NATIONAL HEALTH LABORATORY SERVICE

GeneXpert MTB/RIF

Progress Report

April 2013

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 which stated 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. 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 6 below.

2. Assays performed to date

In summary, a total of 1,301,517 specimens have been processed to date (30 April 2013). In April 119,559 specimens were processed. The total % of *Mycobacterium tuberculosis* complex (MTBC) detected in this cohort was 11.96% (14,298). The percentage positivity has remained on average between 14 -16% country-wide. To date Kwa-Zulu Natal (KZN) has performed the greatest number of tests which is probably as a result of the number of instruments placed (refer to tables 1 & 2).

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Average Rifampicin resistance detection rates have remained around 7% since project inception (Refer to tables 3 & 4).

Table 1 GeneXpert MTB Results by province (01-30 April 2013)

Province	MTB Detected	MTB Not Detected	Test Unsuccessful	Total	% MTB Detected
Eastern Cape	3313	22230	827	**26370**	**12.56**
Free State	1110	9953	76	**11139**	**9.96**
Gauteng	2019	14772	672	**17463**	**11.56**
Kwa-Zulu Natal	2513	17679	927	**21119**	**11.90**
Limpopo	1019	11346	517	**12882**	**7.91**
Mpumalanga	512	3026	128	**3666**	**13.97**
North West	857	6308	324	**7489**	**11.44**
Northern Cape	614	4139	209	**4962**	**12.37**
Western Cape	2341	11819	309	**14469**	**16.18**
Total	**14 298**	**101 272**	**3 989**	**119 559**	**11.96**

Table 2: GeneXpert MTB Results by province (cumulative)

Province	MTB Detected	MTB Not Detected	Test Unsuccessful	Total	% MTB Detected
Eastern Cape	31 205	169 733	5 592	**206 530**	**15.11**
Free State	18 945	127 756	600	**147 301**	**12.86**
Gauteng	21 205	140 172	4 713	**166 090**	**12.77**
Kwa-Zulu Natal	50 661	265 305	12 257	**328 223**	**15.43**
Limpopo	10 156	88 248	2 642	**101 046**	**10.05**
Mpumalanga	8 596	45 690	2 764	**57 050**	**15.07**
North West	11 988	66 784	3 755	**82 527**	**14.53**
Northern Cape	9 886	55 103	2 725	**67 714**	**14.60**
Western Cape	23 681	119 817	1 538	**145 036**	**16.33**
Total	**186 323**	**1 078 608**	**36 586**	**1 301 517**	**14.32**

Table 3: Provincial GeneXpert RIF Results in MTB detected cases (01-30 April 2013)

Province	Inconclusive	Resistant	Sensitive	No Rif Result	Total	% RIF Resistant
Eastern Cape	39	232	3 039	3	**3 313**	**7.00**
Free State	28	64	1 018		**1 110**	**5.77**
Gauteng	32	147	1 830	10	**2 019**	**7.28**
Kwa-Zulu Natal	56	208	2 201	48	**2 513**	**8.28**
Limpopo	11	50	947	11	**1 019**	**4.91**
Mpumalanga	9	60	440	3	**512**	**11.72**
North West	13	42	802		**857**	**4.90**
Northern Cape	4	28	447	135	**614**	**4.56**
Western Cape	49	86	2 206		**2 341**	**3.67**
Total	**241**	**917**	**12 930**	**210**	**14 298**	**6.41**

Table 4: Provincial GeneXpert RIF Results in MTB detected cases (cumulative)

Province	Inconclusive	Resistant	Sensitive	No Rif Result	Total	% RIF Resistant
Eastern Cape	448	2 199	28 283	275	**31 205**	**7.05**
Free State	290	1 128	17 494	33	**18 945**	**5.95**
Gauteng	278	1 416	19 422	89	**21 205**	**6.68**
Kwa-Zulu Natal	803	4 245	45 095	518	**50 661**	**8.38**
Limpopo	145	690	9 192	129	**10 156**	**6.79**
Mpumalanga	125	855	7 524	92	**8 596**	**9.95**
North West	163	902	10 877	46	**11 988**	**7.52**
Northern Cape	134	594	8 861	297	**9 886**	**6.01**
Western Cape	303	1 169	22 206	3	**23 681**	**4.94**
Total	**2 689**	**13 198**	**168 954**	**1 482**	**186 323**	**7.08**

