Ref N.	Year	Setting	Main Findings	Main Conclusions
8	1998	USA	A set of overlapping molecular beacons was used to analyze an 81-bp region of the MTB rpoB gene for mutations that confer resistance to the antibiotic R. In a blinded study of 52 R-resistant and 23 R-susceptible clinical isolates, this method correctly detected mutations in all of the resistant strains and in none of the susceptible strains.	The assay was carried out entirely in sealed PCR tubes and was simple to perform and interpret. This approach can be used to analyze any DNA sequence of moderate length with single base pair accuracy
9	2000	Spain and USA	A rapid closed-tube PCR assay using fluorogenic reporter molecules called molecular beacons to detect reportedly common MTB mutations associated with resistance to H and R was developed. The overall SENS and SPEC of the assay for H resistance were 85 and 100%, respectively, and those for R resistance were 98 and 100%, respectively. R resistance mutations were detected equally well in isolates from both study populations; however, H resistance mutations were detected in 94% of the isolates from Madrid but in only 76% of the isolates from New York (P = 0.02). In New York, H resistance mutations were significantly more common in the MDR isolates (94%) than in single-drug-resistant isolates (44%; P < 0.001). No association between previously described mutations in the kasA gene and H resistance was found. The first mutations that cause H resistance may often occur in sequences that have not been commonly associated with H resistance, possibly in other as yet uncharacterized genes	The molecular beacon assay was simple, rapid, and highly sensitive for the detection of R-resistant MTB isolates and for the detection of H resistance in MDR isolates.
10	2001	USA	The study reports on a highly SENS PCR assay that takes less than 3 h and reliably identifies R-resistant MTB in DNA extracted directly from SS. When 148 MTB clinical isolates of known susceptibility to R were tested, mutations associated with R resistance were detected in 63 of the 65 R-resistant isolates, and no mutations were found in any of the 83 R-susceptible isolates. When DNA extracted directly from the SS of 11 patients infected with R-resistant TB was tested, mutations were detected in all of the samples.	The use of this rapid assay should enable early detection and treatment of drug-resistant TB in clinical settings.
11	2010	Vietnam and Uganda	Analytic tests of MTB DNA demonstrated a limit of detection (LOD) of 4.5 genomes per reaction. Studies using sputum spiked with known numbers of MTB CFU predicted a clinical LOD of 131 CFU/ml. Killing studies showed that the assay's buffer decreased MTB viability by at least 8 logs, substantially reducing biohazards. Tests of 23 different commonly occurring R resistance mutations demonstrated that all 23 (100%) would be identified as R-resistant. An analysis of 20 NTM species confirmed high assay specificity. A small clinical validation study of 107 clinical sputum samples from suspected TB cases in Vietnam detected 29/29 (100%) SS+ C+ cases and 33/39 (84.6%) or 38/53 (71.7%) SS-,C+ cases, as determined by growth on solid medium or on both solid and liquid media, respectively. MTB was not detected in 25/25 (100%) of the C- samples. A study of 64 SS- smear-C+ sputa from retreatment TB cases in Uganda detected 63/64 (98.4%) C+ - cases and 9/9 (100%) cases of R resistance. R resistance was excluded in 54/55 (98.2%) susceptible cases. SPEC rose to 100% after correcting for a conventional susceptibility test error.	This highly sensitive and simple-to-use system can detect MTB directly from SS in less than 2 h.
12	2010	USA	79 MTB isolates and 89 NTM isolates were studied .The Xpert®MTB/RIF assay correctly identified all 79 MTB isolates and correctly excluded all 89 NTM isolates. R resistance was correctly identified in all 37 resistant isolates and in none of the 42 susceptible isolates. Dynamic range was assessed by adding 102 to 107 Colony Forming Units (CFU) of MTB into MTB-negative sputum samples. The assay showed a log-linear relationship between cycle threshold and input CFU over the entire concentration range. Resistance detection in the presence of different mixtures of R-resistant and R-susceptible DNA was assessed. Resistance detection was dependent on the particular mutation and required between 65% and 100% mutant DNA to be present in the sample for 95% certainty of resistance detection	The Xpert®MTB/RIF assay was relatively resistant to contamination by MTBDRplus amplicons and could safely be used in the same laboratory environment. This was the result of inefficient amplification of the MTBDRplus amplicon, which has only limited overlap with the outer priming regions of the Xpert®MTB/RIF assay. The Xpert®MTB/RIF itself contains amplicons within the sealed cartridge, reducing or eliminating the need for the precautions typically associated with other nucleic acid amplification tests.
13	2010	USA	The bioaerosols generated by the Xpert®MTB/RIF assay was compared to AFB microscope slide SS preparation. The Xpert®MTB/RIF assay sample treatment reagent (SR) was also studied for its sterilizing capacity, stability, and effect on assay SENS after prolonged treatment. Neither the sample preparation steps for the Xpert®MTB/RIF assay nor its automated processing produced any culturable bioaerosols. In testing of SR sterilizing capacity, clinical SS samples from strongly SS+ TB patients treated with SR at a 2:1 ratio eliminated MTB growth in all but 1/39 or 3/45 samples cultured on solid or liquid medium, respectively. These few unsterilized samples had a mean 13.1-day delay in the time to C+. SR treatment at a 3:1 ratio eliminated growth in all samples. SR retained a greater than 6-log-unit	These results suggest that benchtop use of the Xpert®MTB/RIF assay limits infection risk to the user.

			killing capacity despite storage at temperatures spanning 4 to 45°C for at least 3 months.	
17	2010	Peru, Azerbaijan, South Africa, and India	Among C+ patients, a single, direct Xpert®MTB/RIF test identified 551/561 patients with SS+ TB (98.2%) and 124 of 171 with SS- TB (72.5%). The test was specific in 604 of 609 patients without TB (99.2%). Among patients with SS- C+ TB, the addition of a second Xpert®MTB/RIF test increased SENS by 12.6% and a third by 5.1%, to a total of 90.2%. As compared with phenotypic DST, Xpert®MTB/RIF MTB/RIF testing correctly identified 200/205 patients (97.6%) with R-resistant bacteria and 504/514 (98.1%) with R-susceptible bacteria. Sequencing resolved all but two cases in favour of the Xpert®MTB/RIF assay.	The Xpert®MTB/RIF test provided sensitive detection of TB and R resistance directly from untreated SS in less than 2 hours with minimal hands-on time.
18	2011	Multicentre study in South Africa, Peru, India, Azerbaijan, Philippines, and Uganda	6648 participants were enrolled. Once-off Xpert®MTB/RIF testing detected 933 (90·3%) of 1033 C-confirmed cases of TB, compared with 699 (67·1%) of 1041 for microscopy. Xpert®MTB/RIF test SENS was 76·9% in SS-, C+ patients (296 of 385 samples), and 99·0% specific (2846 of 2876 non-tuberculosis samples). Xpert®MTB/RIF test SENS for R resistance was 94·4% (236 of 250) and SPEC was 98·3% (796 of 810). Unlike microscopy, Xpert®MTB/RIF test SENS was not significantly lower in patients with HIV co-infection. Median time to detection of TB for the Xpert®MTB/RIF was 0 days (IQR 0—1), compared with 1 day (0—1) for microscopy, 30 days (23—43) for solid C, and 16 days (13—21) for liquid C. Median time to detection of resistance was 20 days (10—26) for line-probe assay and 106 days (30—124) for conventional DST. Use of the Xpert®MTB/RIF test reduced median time to treatment for SS- TB from 56 days (39—81) to 5 days (2—8). The indeterminate rate of Xpert®MTB/RIF testing was 2·4% (126 of 5321 samples) compared with 4·6% (441 of 9690) for C.	The Xpert®MTB/RIF can effectively be used in low-resource settings to simplify patients' access to early and accurate diagnosis, thereby potentially decreasing morbidity associated with diagnostic delay, dropout and mistreatment.
