NATIONAL HEALTH LABORATORY SERVICE

GeneXpert MTB/RIF

Progress Report

August 2012

NATIONAL HEALTH LABORATORY SERVICE

Table of Contents

Background to project	3
Assays performed to date	3
Utilization of instruments within the field	6
Further project phases as defined in the NTCM model	6
Specific GeneXpert Site Progress	7
Training: Laboratory and Clinical	10
Challenges identified during the course of the project to date	12
Literature Update	12
Update on Research Projects	13
TB/HIV Integration	15
Grants Submitted	15
Funding	16
Recent Campaigns	16

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 569,776 specimens have been processed to date (31 August 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).

NATIONAL HEALTH LABORATORY SERVICE

Table 1: GeneXpert MTB Results by province

Province	MTB Detected	MTB Not Detected	Test Unsuccessful	Total	% MTB Detected
Eastern Cape	10,971	56,412	2,299	69,682	15.74
Free State	9,892	60,661	174	70,727	13.99
Gauteng	8,623	58,991	1,542	69,156	12.47
Kwa-Zulu Natal	28,208	128,910	4,853	161,971	17.42
Limpopo	3,835	32,097	463	36,395	10.54
Mpumalanga	5,045	26,618	1,838	33,501	15.06
North West	4,712	24,888	1,546	31,146	15.13
Northern Cape	5,666	29,734	837	36,237	15.64
Western Cape	9,938	50,813	210	60,961	16.30
Total	**86,890**	**469,124**	**13,762**	**569,776**	**15.25**

Table 2: Provincial GeneXpert RIF Results in MTB detected cases

Province	Inconclusive	Resistant	Sensitive	NO Rif Result	Grand Total	% RIF Resistant
Eastern Cape	143	783	9,912	133	10,971	7.14
Free State	123	553	9,191	25	9,892	5.59
Gauteng	88	568	7,957	10	8,623	6.59
Kwa-Zulu Natal	399	2,294	25,116	399	28,208	8.13
Limpopo	60	291	3,452	32	3,835	7.59
Mpumalanga	73	436	4,456	80	5,045	8.64
North West	60	386	4,258	8	4,712	8.19
Northern Cape	72	348	5,217	29	5,666	6.14
Western Cape	106	486	9,342	4	9,938	4.89
Grand Total	**1,124**	**6,145**	**78,901**	**720**	**86,890**	**7.07**

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 as this leads to significant confusion in all aspects of the testing cycle, as well as in some cases being more onerous to TB suspects.

Table 3: Rif Concordance by LPA or DST

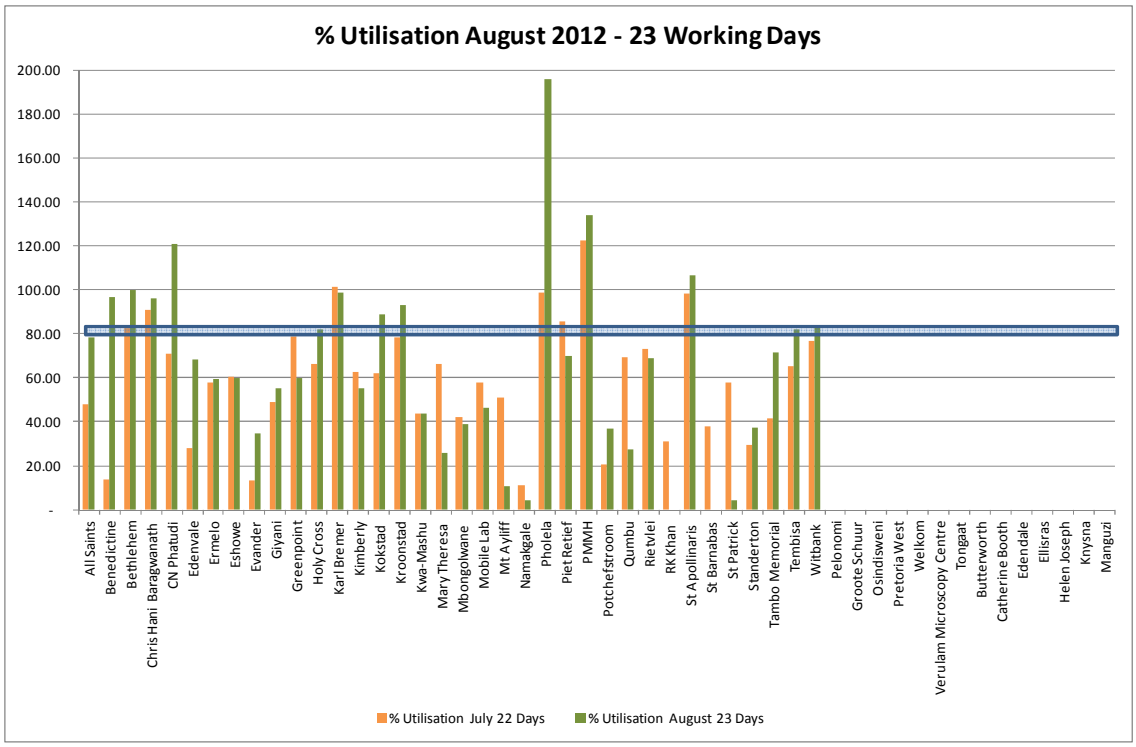
Province	LPA	DST
Eastern Cape	93.3%	12.5%
Free State	83.3%	75.0%
Gauteng	92.3%	88.2%
Kwazulu-Natal	82.2%	93.3%
Limpopo	80.0%	94.4%
Mpumalanga	81.0%	97.2%
North West	100.0%	50.0%
Northern Cape	76.2%	66.7%
Western Cape	95.9%	100.0%
National	**87.2%**	**89.7%**

