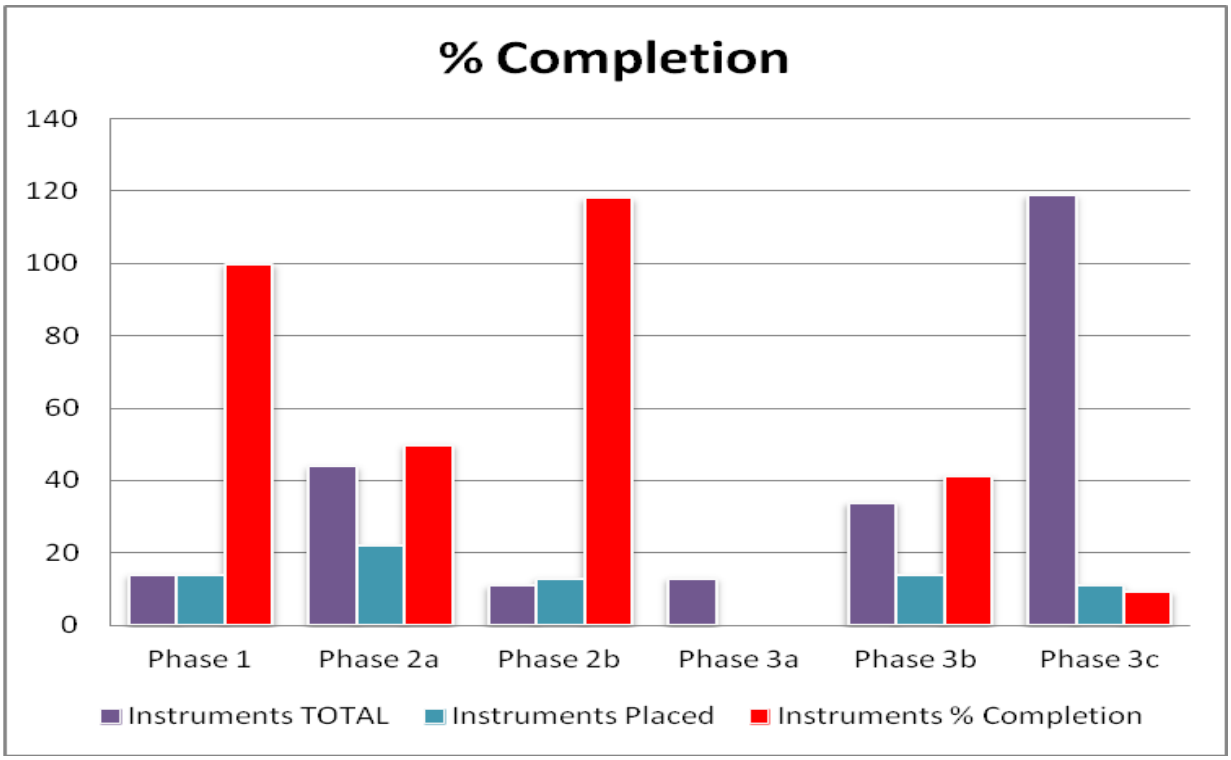
Phase IIb: Full capacitation of high burden districts. In Progress.

Phase IIIa and b: Gates funded study (Gauteng, EC and Free State)

Phase IIIc: ensuring all districts have a minimum of 1 instrument per district

Phase IIId: Completion of all current microscopy and clinic sites

5. Phased Implementation Progress



Phase	GX4	GX16	GX48	TOTAL	Placed	% Completion
Phase 1	20	9	1	30	30	100
Phase 2a	-	14	-	14	14	100
Phase 2b	21	22	1	44	22	50
Phase 3a	1	10	0	11	13	118
Phase 3b	2	11	0	13	0	0
Phase 3c	8	26	0	34	14	41
Phase 3d	38	81	0	119	11	9

To date implementation is 39% complete. On-site deliveries, Installations, instrument verification, training and interfacing of phase 2b instruments and part of phases 3c and d are currently underway. Eleven instruments of varying sizes funded through the URC-USAID were placed at nine sites (refer to table 5). With this placement the Waterberg district will have wide coverage of the GeneXpert

such that every TB suspect in the district has GeneXpert as the first diagnostic test for TB and for rifampicin resistance. Training, verification and LIS interfacing of the instruments are underway.