27	2011	Turkey	253 pulmonary and 176 extrapulmonary specimens obtained from 429 patients were included in the study. 110 (89 C+ and 21 C- for MTB) of the 429 patients were considered to have TB. In pulmonary specimens, SENS of Xpert®MTB/RIF were 100% (27/27) and 68.6% (24/35) for SS+ and SS- specimens respectively. It had lower SENS in extrapulmonary specimens; for SS+ 100% (4/4) and SS- 47.7% (21/44). The test accurately detected the absence of TB in all 319 patients studied. The Xpert®MTB/RIF assay also detected one R-resistant and 88 R-susceptible specimens which were confirmed by phenotypic DST.	The Xpert®MTB/RIF test was a simple method and routine staff with minimal training could use the system. The test appeared to be as SENS as C with SS+ specimens but less SENS with SS- pulmonary and extrapulmonary specimens that included low number of bacilli.
28	2011	Kuwait	Clinical specimens included 206 pulmonary and 29 extra-pulmonary samples .72 (60 pulmonary and 12 extra-pulmonary) samples yielded MTB by C, while 56 (78%) C+ samples (46 pulmonary and 10 extra-pulmonary) were also SS+. Xpert®MTB/RIF showed98% agreement for SS+, C+ samples and 69% agreement for SS-, C+ samples for detection of MTB. Overall concordance with C was 92%.There was 98% and 64% agreement for SS+ and SS- pulmonary specimens, respectively. Xpert®MTB/RIF showed 100% agreement with C for both SS+, C+ and SS-, C+ extra-pulmonary specimens.	The rapidity and simplicity of the closed cartridge Xpert®MTB/RIF test made it a good TB diagnostic test for routine use in reference laboratories of countries with low to intermediate incidence of TB, and may also help in reducing further transmission of infection in such settings
29	2011	Athens, Greece.	The Xpert®MTB/RIF assay was evaluated with microscopically negative and -positive pulmonary- and extrapulmonary specimens. For the pulmonary samples, SENS, SPEC, PPV and NPV were 90.6%, 94.3%, 93.5%, and 91.7%, and for the extrapulmonary samples, these were 100%, 91.6%, 50%, and 100%, respectively. For microscopically negative specimens, the respective values were 86.3%, 93%, 79%, and 95.6%. The assay correctly detected R resistance in all but one specimen, which harboured a mixed population.	The Xpert®MTB/RIF assay was highly effective for TB diagnosis and identification of R resistant strains in smear-negative samples.
30	2011	Singapore	162 respiratory and non-respiratory specimens were compared for the Xpert®MTB/RIF and the Amplified Mycobacterium Tuberculosis Direct (MTD) assay. Based on C as the gold standard, the overall SENS and SPEC for all sample types for the Xpert®MTB/RIF was 90.9 and 89%, respectively, whilst for the MTD assay, the overall SENS and SPEC was 97.3 and 87.1 %, respectively. A higher proportion of total equivocal results were obtained for the MTD assay at 10.5% (17/162) whilst the Xpert®MTB/RIF assay generated 5.5% (9/162) of invalid reads. The limited number of R-resistant isolates (n=4 present in this study hindered a proper assessment of the efficacy of Xpert®MTB/RIF in detecting R resistance.	In the specific laboratory context, the MTD test has a similar performance to the Xpert assay. The MTD test is however a fully manual test. In contrast, the Xpert MTB/RIF is a self-contained, integrated test that offers minimal hands-on time with low potential for PCR contamination. The concurrent detection for R associated mutations is also an added advantage

31	2011	North Carolina, USA	The Cepheid Xpert MTB/RIF research-use-only (RUO) assay and a laboratory-developed test (LDT) targeting IS6110 were evaluated and compared to mycobacterial C as the gold standard in112 specimens from 90 patients, including 89 pulmonary specimens and 23 extrapulmonary specimens. Of the specimens tested, 37 (33%) were C+ for MTB complex; 29 were pulmonary, and 8 were extrapulmonary. Of the C+ specimens, 83% of the pulmonary specimens and 50% of the extrapulmonary specimens were SS+. There was complete concordance between the SS+ C+ specimens, independent of the anatomical site (100% SENS). The SENS of the MTB/RIF RUO assay for SS- specimens was 60% for pulmonary and 75% for extrapulmonary specimens, while the IS6110 LDT SENS were 40% and 0%, respectively. There was also complete concordance among the C- specimens tested. Both assays showed 95% SPEC, with four C- specimens testing as positive. A review of patient records indicated that there was a high likelihood of the presence of MTB complex DNA in the false-positive specimens. Biosafety analysis was performed and showed an acceptable reduction in organism viability using the processing methods described above	Both molecular assays are suitable for the detection of MTB isolates in SS+ pulmonary and extrapulmonary specimens, while SENS of the detection of MTB isolates in SS- specimens was variable.
32	2011	Multisite clinical trial, USA	2,008 samples were tested. Decreasing MTB Ct was associated with increasing SS microscopy grade for smears of concentrated sputum pellets (rs = −0.77) and directly from SS (rs = −0.71). A Ct cut off of approximately 27.7 best predicted SS+ status. The association between MTB Ct and time-to-detection in liquid C (rs = 0.68) and semi-quantitative colony counts (rs = −0.56) was weaker than SS. Tests of paired same-patient SS showed that high-viscosity SS samples contained ×32 more MTB than non-viscous samples. Comparisons between the grade of the acid-fast bacilli SS and Xpert®MTB/RIF quantitative data across study sites enabled the identification a site outlier in microscopy.	Xpert®MTB/RIF quantitation offers a new, standardized approach to measuring bacterial burden in the sputum of TB patients.
33	2011	Primary health care clinic, Johannesburg, South Africa	Consecutive adults with suspected TB attending a primary health care clinic were prospectively enrolled and evaluated for TB according to national guidelines, including assessment for SS- TB by chest X-ray, clinical evaluation, and HIV testing. A single SS sample underwent routine decontamination, AFB SS microscopy, liquid C, and phenotypic DST. Residual sample was batched for molecular testing. 311 participants, HIV prevalence 70% (n = 215), with 120 (38.5%) C+ TB cases. Compared to liquid C, SENS of all the test methodologies, determined with a limited and potentially underpowered sample size (n = 177), were 59% (47%–71%) for SS microscopy, 76% (64%–85%) for MTBDRplus, 76% (64%–85%) for LCTB, and 86% (76%–93%) for , Xpert®MTB/RIF with specificities all >97%. Among HIV+ individuals, SENS of the Xpert®MTB/RIF assay was 84% (69%–93%), while the other molecular tests had SENS reduced by 6%. TB detection among SS-, C+ samples was 28% (5/18) for MTBDRplus, 22% (4/18) for LCTB, and 61% (11/18) for Xpert®MTB/RIF. A few (n = 5) R-resistant cases were detected using phenotypic DST. Xpert®MTB/RIF detected four of these five cases (fifth case not tested) and two additional phenotypically susceptible cases.	The Xpert®MTB/RIF test has superior performance for rapid diagnosis of MTB over existing AFB SS microscopy and other molecular methodologies in an HIV- and TB-endemic region. Its place in the clinical diagnostic algorithm in national health programs needs exploration.