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	ERR	INV	NORES	MTB Result	Total	% Error Total
Eastern Cape	2,074	160	65	67,383	69,682	2.98
Free State	152	16	6	70,553	70,727	0.21
Gauteng	1,336	162	44	67,614	69,156	1.93
Kwa-Zulu Natal	3,741	922	190	157,118	161,971	2.31
Limpopo	379	73	11	35,932	36,395	1.04
Mpumalanga	1,703	121	14	31,663	33,501	5.08
North West	1,410	121	15	29,600	31,146	4.53
Northern Cape	588	226	23	35,400	36,237	1.62
Western Cape	179	18	13	60,751	60,961	0.29
Total	**11,562**	**1,819**	**381**	**556,014**	**569,776**	**2.02**

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 few sites which were affected by the global shortage of cartridges. 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.

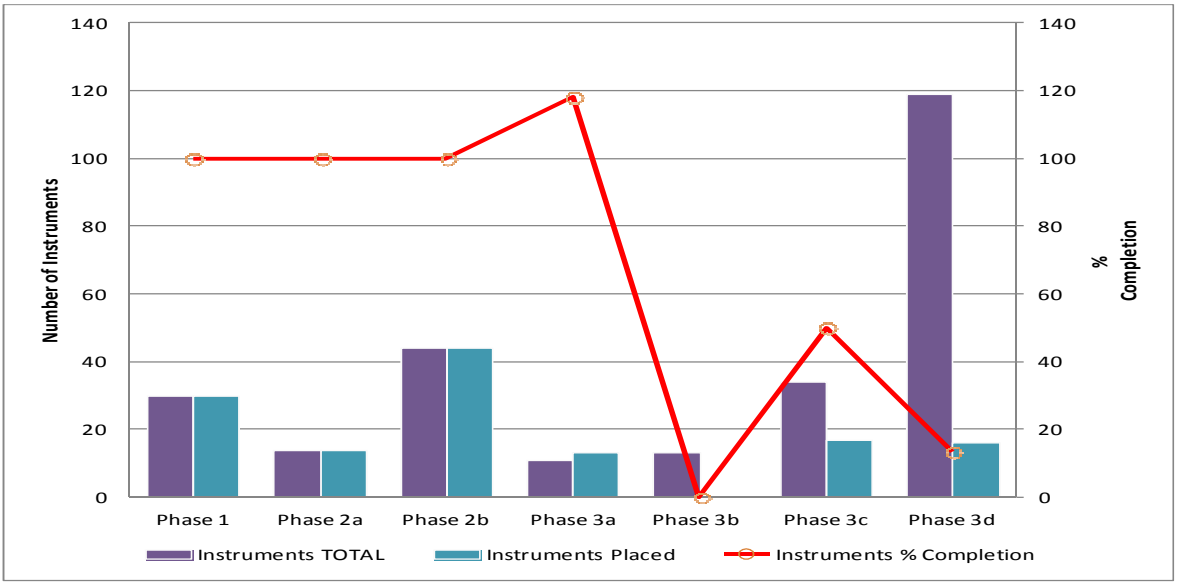
Phase IIb: Full capacitation of high burden districts. In Progress.