Coverage will be 51% by the end of August and 56% by the end of September. Additional districts that will have wide coverage include Eden, Sisonke, Mopani, eThekwini, O.R. Tambo, City of Tshwane, City of Johannesburg, Fezile Dadi, Lejweleputswa, Gert Sibande, Siyanda, City of Cape Town, Nelson Mandela Bay Metro, Thabo Mofutsanyane, Ehlanzeni, Dr Kenneth Kaunda, Namakwa and Pixley Kaseme.

Table 5: URC/USAID Sites

Province	District	Lab	Required Instrument	
			GX4	GX16
EC	Amathole	BUTTERWORTH		1
LP	Greater Sekhukhune	JANE FURSE		1
LP	Waterberg	ELLISRAS	1	1
LP	Waterberg	GEORGE MASEBE	1	
LP	Waterberg	THABAZIMBI	1	
LP	Waterberg	WARMBATHS	2	
LP	Waterberg	POTGIETERSUS		1
NW	Bojanala Platinum	RUSTENBURG		1
NW	Ngaka Modiri Molema (Central)	MAFIKENG		1

Table 6: Phase 2b Placements (Global Fund NDOH)

Province	District	Lab	GX4	GX16
EC	O.R. Tambo	ST ELIZABETH		1
EC	O.R. Tambo	ZITULELE		1
GP	City of Johannesburg	CENTRAL TB	1	1
KZN	eThekwini	Dbn Chest Clinic MC		1
KZN	eThekwini	Inanda CMC		1
KZN	eThekwini	KwaDabeka MC		1
KZN	eThekwini	Kwa-Mashu		1
KZN	eThekwini	Mahatma Ghandi		2
KZN	eThekwini	PineTown MC		1
KZN	uMgungundlovu	Edendale		1
KZN	uMgungundlovu	Imbalenhle Clinic MC		1
KZN	Umzinyathi	Church of Scotland Hospital		1
KZN	Zululand	Benedictine		1
LP	Mopani	KGAPANE		1
LP	Mopani	Namakgale		1

MP	Gert Sibande	EMBHULENI		1
NC	Siyanda	UPINGTON		1
NW	Dr Kenneth Kaunda (Southern)	Potchefstroom		1
WC	City of Cape Town	GROOTE SCHUUR CLINICAL PATH		1
WC	Eden	OUDTSHOORN		1
			1	**21**

Table 7: Phase 2b Placements (Global Fund Right to Care)

Province	District	Lab	Instrument		
			GX4	GX16	GX48
EC	Nelson Mandela Bay Metro	PORT ELIZABETH TB	1	3	
EC	Amathole	EAST LONDON TB			1

Phase 3a Progress

Installations, training an instrument verifications using dried culture spots completed.

Table 8: Phase 3b

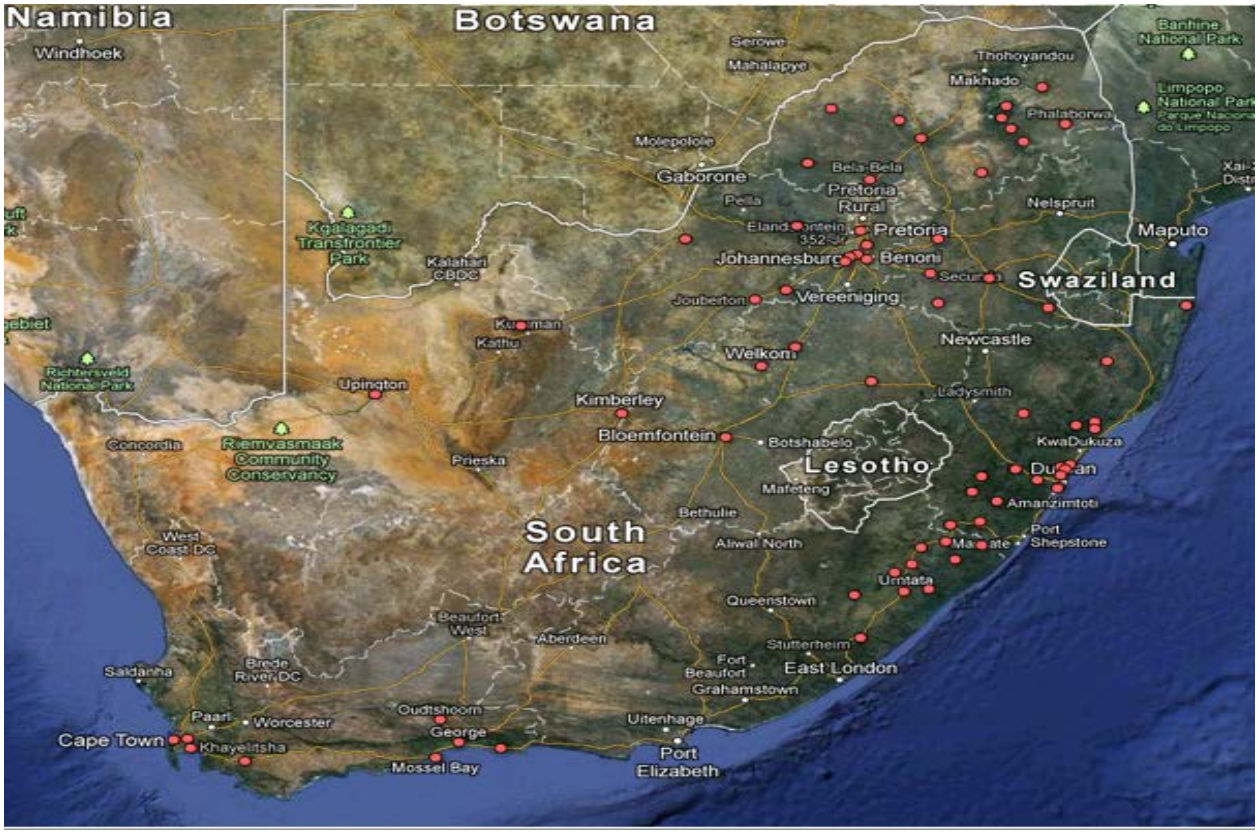
The remaining 10 machines will be placed in September 2012 in the following laboratories:

Province	District	Lab	GX4	GX16	Funding By
EC	Chris Hani	COFIMVABA		1	Gates Control
EC	Chris Hani	QUEENSTOWN	1	1	Gates Control & CDC DOH
EC	Nelson Mandela Bay Metro	UITENHAGE		1	Gates Control
FS	Thabo Mofutsanyane	MANAPO		1	Gates Control
GP	City of Tshwane	JUBILEE		1	Gates Control
GP	City of Tshwane	MAMELODI		1	Gates Control
GP	Ekurhuleni	NATALSPRUIT		1	Gates Control
GP	West Rand	CARLETONVILLE		1	Gates Control
EC	Ukhahlamba	TAYLOR BEQUEST	1	1	Gates Control & CDC DOH
MP	Ehlanzeni	NELSPRUIT		2	Gates Control & CDC DOH

Phase 3c and 3d remain on further release of funding

Pelonomi, Edendale, Christ the King and St. Appolinaris laboratories were fast tracked. This was made possible through a partnership between TB/HIV Care Association who donated two GX4s and PEPFAR CDC (4 GX16 machines).

Figure 1: Current GeneXpert Placement (72 testing centers, 104 analysers, Gx4: 49; Gx16: 54; GX48:1) ***20 clinic placements**



6. Training: Laboratory and Clinical

A total of 119 laboratory staff and 721 health care workers have been trained since December 2011 as summarized in table 7 and 8. This will be an ongoing process to support NDoH training on clinical algorithm. Laboratory staff will receive both clinical and technical training.

Table 9: Laboratory Training

Venue	DATE	Trainer	TOTAL # OF DELEGATES	Outcomes
Christ The King	12 December 11	Veeresh	2	GeneXpert Operation, Maintenance, Troubleshooting and Data Entry
Kokstad	15 December 11	Trevor	2	
St. Apollinaris	13 December 11	Veeresh	3	
Thembisa	15 December 11	Sebaka/Sheila	7	
Pretoria West	10 January 12	Sebaka/Sheila	4	
Mary Theresa	11-12 January 12	Maxine	4	
Mt Ayliff	10 January 12	Trevor	4	

All Saints	11 January 12	Trevor	4	
St Patrick	13 January 12	Maxine	3	
Bethlehem	23 January 12	Trevor	3	
Helen Joseph	23 January 12	Sheila	11	
Witbank	16 January 12	Sheila	10	
Tembisa	20 January 12	Sheila	5	
Tambo Memorial	12 January 12	Sheila/Sebaka	11	
Verulam	03 April 12	Trevor	1	
Osindisweni	11 April 12	Trevor	2	
Rietvlei	11 April 12	Veeresh	3	
Manguzi	19-20 April 12	Veeresh	4	
Letaba	17-18 April 12	Donovan	2	
Pelonomi	17-18 April 12	Trevor	8	
Warmbaths	26 June 12	Fimmie	4	
Mafikeng	20-21 June 12	Donovan	5	
Rustenburg	20 & 25 June 12	Anne	8	
Butterworth	27-28 June	Veeresh	5	
Ellisras	26-27 June	Donovan	3	