34	2011	South Africa	Xpert®MTB/RIF was evaluated using single archived spot-SS samples from 496 South African patients with suspected TB. Overall, Xpert®MTB/RIF detected 95% (95% CI, 88–98%; 89 of 94) of SS+ C+ cases and SPEC was 94% (91–96%; 320 of 339). SENS in SS- cases was 55% (35–73%; 12 of 22) when the analysis was restricted to 1 ml of unprocessed SS and C time-to-positivity of less than or equal to 28 days. Compared with SS microscopy (n = 94), Xpert®MTB/RIF detected an additional 17 cases (n = 111) representing an 18% (11–27%; 111 vs. 94) relative increase in TB case detection. Compared with SS microscopy, the inclusion of Xpert®MTB/RIF -positive C- TB cases (ruled-in by an alternative diagnostic method) resulted in the detection of a further 16 cases (n = 127), thus significantly increasing the TB case detection rate to 35% (95% CI, 26–45%; 94 to 111 vs. 94 to 127; P < 0.01), the overall SPEC to 99.1% (97–100%; 320 of 323; P < 0.001), and SENS in SS- TB to 60% (P = 0.12). Performance was strongly correlated with SS status and C time-to-positivity. In patients infected with HIV compared with patients uninfected with HIV, Xpert®MTB/RIF showed a trend to reduced SENS (P = 0.09) and significantly reduced NPV (P = 0.01). The NPV for RMP resistance was 99.4%.	Xpert®MTB/RIF outperformed SS microscopy, established a diagnosis in a significant proportion of patients with SS- TB, detected many highly likely TB cases missed by C, and accurately ruled out R-resistant TB. Sample-specific factors had limited impact on performance. Performance in patients infected with HIV, especially those with advanced immunosuppression, warrants further study.

35	2011	HIV clinic, South Africa	The accuracy of the X Xpert®MTB/RIF assay for diagnosing TB and drug resistance was assessed in comparison with other tests, including fluorescence SS microscopy and automated liquid C (gold standard) and DST. Of 515 patients enrolled, 468 patients (median CD4 cell count, 171 cells/µl; IQR 102–236) produced at least one SS sample, yielding complete sets of results from 839 samples. MTB was cultured from 81 patients (TB prevalence, 17.3%). The overall SENS of the Xpert®MTB/RIF assay for C+ TB was 73.3% (SPE, 99.2%) compared to 28.0% (SPE, 100%) using SS microscopy. All SS+, C+- disease was detected by Xpert®MTB/RIF from a single sample (SENS, 100%), whereas SENS for SS-, C+ TB was 43.4% from one SS sample and 62.3% from two samples. Xpert®MTB/RIF correctly identified R resistance in all four cases of MDR-TB but incorrectly identified resistance in three other patients whose disease was confirmed to be drug sensitive by gene sequencing (SPEC, 94.1%; PPV, 57%).	In this population of individuals at high risk of TB, intensive screening using the Xpert®MTB/RIF assay increased case detection by 45% compared with SS microscopy, strongly supporting replacement of microscopy for this indication. However, despite the ability of the assay to rapidly detect R-resistant disease, SPEC for drug-resistant TB was sub-optimal.
36	2011	University Center for Chronic Diseases, Groesbeek, The Netherlands	The Authors analysed 89 unprocessed clinical samples (86 SS samples, 1 pleural fluid,1 gastric fluid, 1 bronchial washing). 26 samples were obtained fresh, in clinical routine, 63 samples previously stored at −70°C. SENS for detecting MTB in C+ samples was 93.8% (60/64) and exceeded SS microscopy (40/64, 62.5%). SPEC for detecting MTB was 92.0% (23/25) and for R resistance 100% (8/8). In the 40 SS+ samples that grew MTB, SENS was 100%. One SS+,C- gastric fluid sample was positive with Xpert®MTB/RIF;In 48 SS- samples, SENS was 83.3% (20/24), and SPEC was 95.8% (23/24).The combined SENS for detecting MTB in SS- and SS+ sputum samples was 93.8% (60/64), and SPEC was 92.0% (23/25). The sample set included 8 samples from R-resistant TB patients (4 fresh, 4 frozen; 6 MDR-TB,2 R-monoresistant); all were recognised as R-resistant by the Xpert®MTB/RIF assay. No false positives or -negatives were noted; SENS and SPEC for detecting R-resistance was 100%. No significant differences in SENS and SPEC in fresh (SENS 100%, SPEC 90.1%, n = 26) vs. frozen samples (SENS 91.8%, SPEC 92.9%,n = 63) were detected.	The test was technically simple to conduct and required neither PCR facilities nor biosafety precautions other than those of routine SS handling. These characteristics render it a promising close-to-patient test for TB in various settings. The Xpert®MTB/RIF thus combines all the characteristics required of a close-to-patient test. The analysis was rapid, simple, safe and required hardly any training. This platform is highly promising for close to-patient TB diagnostics.
37	2011	Tanzania	In 292 samples, the diagnostic performance of Xpert®MTB/RIF was compared to standard SS microscopy and C. Xpert®MTB/RIF achieved 88.4% (95%CI = 78.4% to 94.9%) SENS among patients with C+ and 99% (95%CI = 94.7% to 100.0%) SPEC in patients who had no TB. HIV status did not affect test performance in 172 HIV-infected patients (58.9% of all participants). Seven additional cases (9.1% of 77) were detected by Xpert®MTB/RIF among the group of patients with clinical TB who were C- . In 45 sputum specimens that grew NTM the assay's SPEC was 97.8% (95%CI = 88.2% to 99.9%).	The Xpert®MTB/RIF assay was a highly sensitive, specific and rapid method for diagnosing TB which has potential to complement the current reference standard of TB diagnostics and increase its overall sensitivity. Its usefulness in detecting SS and C- patients needs further study. Further evaluation in high burden TB and HIV areas under programmatic health care settings to ascertain applicability, cost-effectiveness, robustness and local acceptance are required.