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	44	100
Phase 3a	1	10	0	11	13	100
Phase 3b	2	11	0	13	0	0
Phase 3c	8	26	0	34	17	50
Phase 3d	38	81	0	119	16	13
TOTAL	**90**	**173**	**2**	**265**	**134**	**51**

To date implementation is 51% complete. Installations, instrument verification, training and interfacing of phase 2b instruments and part of phases 3c and 3d 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 56% by the end of October. 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

Table 8: Phase 3b

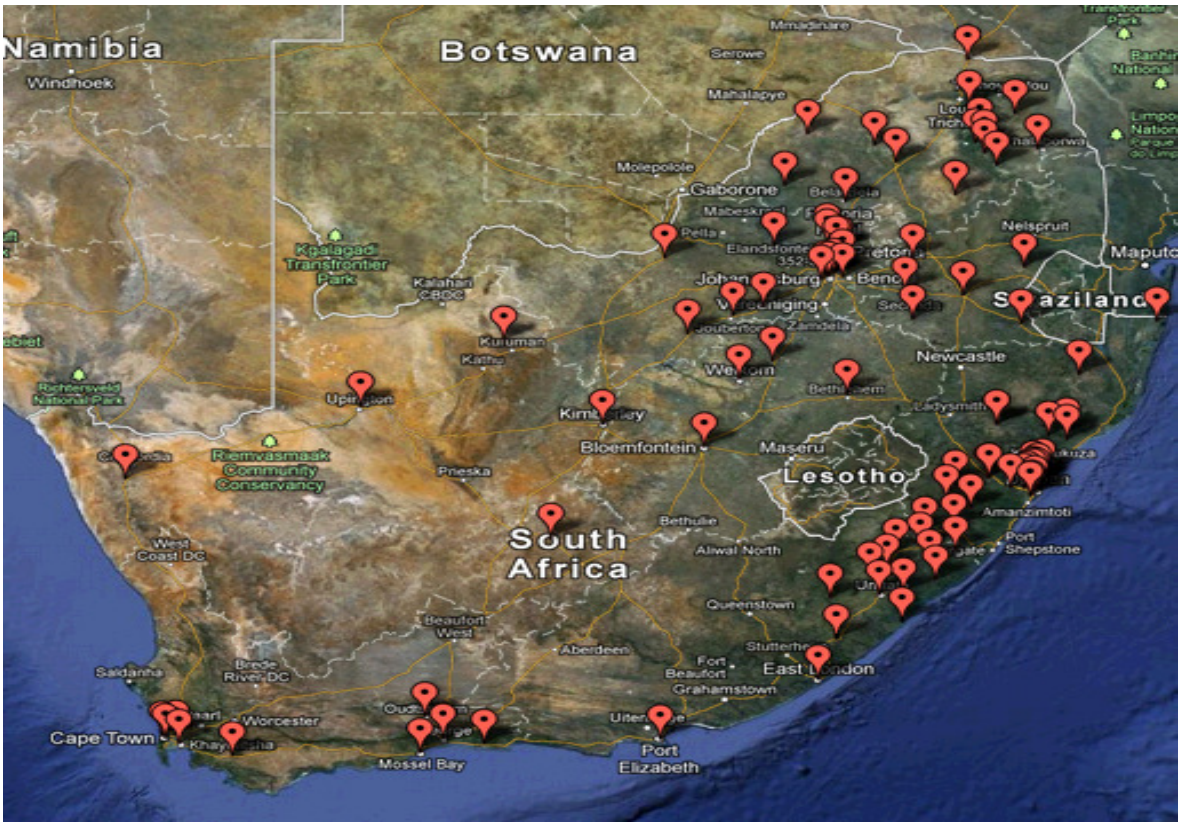
The remaining 10 machines will be placed in October 2012 in the following laboratories:

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	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 (94 testing centers, 134 analysers, Gx4: 59; Gx16: 73; GX48:2) ***20 clinic placements**



6. Training: Laboratory and Clinical

A total of 227 laboratory staff and 1421 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.