Table 10: Clinical Training

Venue	Date	Trainer	Total # of Delegates	Outcomes
Manapo Dept. of Social Services	08 & 09 Feb	Sebaka	28	Background to GeneXpert, TB Testing Algorithm, Recording and Reporting
Siphosensimbi CHC	17 Feb 12	Linda	18	
Phola CHC	20 Feb 12	Linda	8	
eThafeni CHC	23 March 12	Linda	4	
Tembisa Main Clinic	23 March 12	Linda	5	
Vosloorus Poly Clinic	30 March 12	Linda	12	
Dawn Park Clinic	02 April 12	Linda	11	
Mpumalanga District	25 March 12	Elizabeth	40	
Emalahleni Sub-District	12 April 12	Elizabeth	13	
City of Tshwane	19 April 12	Elizabeth	56	

Germiston Clinic	08 May 12	Sylvia	14	
Phola Park CHC	09 May 12	Sylvia	16	
Vosloorus Poly Clinic	10 May 12	Sylvia	16	
Volsloorus Clinic	18 May 12	Sylvia	30	

7. Challenges identified during the course of the project to date

- Finalization of request forms: Incorporate TB testing in the CCMT form if we are to bill using existing channels
- Global shortage of cartridges which has led to delay in rolling out phase 2b

8. Literature Update For GeneXpert

There has been an expansion of the literature with respect to the assay performance. The highlights are summarized in table 11 below:

Table 11: Recent publications (GeneXpert for pulmonary TB and extrapulmonary TB)

Manuscript	Sample population and specimen type (n=…)	Results	
		Sensitivity	Specificity
Taylor N et al, J Clin Micro, 2012	Clinical specimens (paucibacillary): 9 cerebrospinal fluid [CSF], 13 gastric aspirate, 8 tissue, and 17 stool Investigated the utility of flotation procedures (sucrose and NaCl) for concentrating the bacilli before GeneXpert testing	Flotation studies with sucrose or NaCl did not consistently result in lowered cycle thresholds in stool or gastric aspirates, but a cycle reduction of >10 was achieved in two of the three pooled CSF samples. Therefore pre-concentration of CSF samples with sucrose and NaCl may enhance detection of M. tuberculosis by PCR	
Williamson et al, Diagn Micro Infec Dis, 2012	169 specimens tested using the MTB/RIF assay compared to	Found that the assay had 100% sensitivity and specificity for detecting	

	culture as the gold standard to determine false positive Rif calling	M. tuberculosis. However the assay incorrectly assigned rifampicin resistance in 4/13 (31%) of cases	
Peter et al, PloS One, 2012	N=281 HIV-infected hospitalized patients with clinically suspected TB provided on spot urine. Investigated the sensitivity of urine-based methods (Xpert MTB/RIF, LAM strip test and LAM ELISA) in such patients	In sputum-scarce patients, the sensitivity of urine MTB/RIF and LAM ELISA was 40% (95%CI: 22-61) and 60% (95%CI: 39-78), respectively	Urine MTB/RIF specificity was 98%
O'Grady et al, Clin Infec Dis, 2012	N=881 sputums from adult inpatients at a tertiary referral centre in Zambia	Sensitivity of the Xpert® MTB/RIF was 86.1% [80.3-90.4]	Specificity of the Xpert® MTB/RIF assay was 95.0%

9. Update on GeneXpert Research projects:

- Dried Culture Spot (DCS) Verification (n=~500) for Phase IIb of instrument implementation have been manufactured and are being transported with instruments to all sites to ensure the instruments are "fit for purpose" before routine clinical sample testing.
- The following potential EQA materials are being investigated through small pilots (n=10 sites):
    - **i.** DCS EQA panel
    - **ii.** Liquid EQA panel (Vircell)
    - **iii.** Liquid EQA panel from the CDC
    - **iv.** Liquid EQA panel from WHO-
- DCS EQA & verification program development - ACTG (3 sites) and MSF included in program: first batch of verification and pilot EQA material have been shipped to ACTG sites. n=2 site results have been returned