38	2011	France	The Authors evaluated 117 clinical specimens (97 C+ and 20 C- for MTBC) frozen in sediment. The 97 clinical specimens included 60 respiratory and 37 non respiratory specimens, of which 36 were SS+ and 61 were SS-.Among the 97 C+ specimens, 4 had R-resistant isolates. Both methods were highly SPEC and exhibited excellent SENS (100%) with SS+ specimens. SENS of the Xpert®MTB/RIF test with the whole SS- specimens was more reduced than that of the IS6110-TaqMan assay (48 versus 69%, P = 0.005). Both methods exhibited similar SENS with SS- respiratory specimens, but the Xpert®MTB/RIF test had lower SENS with SS- non respiratory specimens than the IS6110-TaqMan assay (37 versus 71%, P = 0.013). Finally, SENS of the Xpert®MTB/RIF test and the IS6110-TaqMan assay were 79% and 84%, respectively with respiratory specimens, and 53% and 78% respectively (P = 0.013), with non-respiratory specimens. The Xpert®MTB/RIF test correctly detected R resistance in SS+ specimens but not in the one SS- specimen.	The Xpert®MTB/RIF test was a simple rapid method well adapted to a routine laboratory that appeared to be as SENS as the IS6110-TaqMan assay with respiratory specimens but less SENS with paucibacillary specimens, such as SS- non respiratory specimens.
39	2011	University hospital Caen, France	91 respiratory and 89 non-respiratory samples were evaluated. Overall, 31 (17.2%) of the 180 samples, including 17 respiratory and 14 non-respiratory (respectively 17 and 12 PCR-positive), yielded MTB on C. SENS and SPEC of PCR were respectively 100% and 100%, and 85.7% and 97.3% for respiratory and non-respiratory samples.	Although the Xpert®MTB/RIF testis validated only for respiratory samples, findings suggested that it could be useful for the diagnosis of extra-pulmonary TB.

40	2011	Western United States	A total of 217 specimens were submitted to evaluate the GeneXpert MTB/RIF assay (for research use only).Overall agreement compared to C was 89% (98% for SS+ and 72% for SS-) for detection of MTB.	Overall, the GeneXpert assay was simple, fast, accurate, and cost-comparative to other commercially available PCR assays for the direct detection of MTB.
41	2011	Spain	64 of 85 (75.3%) SS- respiratory (n = 78) and non respiratory (n = 7) samples with C+ MTB complex (MTC) were detected by the GeneXpert (GX) system using the Xpert®MTB/RIF assay. In addition, GX found rpoB mutations in all six of the R-resistant strains detected. The test was negative in 20 C- and 20 NTM C+ samples (100% SPEC).	The global data obtained in the present study indicate that GX has a high SENS, since all samples analyzed had a low mycobacterial load. The relatively high cost of GX was an important issue that TB control programs should consider prior to implementation of this assay. Its clinical and epidemiological advantages should be weighed against the resources available in each setting. In summary, the GX technique demonstrated a high capacity for detecting MTC and for predicting MDR in SS- clinical samples. Moreover, its rapidity, simplicity, and low laboriousness make the technique a good candidate for routine use in many clinical laboratories whenever the clinical criteria for its application are met.
50	2011	South Africa	452 children (median age 19·4 months, IQR 11·1—46·2) had at least one induced sputum specimen; 108 children (24%) had HIV infection. 27 children (6%) had a positive SS result, 70 (16%) had a positive C result, and 58 (13%) had a positive Xpert®MTB/RIF test result. With mycobacterial C as the reference standard, Xpert®MTB/RIF tests when done on two induced sputum samples detected twice as many cases (75.9%, 95% CI 64.5—87.2) as did SS microscopy (37.9%, 25·1—50·8), detecting all of 22 SS+ cases and 22 of 36 (61·1%, 44.4—77.8) SS- cases. For SS- cases, the incremental increase in SENS from testing a second specimen was 27.8% for Xpert®MTB/RIF , compared with 13·8% for C. SPEC of Xpert®MTB/RIF was 98.8% (97.6—99.9). Xpert®MTB/RIF results were available in median 1 day (IQR 0—4) compared with median 12 days (9—17) for C (p<0·0001).	Xpert®MTB/RIF testing of two induced sputum specimens was warranted as the first-line diagnostic test for children with suspected pulmonary TB.
51	2011	South Africa	The Authors compared the turn-around-time, detection-threshold, dynamic range, reproducibility, relative discriminative ability, of 4 mycobacterial load determination techniques: automated liquid culture (BACTEC-MGIT-960), [3H]-uracil incorporation assays, luciferase-reporter construct bioluminescence, and quantitative PCR(Xpert -MTB/RIF) using serial dilutions of Mycobacterium bovis and Mycobacterium tuberculosis H37RV. Mycobacterial colony-forming-units(CFU) using 7H10-Middlebrook solid media served as the reference standard. All 4 assays correlated well with the reference standard, however, bioluminescence and uracil assays had a detection threshold ≥1×103 organisms. By contrast, BACTEC-MGIT-960 liquid C, although only providing results in days, was user-friendly, had the lowest detection threshold (<10 organisms), the greatest discriminative ability (1 vs. 10 organisms; p = 0.02), and the best reproducibility (coefficient of variance of 2% vs. 38% compared to uracil incorporation; p = 0.02). Xpert®MTB/RIF correlated well with mycobacterial load, had a rapid turn-around-time (<2 hours), was user friendly, but had a detection limit of ~100 organisms.	Choosing a technique to quantify mycobacterial burden for laboratory or clinical research depends on availability of resources and the question being addressed. Automated liquid culture had good discriminative ability and low detection threshold but results were only obtained in days. Xpert®MTB/RIF provided rapid quantification of mycobacterial burden, but had a poorer discrimination and detection threshold.
52	2011	South Africa	20 cases with confirmed TB pleural effusion. Xpert®MTB/RIF SENS and SPEC in pleural fluid was 25% and 100%, respectively. All positive Xpert®MTB/RIF results were also pleural fluid C+.	Xpert®MTB/RIF testing in pleural fluid samples is feasible, but of low SENS and linked to a positive pleural fluid C. There is an indication for high SPEC, which must be verified with larger studies including more patients with a pleural effusion due to other causes than TB. Before this is attempted the methods for collection, storage and preparation of pleural fluid samples need to be optimized in order to increase Xpert®MTB/RIF SENS on pleural fluid.

53	2011	Spain	340 non-respiratory samples were processed using two real-time PCR assay kits: Xpert®MTB/RIF and Cobas TaqMan MTB. SENS and SPEC of the Xpert assay were 95% and 100%, respectively, compared to 78% and 98% for the Cobas assay.	Both molecular techniques represent an important contribution to the detection of MTB, since they can provide results in a matter of hours, whereas the reference C method takes days. Real-time PCR techniques afford greater SENS and SPEC and a much-reduced response time,as well as enabling visualization of amplification curves. One limitation of these techniques is that, in detecting MTB DNA, they cannot distinguish between viable and nonviable microorganisms. For that reason, although these assays are semi-quantitative, they should not be used for monitoring patient progress or treatment efficacy
54	2011	South Africa	To determine the diagnostic utility of the Xpert® 52 MTB/RIF, FNAB (fine needle aspiration biopsy) were collected from 50 consenting patients by aspirating TB lymphadenitis..Aspirates underwent C (MGIT 960), genotypic DST (Genotype MTBDR*plus* assay) and Xpert MTB/RIF. Compared to the reference standard, Xpert MTB/RIF correctly identified 29 out of 30 TB cases (sensitivity 96.7%, 95%CI, 86.6-100). The possible "false negative" result had a prolonged transit interval of 9 days before Xpert MTB/RIF testing, which may have affected the result. Xpert MTB/RIF was positive in two cases with negative cytomorphology and culture (specificity 88.9%, 95%CI, 69.6-100). The Xpert MTB/RIF test was positive in all 6 smear negative culture positive cases and correctly identified the 1 of the 2 R resistant cases. The average time to result for microbiological C was 18.5 days (range 9-55 days), while the Xpert MTB/RIF test result was available within 2 hours of commencing the test.	This study demonstrated the excellent diagnostic accuracy of the Xpert® MTB/RIF test in patients with TB lymphadenitis.