3. Rif Condordance

Rifampicin concordance is good for both LPA and culture. There is significant regional variation in Rifampicin mono-resistance. The national average is 12% for DST and 17% for LPA. This could be attributed to a number of factors such as geographical variation, laboratory variation, 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 the TB patients themselves.

Table 5: Rif Concordance by LPA or DST

Province	Rif Resistant Cases	GeneXpert Confirmation & Rif Concordance									
		DST					LPA				
		Confirmed		Rif Concordance		Pre-analytical	Confirmed		Rif Concordance		Inderterminate
		#	%	#	%		#	%	#	%	
Eastern Cape	1153	47	4.1%	10	21.3%	0	46	4%	45	97.8%	1
Free State	724	15	2.1%	7	46.7%	11	79	11%	64	81.0%	14
Gauteng	895	21	2.3%	16	76.2%	21	90	10%	84	93.3%	2
Kwazulu-Natal	2726	686	25.2%	652	95.0%	0	631	23%	509	80.7%	28
Limpopo	380	28	7.4%	27	96.4%	1	44	12%	39	88.6%	0
Mpumalanga	514	81	15.8%	78	96.3%	1	131	25%	111	84.7%	2
North West	435	8	1.8%	7	87.5%	2	50	11%	47	94.0%	6
Northern Cape	343	24	7.0%	17	70.8%	8	55	16%	47	85.5%	8
Western Cape	782	1	0.1%	0	0.0%	3	235	30%	234	99.6%	0
National	7 952	911	11.5%	814	89.4%	47	1 361	17%	1 180	86.7%	61