- TBGxMonitor™ (www.tbgxmonitor.com) automated GeneXpert Verification and EQA reporting platform has been upgraded to include full EQA report processing. Both Verification and EQA components have been completed. Next development phase (3) to include EQA qualitative and quantitative evaluation and reporting of sites. Interim upgrade specification (phase 2.5) has been received by the developers and will have be completed by 3 August. Preliminary specifications for phase 3 have been received by the developers.
- Alternative specimen preparation protocols:
    i. Protocols being developed for Pediatric TB diagnosis and Extra-pulmonary TB diagnosis
        1. Paediatric study at Rahimma Moosa Mother and Child Hospital: n=400 TB suspects have been recruited to the study for comparison of Xpert MTB/RIF assay to smear and culture on paed specimens. Awaiting culture results for comparison.
        2. Protocols under development for EPTB: A GeneXpert room has been refurbished at the Braamfontein TB referral lab for the study. A laboratory technician has been recruited and trained. The R&D GeneXpert has been placed for study commencement.
    ii. Sputum heat inactivation: to determine whether heat inactivation can be used prior to Xpert testing to render it safe for further manipulation (n=121) – study will continue.
    iii. Protocol under development to test residual SR buffered Xpert specimens on the line probe assay for DST resulting – study ongoing.
- Connectivity: Collaboration with Cepheid ongoing
    i. Remote connectivity – System deployed at 37 sites by Cepheid (newly placed instruments) with 6 sites reporting live on the system. More than 5,000 results reported to date. Cepheid pre-install the system on the instruments before delivery to sites. NHLS IT to install on all existing sites (remotely).

ii. Remote Calibration – Second pilot study currently initiated with the recommendations and feedback received from phase 1 of the pilot. Number of sites and cartridges to be confirmed.

10. HIV/TB Integration

- Grand Challenges Canada project: Multiple POC HIV/TB integration feasibility project
  - Phase I complete
  - Phase II: Evaluation of nurse operated POC versus routine lab completed at HJH Themba Lethu clinic (n=326) complete.
  - Site visits completed (n=12) and selection of first site (Grace Mokgomo, North West Province) for randomized controlled trial (RCT) has been finalized and staff trained.
  - RCT: The study site has been initiated and ~n=20 patients recruited into the study; n=10 randomized to standard of care and n=10 randomized to POC arm.
  - A second study site, Botshabelo will be initiated mid-august and the 3rd site in September
  - A sub-study to investigate feasibility and patient acceptance of multiple finger sticks for POC testing has been completed at Tshwane District Hospital (n=300). Results are being analysed. An abstract has been submitted to ASLM, Cape Town 2012.
- Connectivity:
  - Conworx (POCcelerator) and LDS (AegisPOC) to be trialed in 2 sites during RCT
  - HemoCue project at CMJAH – network installation for the Hemocue's has been completed. Awaiting installation of offline version of TrakCare by NHLS.

11. Grants Submitted

None

12. Funding

Table 12: Total and Percentage Contribution to date by Donor

Donor	Amount	% Contribution
NDoH	47,000,000	36.13
Bill & Melinda Gates Foundation	14,071,620	10.82
TB Reach	2,783,128	2.14
MSF	1,758,200	1.35
FIND	870,000	0.67
USAID	4,785,580	3.68
CDC NHLS 2010/11	28,886,600	22.20
CDC NDoH	1,400,000	1.08
CDC NHLS 2011/12	2,710,000	2.08
Dr. Niebauer	390,000	0.30
Gobal Fund NDOH	20,000,000	15.37
Global Fund RTC	5,442,000	4.18
Subtotal	**130,097,128**	**100**

CDC has contributed 25, 36% towards the program to date.

13. Recent Campaigns

NHLS together with the National Department of Health (HIV and AIDS and STIs Chief Directorate), as well as other key Government Departments and Partners participated in the HCT campaigns in support of the deputy minister in Qwa-Qwa stadium on 10th of May and Pimville, Soweto on 13th of May 2012. The NPP GeneXpert team, with the generous assistance of Cepheid SA, managed to install two GeneXpert 16 instruments at each site for rapid detection of MTBC and Rifampicin. Forty patients were tested for MTBC in Qwa-Qwa and 33 in Pimville. Results were released to patients on the day.

Another campaign was held in Brits on the 4th of July 2012. The National Priority Programme CD4 team, with the generous assistance of Beckman Coulter, managed to secure a local mobile unit into which one XL flow cytometers and three GX16 instruments were housed. The instruments were successfully installed, validated and verified for accuracy on the day preceding events, with confirmatory quality control measures passed on the day of testing. In total, 61 patients were tested for an absolute CD4 count and 18 for TB using the GeneXpert. Test results were released to local coordinators for follow up of patients.