55	2011	India	547 extrapulmonary specimens were split and processed simultaneously for both C (solid and liquid) and Xpert®MTB/RIF testing. For culture, SENS was low, 53% (150/283 specimens). Xpert®MTB/RIF SENS and SPEC results were assessed in comparison to a composite reference standard made up of SS and C results and clinical, radiological, and histological findings. SENS of the Xpert®MTB/RIF assay was 81% (228/283 specimens) (64% [89/138] for SS- cases and 96% [139/145] for SS+ cases), with 99.6% SPEC. SENS was found to be high for the majority of specimen types (63 to 100%) except for cerebrospinal fluid, with SENS of 29% (2/7 specimens). The Xpert®MTB/RIF correctly identified 98% of phenotypic R--resistant cases and 94% of phenotypic R-susceptible cases. Sequencing of the 6 discrepant samples resolved 3 of them, resulting in an increased specificity of 98%.	The results of this study suggested that the Xpert®MTB/RIF test also showed good potential for the diagnosis of extrapulmonary TB and that its ease of use made it applicable for countries where TB is endemic.
56	2011	Germany	521 non-respiratory specimens were comparatively investigated with the Xpert®MTB/RIF assay and conventional liquid and solid C methods. 20 (3.8%) of the 521 specimens gave no interpretable result. Whereas SENS of the X Xpert®MTB/RIF with tissue specimens was 69.0% (20 out of 29 C+ cases detected), 100% SENS was found with urine and stool specimens. The combined SENS and SPEC of the Xpert®MTB/RIF assay were calculated to be 77.3% and 98.2%, respectively.	The Xpert®MTB/RIF assay could be applied to extrapulmonary specimens with a high SENS and SPEC, which, coupled with its speed and simplicity, made this technique a very useful tool for the diagnosis of extrapulmonary TB.
57	2011	CapeTown, South Africa	In this cost-analysis study the Authors assessed the accuracy and/or laboratory-associated cost-of-diagnosis of SS microscopy,chest-radiography, and interferon-γ-release assays (IGRAs; T-SPOT-TB and QFT-GIT), combined with a single Xpert-MTB/RIF, for the diagnosis of TB in 480 suspects. When conducted prior to Xpert®MTB/RIF testing: (i) SS-microscopy followed by Xpert®MTB/RIF (if SS-) had the lowest cost-of-diagnosis of any strategy investigated; (ii) a combination of SS-microscopy, chest-radiography (if SS-) and Xpert®MTB/RIF (if imaging compatible with active-TB) did not further reduce the cost per TB case diagnosed; (iii) a normal chest radiograph ruled-out TB in 18% of suspects [57/324; NPV 100%(57/57)]. When downstream adjunct tests were applied to Xpert®MTB/RIF -negative individuals: (i) radiology ruled-out TB in 24% [56/234; NPV 100%(56/56)]; (ii) SS-microscopy ruled-in TB in 21%(7/24) of C+ individuals; (iii) IGRAs were not useful in either context.	In resource-poor settings, SS-microscopy combined with Xpert®MTB/RIF had the highest accuracy and lowest cost-of-diagnosis compared to either technique alone. In Xpert®MTB/RIF-negative individuals, chest radiography had poor rule-in value but couldreliably rule-out TB in ~1 in 4 of such cases. These data informed the programmatic utility of Xpert®MTB/RIF in high burden settings. Detailed cost-effectiveness analyses are required.
58	2011	South Africa	A pilot program using dried culture spots (DCS) of inactivated MTB is described. Of 274 DCS results received, 2.19% generated errors; the remaining yielded 100% correct MTB detection. Probe A cycle threshold (Ct) variability of three DCS batches ≤3.47Ct. Longer-term DCS stability is on-going.	This study provided preliminary demonstration through the use of inactivated MTB coupled with easier transportation of DCS material that an EQA program can be safely provided. Future design of an

				Xpert®MTB/RIF EQA program could be similarly based on line probe assay programs using one pan-susceptible strain, one R-monoresistant strain with a common rpoB mutation, one MDR strain, one NTM strain and a negative control each placed on a DCS card and distributed 3/6 monthly.
59	2012	Italy	Review of the findings by Tortoli et al (Ref 72) done by a per-sample analysis of 268 diagnoses of extrapulmonary TB (EPTB) at a range of anatomic sites (SENS: 81.3%; 95% CI: 76.2–85.8) and data for 1206 samples in which EPTB was excluded (SPEC: 99.8%; 95% CI: 99.4–100).	The AA conclude that this study (Ref 72) was an important addition to the growing body of literature demonstrating the utility of Xpert®MTB/RIF for EPTB diagnosis when applied to diverse types of clinical samples.
60	2012	USA	The Authors determined the lower limit of detection (LOD) of the GeneXpert MTB/RIF assay with non-respiratory specimens and investigated the utility of flotation procedures for concentrating the bacilli. Clinical specimens (9 CSF, 13 gastric aspirate, 8 tissue, and 17 stool) were spiked with single-celled MTB and the LOD of the GeneXpert was determined. Flotation studies were conducted with sucrose and NaCl and the cycle thresholds of the MTB/RIF assay were compared between treated and untreated samples.There was no significant difference between the LOD of the Xpert®MTB/RIF with saline (median 33 CFU/ml) and CSF (median 25 CFU/ml) ($P > 0.05$) or gastric aspirate samples (median 58 CFU/ml) ($P > 0.05$). The LOD with spiked tissue (median 1,525 CFU/ml) and stool samples (median 6,800 CFU/ml) was significantly elevated compared to saline ($P \leq 0.05$ and ≤ 0.0005, respectively). Flotation studies with sucrose or NaCl did not consistently result in lowered cycle thresholds in stool or gastric aspirates but >10 cycle reduction was achieved in two of the three pooled CSF samples.	Unlike with tissue and stool samples, there was no significant PCR inhibition in the Xpert®MTB/RIF assay with CSF and gastric aspirates. Although pre-concentration of CSF samples with sucrose and NaCl may enhance detection of MTB by PCR, further advances are needed to concentrate the bacilli and eliminate PCR inhibitors in paucibacillary non-respiratory samples.
61	2012	South Africa	The Authors determined the diagnostic yield of the Xpert MTB/ RIF assay for TB when testing small volumes of urine from ambulatory HIV-infected patients before starting ART therapy in South Africa. Among 602 patients recruited, 535 produced at least 1 sputum sample and a specimen of urine. Sputum C results were available from 516 patients and these yielded 85 diagnoses of C+ TB. The remainder (n = 431) were sputum C negative.Compared with a gold standard of sputum culture, SENS of urine Xpert®MTB/RIF among those with CD4 cell counts of <50, 50–100, and >100 cells per microliter were 44.4%, 25.0%, and 2.7% ($P = 0.001$), respectively.	Urine Xpert®MTB/RIF testing provided a means of rapid TB diagnosis in patients with advanced immunodeficiency and poor prognosis. These data were indicative of high rates of TB dissemination and renal involvement in this clinical population.