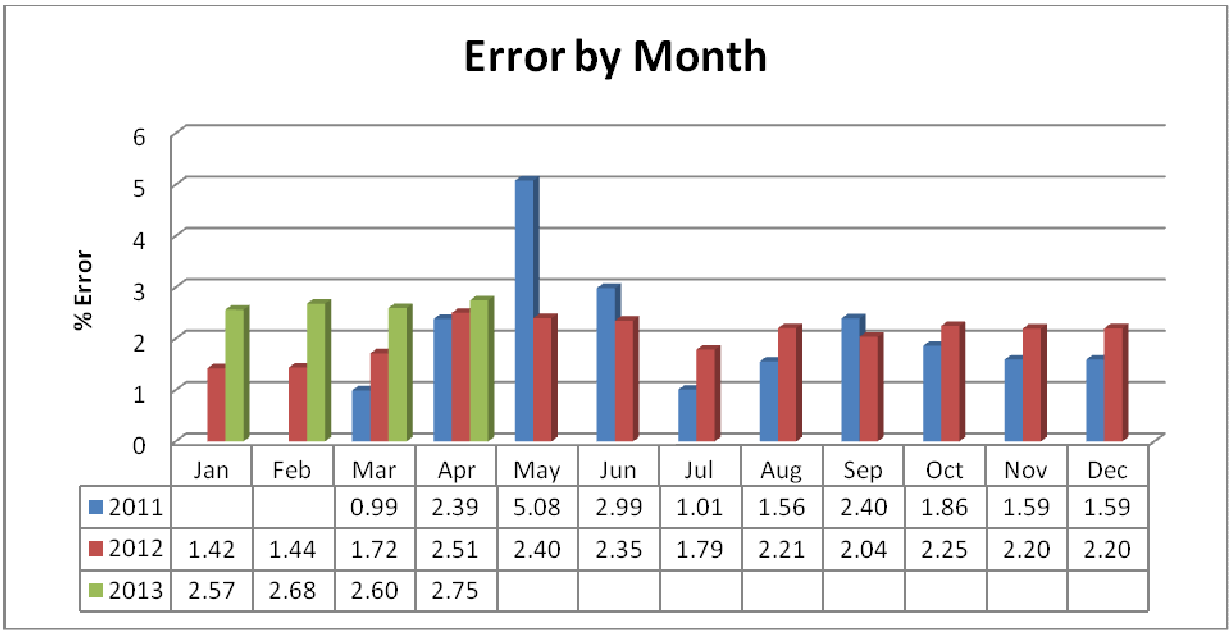
4. Errors

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 6: Number of Unsuccessful Tests and Reasons

Province	Error	Invalid	No Result	No Raw Result	MTB Result	Total	% Error
Eastern Cape	4 755	472	346	19	200 938	**206 530**	**2.30**
Free State	491	56	42	11	146 701	**147 301**	**0.33**
Gauteng	4 126	420	165	2	161 377	**166 090**	**2.48**
Kwa-Zulu Natal	9 723	1 562	968	4	315 966	**328 223**	**2.96**
Limpopo	2 238	322	81	1	98 404	**101 046**	**2.21**
Mpumalanga	2 542	184	36	2	54 286	**57 050**	**4.46**
North West	3 395	257	103		78 772	**82 527**	**4.11**
Northern Cape	970	279	45	1 431	64 989	**67 714**	**1.43**
Western Cape	1 397	113	28		143 498	**145 036**	**0.96**
Total	**29 637**	**3 665**	**1 814**	**1 470**	**1 264 931**	**1 301 517**	**2.28**