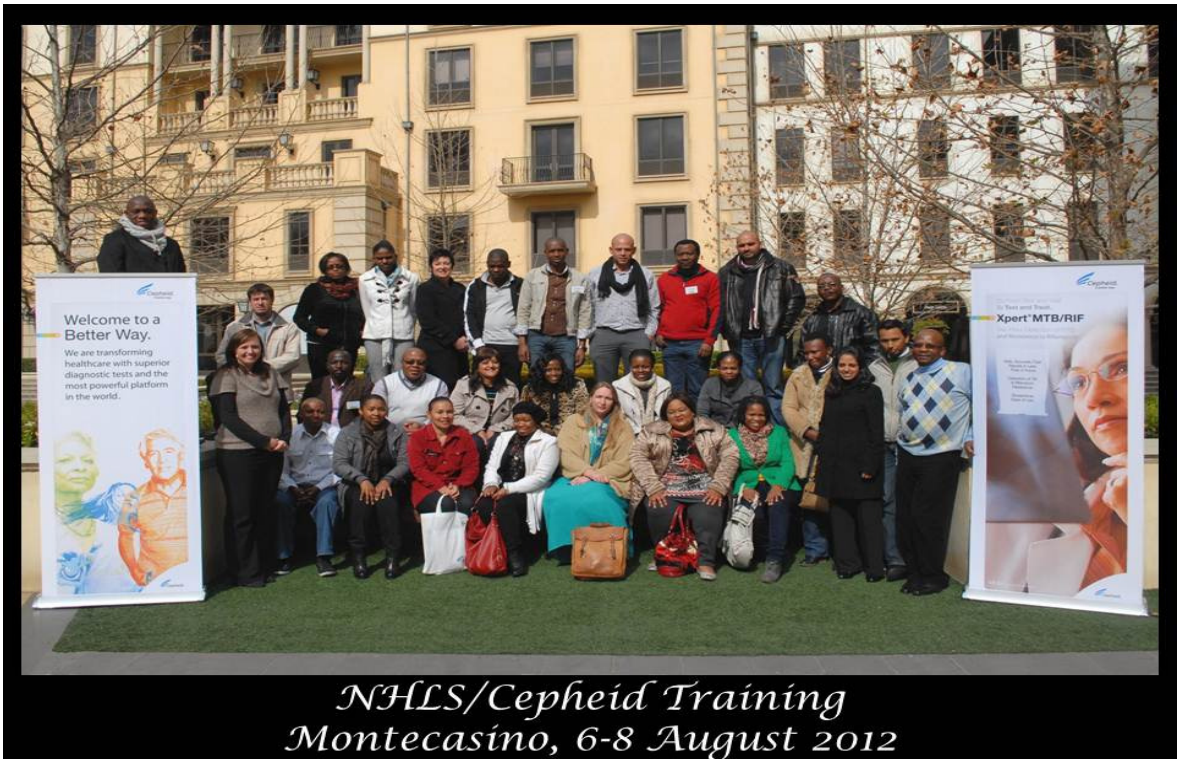
In addition the GeneXpert train the trainer course was held from 06-08 August 2012 at the Pivot, Montecasino. The training was designed for individuals who are technically involved in carrying out the Xpert MTB/Rif assay. The trained individuals are to be available thereafter to train existing or newly implemented laboratories in their regions. The training was collaboration between the NHLS and Cepheid SA. A total of 27 participants from each region attended the training.

Training covered the following areas:

Day 1: Cepheid presentation, complaint handling, service and Calibration handling, GX technology, Real-time PCR, installation precaution and of installation.

Day 2: Software 4.3 features, Maintenance, Xpert MTB/RIF assay, how to solve a problem with GeneXpert, Xpert MTB RIF sampling, Xpert MTB/RIF assay lab work/What information to provide to Lab technicians.

Day 3: Focus on Xpert MTB/RIF algorithm / focus on troubleshooting / quiz, correction / general discussion.