62	2012	Spain	Among 108 SS- extrapulmonary samples showing a C+ for MTB complex (43 body fluids and 65 non liquid specimens), 63 (58.3%) were positive with the Xpert®MTB/RIF assay . SENS was quite low for samples from sterile locations (especially for pleural fluids: 26.9%) but high for some non-liquid samples, like abscess aspirates (76.5%).	Xpert®MTB/RIF may be a useful tool to be considered for extrapulmonary TB diagnosis.
63	2012	University Teaching Hospital Lusaka, Zambia	C+ TB was found in 201/881 patients (22.8%). Xpert®MTB/RIF SPEC was 95.0% (95% CI, 92.4%–96.8%); SENS was 86.1% (95% CI, 80.3%–90.4%). In SS-, C+ cases, the assay had 74.7% SENS (95% CI, 64.6%–82.8%), identifying 71 additional TB cases that were not detected by SS. 18/111 patients with TB who were tested (16.2%) had MDR- TB. SENS and SPEC of Xpert®MTB/RIF for detecting C+, Resistant TB was 81.3% (95% CI, 53.7%–95.0%) and 97.5% (95% CI, 90.4%–99.6%), respectively.	Xpert®MTB/RIF performed better than SS in an inpatient setting in a country where TB and HIV infection are highly endemic. Assessment of its usefulness and cost-effectiveness for increased detection of TB cases missed by SS and for concomitant screening for MDR-TB among adult inpatients attending tertiary care referral centres in other countries with a high burden of TB and HIV infection is warranted.
64	2012	Different Hospitals, sub-Saharan Africa	42% (116/242) of patients had C+ TB. 18% (20/54) were SS scarce. In SS-scarce patients, SENS of urine Xpert®MTB/RIF MTB/RIF and LAM ELISA test was 40% (95%CI: 22–61) and 60% (95%CI: 39–78), respectively. Urine Xpert®MTB/RIF SPEC was 98% (95%CI: 95–100). Combined SENS of urine LAM ELISA and Xpert®MTB/RIF was better than Xpert®MTB/RIF alone [Xpert®MTB/RIF and LAM: 70% (95%CI: 48–85) vs. Xpert®MTB/RIF: 40% (95%CI: 22–61), $p = 0.03$]. Significant predictors of urine Xpert®MTB/RIF positivity were CD4<50 cells/ml ($p = 0.001$), elevated protein-to-creatinine ratio ($p<0.001$) and LAM ELISA positivity ($p<0.001$). Urine centrifugation and pelleting significantly increased the SENS of Xpert®MTB/RIF over unprocessed urine in paired samples [42% (95%CI: 26–58) vs. 8% (95%CI: 0–16), $p<0.001$]. Urine Xpert®MTB/RIF -generated CT (cycle-threshold)values correlated poorly with markers of bacillary burden (SS grade and time-to-positivity).	This preliminary study indicates that urine-based Xpert®MTB/RIF , alone or in combination with LAM antigen detection, maypotentially aid the diagnosis of TB in HIV-infected patients with advanced immunosuppression when SS-based diagnosis is not possible. Concentration of urine prior to Xpert®MTB/RIF testing significantly improves SENS.

65	2012	City Hospital Auckland, New Zealand	All AFB SS+ respiratory specimens, were processed and analysed by Xpert® MTB/RIF assay using C (MGIT 960 system) as the gold standard, A total of 169 specimens (89 SS+ respiratory specimens; 9 smear-positive extra-pulmonary specimens and 71 positive MGIT liquid C vials) from 169 patients were analysed. With the use of culture as the "gold standard", the overall SENS and SPE of the MTB/RIF assay for the detection of MTB were 100% (141/141) and 100% (28/28), respectively. The MTB/RIF assay detected R resistance in 13/169 (7.7%) specimens. However, using standard phenotypic methods, R resistance was detected in only 7/13 (54%) isolates. In 2 of the remaining 6 isolates, amplification and sequencing of the rpoB gene revealed mutations associated with increased but low-level R resistance.	Similar to previous studies, the Authors found the MTB/RIF assay highly SENS and SPE for the detection of MTB, when used for both smear-positive pulmonary and extrapulmonary specimens as well as for isolates in liquid C media. However, the Authors found that the assay was less reliable for the detection of R resistance, producing false-positive results in 4/13 (31%) specimens. Further work is therefore required to evaluate the performance of the MTB/RIF assay for the detection of R resistance in a range of clinical settings and on a range of specimen types.
66	2012	Supra-national Reference Laboratory, Milan, Italy	An innovative approach allowed selective amplification of DNA derived from viable MTB in clinical specimens, which was useful for monitoring mycobacterial load in pulmonary TB patients during anti-TB treatment. The protocol was based on pre-treatment of samples with propidium monoazide (PMA).PMA did not significantly affect PCR yield of specimens collected at time zero, confirming that the SS positivity of these samples was mostly due to highly damaged bacteria. Moreover, Delta Ct (difference in amplification yield between samples with and without PMA pre-treatment) calculated between t0 and t1 in PMA-untreated samples was found to be too low to represent a real decrease in bacterial load due to therapy. All patients were successfully treated and cured at the end of therapy, consistent with the reduction of live bacteria detected by the PMA assay.	Quantitative molecular techniques combined with the PMA method could be an alternative to SS and C for monitoring early treatment response and for preliminary evaluation of personalised regimens. The use of this assay can allow earlier evaluation of treatment efficacy, showing a clear decrease in the vital mycobacterial load. Absence of the response to therapy might also be promptly identified by the test allowing a regimen change and limiting the spread of infection and further resistance development
67	2012	HIV service, South Africa	523/602 patients screened had ≥1 Xpert®MTB/RIF and C result, yielding 89 C+ TB diagnoses. Of these, 37 (42%) of TB patients were Xpert®MTB/RIF -negative when a single SS sample was tested, compared with 25 (28%) when 2 samples were tested. Compared to patients with Xpert®MTB/RIF -positive TB, those with Xpert®MTB/RIF -negative TB (using either definition) had substantially higher CD4 cell counts, lower plasma viral loads, higher hemoglobin concentrations, and higher body mass index. Their TB was also less advanced, with a lower frequency of prolonged cough (≥2 weeks), less extensive radiographic abnormalities, and a lower frequency of detectable lipoarabinomannan antigenuria and mycobacteriuria. Xpert®MTB/RIF -negative cases were all SS- with prolonged time to C positivity (median, 21 days). Despite greater delays in starting TB treatment, Xpert®MTB/RIF -negative patients were less likely to die during follow-up.	Compared to patients with Xpert positive TB diagnosed during pre-ART screening, Xpert negative cases had less advanced immunosuppression and less advanced TB and did not have adverse outcomes despite substantial delays in starting TB treatment.