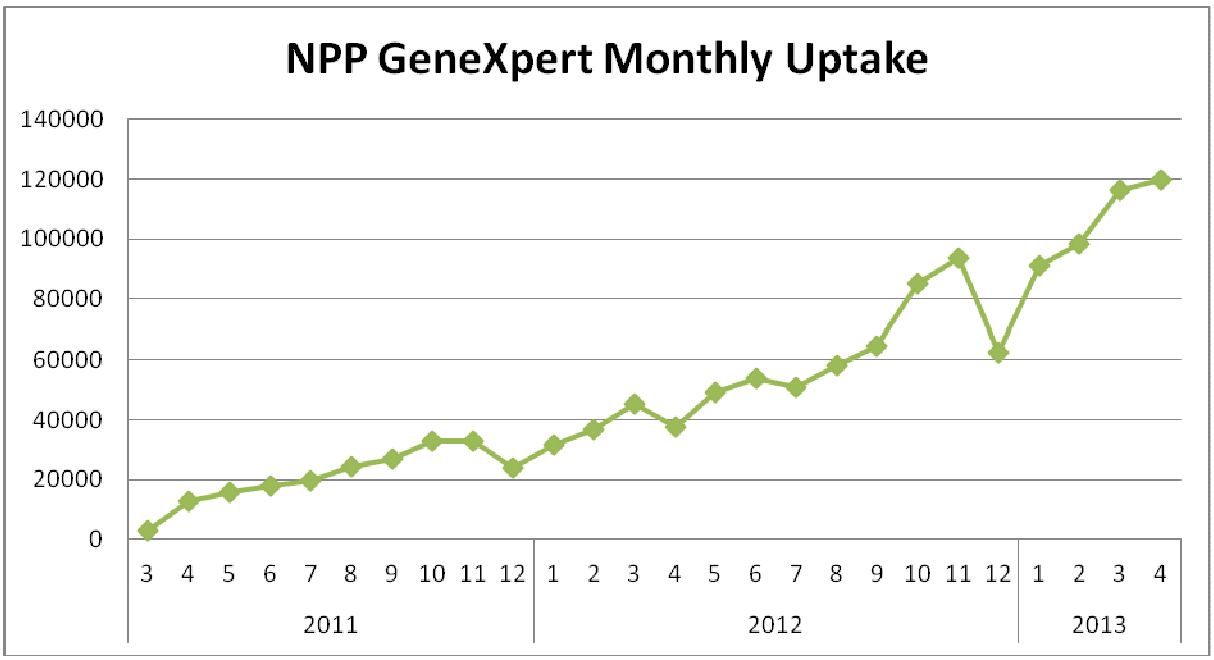
Figure 1: GeneXpert Error by Month



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2011			0.99	2.39	5.08	2.99	1.01	1.56	2.40	1.86	1.59	1.59
2012	1.42	1.44	1.72	2.51	2.40	2.35	1.79	2.21	2.04	2.25	2.20	2.20
2013	2.57	2.68	2.60	2.75								

5. Monthly uptake since implementation started

Figure 2: GeneXpert Monthly Uptake



Monthly uptake increased steadily since program inception. The main reason for interruptions is due to the variation in work practices which is expected during the December period. In addition, there was a global shortage in the supply of Xpert MTB/RIF® cartridges in the months of July, October and November 2012. This was resolved in December 2012. Another shortage was experienced in March. The stock supply was stabilized in April. In addition Cepheid re-introduced the supply of 50 kit cartridges to high volume sites.

6. 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

Phase IIb: Full capacitation of high burden districts. Completed

Phase IIIa and b: Gates funded study (Gauteng, EC and Free State). Phase 3a Completed