Back: Sebaka Molapo (NPP), Donovan Miller (Cepheid), Mankwana Titus (Mafikeng), Wilhemina Mbodi (TAD), Anna Bench (George), Nkateko Shilenge (Letaba) ,Samuel Mohlahlo (Ermelo) , Floyd Olsen (NPP), Martin Xaba (KZN), Kavish Maharaj (NPP), Sello Matsho (Tembisa)

Middle: Justice Mzimela(Prince Mshiyeni), Kabelo Finger (Pelonomi), Lata Bhagoobhai (Baragwanath), Ruth Kwachenera (NPP), Lethabo Theko (Rustenburg), Morifi Matlou (Kgapane), Lerato Kekana (TAD), Igsaan Noordien (Western Cape),

Front: Fimmie Steyn (Cepheid), Sydwell Mvo (Umtata), Puleng Marokane (NPP), Nuraan Paulse (Kimberley), Sylvia Ntsimane (NPP), Caren Pretorius (Thabazimbi), Sipokazi Jizana (Eastern Cape), Boitumelo Maitshotlo (Region F), Indira Soundiram (Cepheid), Vincent Africa Cepheid

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
Balcells 2012, Int J Tuberc Lung Dis	N=166 respiratory specimens in 5 hospital settings in Chile. Compared smear microscopy to Xpert	Diagnostic sensitivity increased from 66.7% (95%CI 39.1-86.2) for acid-fast smear to 91.7% (95%CI 64.6-98.5) for Xpert MTB/RIF	Specificity: Smear = 98.6% Xpert = 99.3%
Dorman et al, 2012, PloS One	6893 sputum specimens Aim was to determine performance of Xpert and cost.	Xpert = 62.6% (95% confidence interval [CI] 55.2, 69.5)	99.6%
		For the testing scenario of 7000 specimens with 2.7% of specimens culture positive for MTB, costs were $165,690 for Xpert and $115,360 for the package of microscopy plus culture	
Zar et al, 2012 Clin Inf Dis	N=535 children Of which 396 had Paired specimens (Nasopharyngeal aspirates (NPA) and induced sputums (IS)) were tested using smear, liquid culture and Xpert	The sensitivity of two Xpert tests was similar for IS and NPAs [(45/63) 71% vs (41/63) 65%, P = .444)	Xpert specificity was 99.1% (98.1-100) for IS and 98.2% (96.8-99.6)

			for NPAs.
Ntinginya et al, 2012, Int J Tuber Lung Dis	Active case-finding strategy, using two spot sputum samples collected within a 1-hour interval from household contacts of smear-positive TB index cases n= 219 enrolled contacts	Xpert = 100% Smear = 60%	Not stated
Alvarez-Uria et al, 2012, Tuberc Resp Treat	Compared the performance of light-emitting diode (LED) auramine fluorescent microscopy and the Xpert assay	Xpert outperformed LED fluorescent microscopy in all type of specimens, especially in cerebrospinal fluid where the number of positive results was increased 11 times.	
Carriquiry G et al, 2012, Plos One	N=131 HIV-positive patients with high clinical suspicion of TB were enrolled from two tertiary hospitals in Lima, Peru	Sensitivity of MTB/RIF = 97.8% (95% CI 88.4-99.6) (44/45)	specificity was 97.7%
Barnard et al, 2012, J Clin Micro	Evaluated the diagnostic performance of the Xpert®MTB/RIF and Genotype® MTBDRplus (version 2) assays on n= 282 smear-positive and smear-negative patient specimens submitted to a high throughput diagnostic laboratory.	Sensitivity of the Genotype® MTBDRplus (v2.0) = 73.1% Xpert®MTB/RIF assay = 71.2%	100% for both assays

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. Rwanda to receive both EQA and Verification Material to aid in their initial setup.
- 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. Phase 2.5 upgrade has been completed with minor additions to the system. The next major upgrade (phase 3) scope has been finalized and development is commencing. Phase 3 includes EQA qualitative and quantitative evaluation and reporting of sites. Phase 4 scope of work has been generated. Awaiting finalization of specification.
- Alternative specimen preparation protocols:
    i. Protocols being developed for Extra-pulmonary TB diagnosis
        1. 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. The study commenced in the last week of August, investigating 0.5ml of un-centrifuged or concentrated EPTB specimens. The activity is ongoing.
    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 on 68 sites by Cepheid and the NHLS (. More than 83,000 results reported to date. Cepheid pre-install the

system on the instruments before delivery to sites. NHLS have been responsible for installing (remotely) on existing sites.

ii. Remote Calibration – Second pilot study completed at 6 sites with Cepheid. Feedback from the users to Cepheid has been received and document. Expected launch of the remote calibration product (barring any issues) is December 2012.

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=109 patients recruited into the study; n=52 randomized to standard of care and n=57 randomized to POC arm.
  - A second study site, Botshabelo has been initiated mid-august and the 3rd site is expected to be launched by the end of 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. AegisPOC is currently being tested and instruments pre-configured before delivery to the site.

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.