68	2012	Primary care clinic, Cape Town, South Africa	Lawn and colleagues described the clinical characteristics of patients with pulmonary TB who tested negative by Xpert®MTB/RIF but had radiographically less advanced TB and fewer adverse outcomes compared with those who tested positive, prior to commencing antiretroviral therapy in Cape Town, South Africa (ref 67). In a primary care clinic based in the same city, and using archived samples from a prospectively recruited cohort, Theron et al. demonstrated Xpert®MTB/RIF –negative TB patients to have a significantly diminished SS bacillary load compared with those who tested positive. They also described the use of different tests, including chest radiography, when further investigating individuals. Theron's et al data imply that, contrary to what was suggested by Lawn and colleagues, Xpert®MTB/RIF was more likely to miss HIV-infected individuals with advanced immunosuppression. Given the small sample sizes of both studies, and the conflicting findings, the impact of declining CD4 count on sputum-based Xpert®MTB/RIF still remains unclear.	The findings from both studies need to be considered preliminary and context-specific, and further work, especially focusing on the outcome-related impact of extrapulmonary or disseminated TB in patients who have paucibacillary SS, is required.
69	2012	South Africa	Xpert®MTB/RIF –generated cycle-threshold (CT) values have poor clinical utility as a rule-in test for SS positivity (cut-point ≤20.2; SENS 32.3%, SPEC 97.1%) but moderately good rule-out value (cut-point >31.8; NPV 80.0%). Thus, 20% of individuals with CT values >31.8 were erroneously ruled out as SS-. This group had a significantly lower SS bacillary load relative to correctly classified SS+ patients (CT ≤ 31.8; P < .001).	Xpert®MTB/RIF -generated average CT values >31.8 provide moderately good rule-out value for SS positivity. Whether individuals falling above this cut-point, compared with those below, will display reduced disease transmission requires prospective validation. These data have public health implications for the roll-out of Xpert®MTB/RIF and inform public health and contact tracing strategies.

70	2012	Primary care clinic, Johannesburg, South Africa,	An unexpected case of R resistance was investigated on Xpert®MTB/RIF using repeat Xpert®MTB/RIF , SS microscopy, MTBDRplus® assay, C, DST, spoligotyping and rpoB gene sequencing. A false-positive result was most likely, given the wild type rpoB gene sequence and exclusion of both mixed infection and mixture of drug-susceptible and drug-resistant populations.	This report highlights the need for health care workers' understanding of assay performance characteristics when decentralising the diagnosis of drug-resistant TB. These issues should not, however, diminish enthusiasm for the Xpert®MTB/RIF assay.
71	2012	Tanzania	28/164 children (17.1%) had confirmed TB. Xpert®MTB/RIF detected 100% (95% CI, 59.0%–100%) of SS+ cases and 66.6% (95% CI, 43.0%–85.4%) of C+ but SS- cases. In the per-sample analysis, Xpert®MTB/RIF displayed a similar SENS (54.7% [95% CI, 42.7%–66.2%]) compared with C methods. Xpert®MTB/RIF detected 3-fold more confirmed TB cases than SS microscopy but with equal rapidity. Four additional cases (8.5%) with clinical TB but negative C were diagnosed by Xpert®MTB/RIF . Testing second and third samples increased SENS by 20% and 16%, respectively. When TB was reliably excluded, Xpert®MTB/RIF specificity was 100%. HIV infection did not affect diagnostic accuracy of Xpert®MTB/RIF. .	Xpert®MTB/RIF was easy to perform and displayed similar diagnostic accuracy as C methods in children with suspected TB. Rapid turnaround times should reduce treatment delay and improve patient outcome, although SENS remains suboptimal and access was dependent on local laboratory infrastructure.
72	2012	Italy	1,476 consecutive extra-pulmonary clinical specimens including both paediatric (494) and adult samples were investigated with Xpert®MTB/RIF. In comparison with a reference standard consisting of combination of C and clinical diagnosis of TB, an overall SENS and SPEC of 81.3% and 99.8%were found for Xpert®MTB/RIF while the SENS of microscopy was 48%. For biopsies, urines, pus and cerebrospinal fluids SENS exceeded 85% while it was slightly under 80% for gastric aspirates. SENS was lower than 50% for cavitary fluids. High SENS and SPEC (86.9% and 99.7% respectively) were also obtained for paediatric specimens.	Although the role of C remains central in the microbiological diagnosis of EPTB, the SENS of Xpert®MTB/RIF in rapidly diagnosing the disease made it a much better choice compared to SS microscopy. The ability of ruling out the disease still remained suboptimal.
73	2012	South Africa	535 children [median age 19 months, 117 (21·9%) HIV-infected] underwent one induced sputum (IS) and nasopharyngeal aspirate (NPA); 396 had two paired specimens. A positive SS, Xpert®MTB/RIF or C occurred in 30 (5.6%), 81 (15.1%) and 87 (16.3%) respectively. C yield was higher from IS (84/87, 96.6%) vs NPA (61/87, 70.1%, p<0.001). Amongst children with two paired specimens, 63 C confirmed cases occurred [60 (95.2%) IS vs. 48 (76.2%) NPA, p=0.002]. SENS of two Xpert®MTB/RIF tests was similar on IS and NPAs (45/63, 71% vs 41/63, 65%, p=0.444); SENS of SS was lower on IS (21/63, 33%) and NPA (16/63, 25%). Incremental yield from a second IS was 9 cases (17.6%) by C and 9 (25%) by Xpert®MTB/RIF ; a second NPA increased C yield by 10 (26.3%) and Xpert®MTB/RIF 11 (36.7%). Xpert SPEC was 99.1% (98.1 - 100) on IS and 98.2 (96.8 - 99.6) on NPAs. Xpert®MTB/RIF provided faster results than culture (median 0 vs 15 days, p<0·001).	Xpert®MTB/RIF on 2 NPAs was useful in children with suspected PTB particularly in settings where IS and culture were not feasible.
74	2012	Zambia	A model proposed to integrate TB and HIV screening, diagnosis, and treatment into existing antenatal care using Xpert®MTB/RIF technology (as per WHO recommendations).	Pilot studies were urgently required to evaluate strategies for the integration of TB screening into antenatal clinics using new diagnostic technologies, in order to reduce morbidity and mortality for both the mother and child, particularly in women who are co-infected with HIV.
98	2012	South Africa	In this diagnostic sub-study of a TB prevalence survey conducted in gold mining companies in South Africa, 6,893 participants provided a SS specimen. 187/6893 (2.7%) were positive for MTB in C, 144/6893 (2.1%) were positive for MTB by Xpert®MTB/RIF , and 91/6893 (1.3%) were positive for AFB by microscopy. SENS, SPEC, PPV and NPV for detection of MTB by Xpert®MTB/RIF were 62.6% (95% CI 55.2, 69.5), 99.6% (99.4, 99.7), 81.3% (73.9, 87.3), and 98.9 (98.6, 98.8); agreement between Xpert®MTB/RIF and C was 98.5% (98.2, 98.8). SENS of microscopy was 17.6% (12.5, 23.9). When individuals with a history of TB treatment were excluded from the analysis, Xpert®MTB/RIF SPEC was 99.8 (99.7, 99.9) and PPV was 90.6 (83.3, 95.4) for detection of MTB. For the testing scenario of 7000 specimens with 2.7% of specimens C+ for MTB, costs were $165,690 for Xpert®MTB/RIF and $115,360 for the package of microscopy plus C.	In the context of a TB prevalence survey, the Xpert®MTB/RIF diagnostic yield was substantially higher than that of microscopy yet lower than that of liquid C. Xpert may be useful as a sole test for TB case detection in prevalence surveys, particularly in settings lacking capacity for liquid C.