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

Phase IIId: Completion of all current microscopy and clinic sites: In Progress

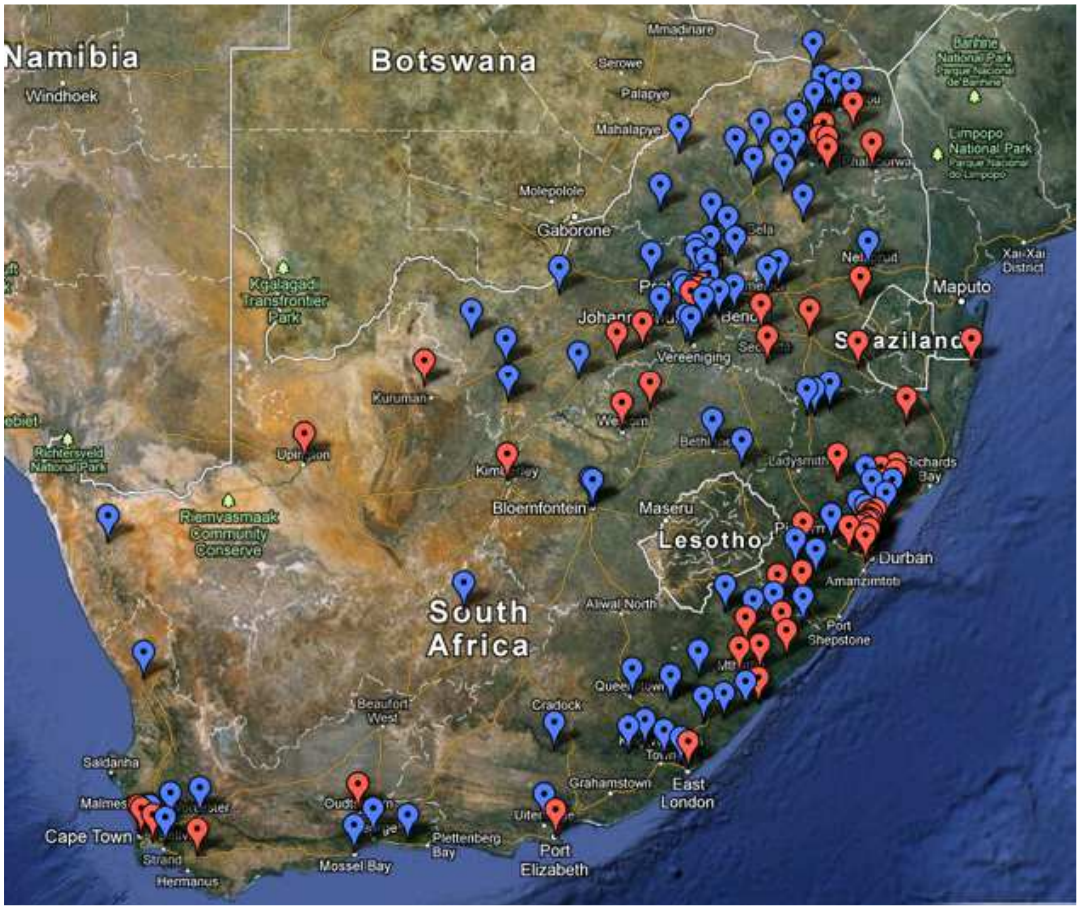
7. Phased Implementation Progress

Table 7: Phased Implementation Progress

Phase	GX4	GX16	GX48	TOTAL	Placed	% Completion
Phase 1/2a	7	30	1	38	38	100
Phase 2b	22	23	1	46	46	100
Phase 3a	3	10	0	13	13	100
Phase 3b	2	11	0	13	13	100
Phase 3c	6	28	0	34	30	88
Phase 3d	41	83	0	124	63	51
TOTAL	**81**	**185**	**2**	**268**	**203**	**76**

To date implementation is 76% complete.

Figure 3: Current GeneXpert Placement (142 testing centers, 203 analysers, Gx4: 65; Gx16: 136; GX48:2) ***20 clinic placements**



8. Training: Laboratory and Clinical

A total of 664 laboratory staff and 2,159 health care workers have been trained since December 2011. This will be an ongoing process to support NDoH training on clinical algorithm. Laboratory staff received both clinical and technical training.

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

- Delay in training health care workers, especially doctors whose availability is limited, on clinical algorithm: is being addressed
- Global shortage of GXP cartridges: addressed
- Rollout of EGK to avoid duplications

- Laboratories using GXP for monitoring treatment (and not just diagnosis): is being addressed through training

10. 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 8: Recent publications (GeneXpert for pulmonary TB and extrapulmonary TB)

Manuscript	Sample population and specimen type (n=…)	Results	
		Sensitivity	Specificity
Kayigire et al 2013, J Clin Micro	Groups of 15 patients were treated with 6 different antituberculosis agents or regimens. Patients collected sputum for 16 h overnight at baseline and at days 7 and 14 after treatment initiation to determine the value of a quantitative PCR assay for early bactericidal activity determination	The best discrimination between group effects was found with culture time to positivity at day 7 and day 14 ($F = 9.012$, $P < 0.0001$, and $F = 11.580$, $P < 0.0001$), followed by log CFU ($F = 4.135$, $P = 0.0024$, and $F = 7.277$, $P < 0.0001$). CT was not significantly discriminative	
Deggim et al, 2013, J Clin Micro (abstract only)	The performance of the Xpert MTB/RIF assay as a primary screening test for urgent clinical specimens was evaluated during a two-year period.	The results show that replacing smear microscopy with the Xpert MTB/RIF assay facilitates laboratory handling and improves sensitivity and specificity of Mycobacterium tuberculosis detection	
Lee et al, 2013, Int J Tuber Lung Dis	N=132 patients suspected PTB for whom the Xpert MTB/RIF assay was performed on bronchoscopy specimens records were retrospectively reviewed the records of patients with	The sensitivity of the Xpert assay using bronchial washing or bronchoalveolar lavage (BAL) fluid for the diagnosis of PTB was 81.6%	Specificity was 100%
		The PPV and NPV were 100% and 92.1%, respectively	

Van Rie et al, 2013, Eur J Clin Microbiol Infect Dis.	N=344 adult Fine Needle Aspirates	The sensitivity was high [93.3 %, 95 % confidence interval (CI) 87.6-96.6] and increased with decreasing CD4 count (from 87.0 % for CD4 >250 to 98.6 % for CD4 <100 cells/mm^3)	The specificity of a single Xpert was suboptimal at 88.2 %
		All Xpert-positive patients initiated treatment within one day, compared to 70 % of culture-positive but Xpert-negative and 13 % of culture- and Xpert-negative but cytology-positive patients	
Feasey et al, 2013, J Clin Micro	104 blood samples from HIV infected patients	Xpert showed a sensitivity of 21% in blood samples	specificity of 100%
Banada et al, 2013, J Clin Micro	One through 20 ml of blood were spiked with 0.25 to 10 colony forming units (CFU)/ml of the MTB surrogate M. bovis BCG. Multiple replicates of each sample were processed by a new lysis-centrifugation method, and tested with the Xpert® MTB/RIF assay	In the 20 ml samples, BCG was detected in blood spiked with 10, 5, 1, and 0.25 CFU/ml 100%, 100%, 83%, and 57% of the time, respectively, compared to 100%, 66%, 18% and 18%, respectively in 1 ml blood samples	

11. Update on GeneXpert Research projects:

- ~600 Dried Culture Spot (DCS) for verification of GeneXperts are being rolled out for quarter 2 of implementation.
    - **i.** A manuscript is in progress
- DCS for EQA program: EQA panels have been prepared for =144 NHLS sites (64 Gx4; 133 Gx16; 2 Gx48). These have been shipped out. Results are pending.
- DCS for ACTG sites: EQA panels are ready for shipment to n=23 international ACTG sites (27Gx4; 2Gx16; 1Gx80). This includes all three EQA rounds for the whole year.

- The following potential EQA materials where investigated through a pilot, feasibility study (n=11 sites):
    - **i.** DCS EQA panel
    - **ii.** Lypohilised EQA panel (VIRCELL™)
    - **iii.** Dried Tube Spot EQA panel from the CDC
    - **iv.** Simulated sputum EQA panel from WHO-GLI
    - **v.** Liquid panel from Maine Molecular Diagnostics (MMQCI™)

  The results were presented at the 5th GLI meeting in France and summarized below:

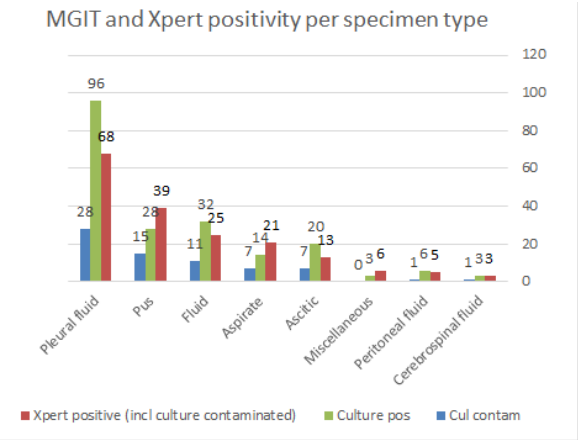
- MMQCI panel, which was the only panel that required cold storage which contributed to a lower score.
- All panels where received in good condition and therefore good for shipping across distances, and all showed compatibility with the Xpert testing process.
- No panel caused any PCR inhibition.
- Matrix requirement (liquid or dry) did not appear to be a distinguishing criterion as had reduced scores on: insufficient volumes; need for extra consumables; ability to transfer to the Xpert cartridge.
- Minimal variation in probe Ct may be more attractive for monitoring RIF call rates using differences in probe drop out or probe delayed hybridisation.

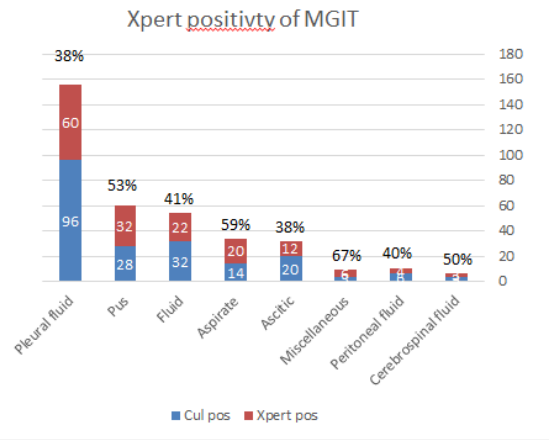
  Factors such as SOP clarity, label bar-code scanning, and use of the web based program highlight the need for any EQA program to be accompanied by training and ongoing improvements.