106	2012	Central hospital laboratory, South Korea	Xpert®MTB/RIF detected MTB in 71 (100%) specimens (32 SS+, 39 SS-). 100% (62/62) concordance with drug resistance confirmed by phenotypic method and 98.4% (61/62) concordance with sequencing. One specimen containing approximately 50% of mutant p.His526Tyr was falsely interpreted as wild-type. The minimal detection ratio was 5:1 of mutant vs. wild-type cells. The median time saved was 18.5 days (range 9-30) for the diagnosis of TB and 81.5 days (65-136) for R susceptibility in SS-, C+ patients.	Xpert®MTB/RIF showed: high SENS in detecting MTB with information on R resistance; rapid time to diagnosis compared to conventional tests. Location and number of mutations may affect test sensitivity.
107	2012	Central hospital laboratory, South Africa	Xpert®MTB/RIF was compared vs.Genotype® MTBDRplus (version 2) on SS+ and SS- patient specimens. 282 consecutive specimens were tested by the two new molecular assays and routine diagnostics. Both assays showed similar diagnostic performance characteristics. SEN of the Genotype® MTBDRplus (v2.0) and Xpert®MTB/RIF assay for the detection of C- MTB was 73.1% and 71.2% respectively; SPEC for both assays was 100%. Both assays diagnosed MTB in 57–58% of SS-cases suggesting that the performances depend on bacillary load. Detection of MTB in C- specimens confirmed that molecular-based assays should not be used for treatment monitoring. SENS and SPEC for R resistance detection was 100% in both assays. Genotype® MTBDRplus (v2.0) assay provided additional information on H susceptibility.	The Genotype® MTBDRplus (v2.0) assay will complement the Xpert®MTB/RIF screening assay by validating R susceptibility, providing information on H susceptibility and providing pharmacogenetic information useful in guiding treatment.
108	2012	Reference centre, Lima, Peru	Detection of TB by Xpert®MTB/RIF was compared to a composite reference standard of Löwenstein-Jensen (LJ) and liquid culture. Detection of R resistance was compared to the LJ proportion method. 131 patients were included, the median CD4 cell count was 154.5 cells/mm3 and 45 (34.4%) had TB. For TB detection among HIV patients, SENS of Xpert®MTB/RIF was 97.8% (95% CI 88.4–99.6) (44/45); SPEC was 97.7% (95% CI 91.9–99.4) (84/86); PPV was 95.7% (95% CI 85.5–98.8) (44/46); NPVwas 98.8% (95% CI 93.6–99.8) (84/85). Xpert®MTB/RIF detected 13/14 SS- TB cases, outperforming smear microscopy [97.8% (44/45) vs. 68.9% (31/45); p = 0.0002]. For R resistance detection, SENS of Xpert®MTB/RIF was 100% (95% CI 61.0–100.0) (6/6); SPEC was 91.0% (95% CI 76.4–96.9) (30/33); PPV was 66.7% (95% CI 35.4–87.9) (6/9); NPV was 100% (95% CI 88.7 –100.0) (30/30).	In HIV patients with a high clinical suspicion of TB, Xpert®MTB/RIF performed well for TB diagnosis and outperformed smear microscopy.
109	2012	District hospital, India	Performances of LED auramine fluorescent microscopy and Xpert®MTB/RIF for diagnosis of TB in HIV-infected patients were compared. Although at higher cost, Xpert®MTB/RIF outperformed LED fluorescent microscopy in all type of specimens, especially in cerebrospinal fluid where the number of positive results was increased 11 times. Pleural fluid, ascitic fluid, pus, and stool specimens also yielded positive results with the Xpert®MTB/RIF assay. When collecting two additional early-morning sputum samples, the increase in the number of positive results with the Xpert®MTB/RIF assay was lower than previously reported for HIV-infected patients. R resistance was observed in 2.2% of the cases.	Xpert®MTB/RIF assay can improve the rapid diagnosis of TB meningitis and other types of extrapulmonary tuberculosis in HIV-infected patients.
110	2012	Central Hospital, Tanzania	Among 219 enrolled contacts, the yield of active TB was 2.3%. SENS of SS microscopy was 60% (95%CI 14.7-94.7), SENS of Xpert®MTB/RIF MTB/RIF was 100% (95%CI 47.81-100.0).	As all C+ cases tested positive by Xpert®MTB/RIF on the first submitted sample, the evaluation of one sample only could be sufficient for TB diagnosis in this context.
111	2012	Reference Hospital Santiago, Chile	166 subjects were enrolled; 50.6% provided two sputum samples, 33.1% only one sputum sample and 16.3% a mouth wash sample. The prevalence of TB was 8.1% (13/160). Diagnostic SENS increased from 66.7% (95%CI 39.1-86.2) for SS to 91.7% (95%CI 64.6-98.5) for Xpert®MTB/RIF , with comparable SPEC at 98.6% (146/148, 95%CI 95.2-99.6) and 99.3% (147/148, 95%CI 96.3-99.9). Xpert®MTB/RIF allowed early detection of R resistance in 16.6% of cases, with rapid adjustment to MDR-TB treatment.	Xpert®MTB/RIF provided earlier TB diagnosis in 25% more cases than SS alone. Its implementation should be considered for TB diagnosis in HIV-positive patients even outside TB-endemic areas.
112	2012	South Africa	Xpert®MTB/RIF testing of two induced sputum specimens detected approximately 75% of children with C-confirmed disease. Urine lipoarabinomannan has shown promise as a rapid diagnostic in a subgroup of HIV-infected severely immunocompromised adults, but there have been no data in children so far.	The availability of Xpert®MTB/RIF was an important advance that could improve case detection in children and enable rapid detection of mycobacterial drug resistance.

Annex 1: Synopsis of the available studies on Xpert®MTB/RIF presented in the order they appear in the text for the following areas: assay evaluation, assay development, detection of extrapulmonary TB, detection of paediatric TB, diagnostic algorithms, use in prevalence surveys and quality assurance.

Legend:

AFB: Alcohol-acid fast bacilli
ART: Antiretroviral therapy
C+/-: Culture positive/negative
CFU: Colony Forming Unit
CI: Confidence Intervals
CT: Cycle-threshold
EQA: External quality assurance
H: Isoniazid
IQR: Interquartile range
LED: Light-emitting diode
MDR-TB: Multidrug-resistant TB
MTB: *Mycobacterium tuberculosis*
NPV: Negative predictive value
NTM: Non-tuberculous mycobacteria
PPV: Positive predictive value
R: Rifampicin
SENS: Sensitivity
SPEC: Specificity
SS+/-: Sputum smear positive/negative
TB: Tuberculosis
XDR-TB: Extensively drug-resistant TB