- 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. The next major upgrade Phase 3 has been completed and is currently live. The new site automatically releases all reports in real-time for both Verification and EQA. Currently the site has processed over 100 EQA reports

for NHLS laboratories in the first GeneXpert EQA round for 2013 which is still ongoing.

- Alternative specimen preparation protocols:
    i. Protocols being developed for TB diagnosis in children
    ii. Protocols under development for EPTB: Preliminary data has been presented to the NDoH as well as the GLI of the WHO. Preliminary data also has been submitted to a WHO initiated meta-analysis. Overall sensitivity of Xpert compared to MGIT=55.9% (CI 48.8; 62.8), Absolute number: 159 new cases (18% of total referrals). The bar charts summarise the positivity and culture contamination for various tissue types.





- Connectivity: Collaboration with Cepheid ongoing
    i. Remote connectivity – System deployed on more than 100 sites by Cepheid and the NHLS. More than 340,000 results reported to date. The current pilot system cannot handle the additional testing capacity which will be addressed in the full product version. Discussions are currently under way to include the remainder of the NHLS sites on the system, purge the data and begin monitoring again to assist in the evaluation of the ongoing rollout.
    ii. The first point of care site (Botshabelo Clinic, North West Province) has gone live on the Cepheid Dashboard with an additional 2 sites to be connected.

These sites are using Metacom-sponsored routers (3G) connection for reporting.

12. 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.
        - Manuscript in progress.
    - RCT: ~n=452 patients (POC arm =226; SOC =226) recruited into the study.
    - Sub-study to investigate feasibility and patient acceptance of multiple finger sticks for POC testing: Completed. Awaiting re-submission.
    - Sub-study 1: to investigate various blood specimen storage and transport options: This study will compare viral load testing on Dried Blood Spots (DBS) to new technologies/alternatives such as Hemaform plates, Primestore tubes and a thicker DBS cards.
        - Protocol has been developed. Ethics clearance has been obtained.
        - Patient recruitment will begin next week at Themba Lethu Clinic
    - Sub-study 2: to investigate volumes of blood collected from a finger stick for point of care testing:
        - This is in collaboration with Northwestern University and a medical student has been sent to assist.
        - The study has begun with patient recruitment at Themba Lethu Clinic n=100
- Connectivity:
    - Conworx (POCcelerator) and LDS (AegisPOC) to be trialed in 2 sites during RCT. AegisPOC was installed at the first connectivity on 15 September, 2012. The Conworx solution was installed on the 14th of December, 2012. An antennae was installed and sufficiently boosted the signal. Conworx now running routinely at Tigane Clinic. Internet outage has prevailed at Tigane clinic due to a firewall fault on the supplied router. The service provider is correcting the routine issue which should restore connection to the site.

- A preliminary evaluation and comparison of the systems is about to commence as part of the study outputs. The proposed evaluation includes the option to switch the control site (paper-based) to one of the live systems in order to document and measure the impact on workflow before and after the installation of the system.

13. Grants Submitted

None

14. Funding

Table 9: Total and Percentage Contribution to date by Donor

Donor	% Contribution
NDoH	24.04
Bill & Melinda Gates Foundation	7.20
TB Reach	1.42
MSF	0.90
FIND	0.45
USAID	2.45
CDC NHLS 2010/11	14.78
CDC NDoH	0.72
CDC NHLS 2011/12	1.39
Dr. Niebauer	0.20
Gobal Fund NDOH	40.91
Global Fund RTC	2.78
CDC NDoH	2.77
Subtotal	**100**

CDC has contributed 19, 65% towards the program to date.

15. Recent Campaigns

- None in April