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Diagnostic Testing and Airborne-Isolation Outcomes After Implementation of an Xpert-First Reflex- Smear Policy: A Before-and-After Study

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Research Article

Keywords: Xpert, Xpert MTB/RIF Ultra, Pulmonary Tuberculosis, TB-PCR, Diagnostic stewardship

Posted Date: November 18th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11069795/v1

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Additional Declarations: No competing interests reported.

Abstract

Background:

The value of reducing routine acid-fast bacilli (AFB) smear testing where molecular testing is already widely used remains uncertain. We evaluated diagnostic testing and airborne-isolation outcomes after implementation of an Xpert-first, reflex-smear policy for suspected pulmonary tuberculosis (TB).

Methods:

We conducted a single-centre retrospective before-and-after study of adults evaluated for suspected pulmonary TB. The primary analysis compared 1 November 2023–31 October 2024 with 1 December 2024–30 November 2025, excluding November 2024 as the transition month. Extended follow-up continued through 31 March 2026. The revised policy reserved AFB smears for Xpert-positive specimens and permitted de-isolation after two negative Xpert results without awaiting negative smears. The primary outcome was AFB smears per 100 eligible admission episodes. Analyses accounted for patient clustering but were not adjusted for baseline covariates.

Results:

The primary analysis included 3,408 admission episodes (1,580 pre-implementation; 1,828 post-implementation). AFB smear utilization decreased from 121.5 to 59.8 per 100 episodes (rate ratio 0.49, 95%CI 0.46–0.52; P < 0.001). Culture-confirmed TB among episodes undergoing mycobacterial culture remained similar (4.5% vs 4.4%; P = 0.881), as did Xpert and culture utilization. Median airborne-isolation duration decreased from 54.6 to 43.3 hours (median difference − 11.2 hours, P < 0.001), and isolation > 48 hours decreased from 56.8% to 41.1%. Hospital length of stay and intensive care unit admission were similar between periods. Reduced AFB smear utilization persisted through March 2026 (121.5 vs 56.5 per 100 episodes; rate ratio 0.47, 95%CI 0.44–0.49), with similar culture-confirmed TB detection rates (4.5% vs 4.2%). Among 169 culture-confirmed TB episodes, the proportion without a smear was higher post-implementation (1.5% versus 10.9%), although the estimate was imprecise.

Conclusions:

In this uncontrolled before-and-after study, implementation of an Xpert-first policy was associated with lower AFB smear utilization and shorter airborne isolation. The uncontrolled design and unmeasured pathway adherence limit causal interpretation. Safety equivalence was not established. Therefore, continued monitoring of smear omission and clinical outcomes is warranted.

Background

Pulmonary tuberculosis (TB) remains an important diagnostic and infection-control challenge in hospitalized patients. Patients with suspected pulmonary TB commonly require airborne isolation while microbiological investigations are completed, consuming negative-pressure room capacity and laboratory resources, although only a minority ultimately have culture-confirmed TB.[1] Conventional acid-fast bacilli (AFB) smear microscopy provides a rapid indication of mycobacterial burden but has limited sensitivity for pulmonary TB and cannot reliably distinguish the *Mycobacterium tuberculosis* (MTB) complex from nontuberculous mycobacteria.[2] Despite these limitations, AFB smear status remains operationally relevant because it contributes to infectiousness assessment and local infection-control policy decisions regarding initiation of contact tracing.[3, 4] Nucleic acid amplification tests (NAATs), including Xpert MTB/RIF ultra, provide rapid and specific detection of the MTB complex and rifampicin resistance. Current World Health Organization recommendations support rapid molecular diagnostic tests as initial tests for adults with signs or symptoms of pulmonary TB, and American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention (ATS/CDC/IDSA) guidance similarly recommends NAAT on the initial respiratory specimen.[2, 5]

Previous studies have demonstrated potential health-system benefits of molecular-first testing. Lippincott et al. reported projected airborne-isolation durations of 68.0 hours with sputum smear microscopy, 20.8 hours with one Xpert test, and 41.2 hours with two Xpert tests.[6] Chaisson et al. subsequently estimated that replacing serial sputum AFB smears with a single Xpert test could reduce unnecessary isolation by approximately 35–45 hours.[7] Similarly, Cowan et al. found that Xpert-based strategies were more efficient at reducing airborne-isolation duration and were more cost-effective than serial smears among hospitalized patients with presumptive pulmonary TB.[8]

However, much of the existing literature has focused on diagnostic performance or the use of molecular testing to accelerate pulmonary TB exclusion and discontinuation of respiratory isolation, rather than on diagnostic stewardship of AFB smear utilization itself. Diagnostic stewardship involves modifying the ordering, performance, or reporting of diagnostic tests to improve diagnosis and treatment, with interventions intended to support the right test for the right patient at the right time.[9] In this study, diagnostic stewardship was operationalized as a deliberate modification of the pulmonary TB testing pathway: 1) sputum Xpert was prioritized as the initial rapid molecular test; 2) under the intended pathway, AFB smear microscopy was reserved for Xpert-positive episodes through a laboratory reflex workflow; and 3) mycobacterial culture was retained to detect Xpert-negative TB and provide isolates for phenotypic drug-susceptibility testing. The objective was to reduce routine or potentially redundant AFB smear testing while maintaining molecular testing and culture-based TB detection and examining selected clinical outcomes. The effects of this approach on microbiological detection and selected safety outcomes in routine practice remain uncertain.

In Singapore, Chew et al. demonstrated the clinical utility of Xpert among hospitalized patients with suspected pulmonary TB, including smear-negative disease (sensitivity 75.0%, specificity 99.5%, positive predictive value 94.7%, and negative predictive value 97.0%), but did not evaluate implementation of an Xpert-first/reflex-smear pathway or its effects on laboratory utilization and health-system outcomes.[10]

In a pragmatic before-and-after implementation study of 621 hospitalized patients, Chaisson et al. reported a shorter time to isolation discontinuation (2.9 vs 2.5 days), shorter hospital length of stay (LOS) (6.0 vs 4.9 days), sustained molecular-test adoption, and lower hospital costs after Xpert implementation, with estimated savings of US$13,347 per isolated TB-negative patient.[11] However, these studies primarily evaluated molecular testing as a means of accelerating TB exclusion and de-isolation rather than reducing AFB smear utilization as a primary diagnostic-stewardship outcome.

We therefore evaluated implementation of an Xpert-first diagnostic strategy with reflex AFB smear testing at a large tertiary hospital in Singapore. We assessed: 1) its association with AFB smear utilization, 2) whether any reduction was sustained during extended follow-up, 3) changes in airborne-isolation and hospital resource utilization, and 4) selected safety outcomes among admission episodes with culture-confirmed TB.

Methods

Study design and setting

We conducted a single-centre retrospective before-and-after cohort study at Ng Teng Fong General Hospital (NTFGH), a 700-bed tertiary-care teaching hospital in Singapore. Study subjects were identified using routinely collected clinical data from the Epic electronic medical record and microbiology laboratory records. The hospital admission episode was the unit of analysis. Individual patients could contribute more than one eligible admission episode, including episodes in both the pre- and post-implementation periods; statistical analyses therefore accounted for within-patient correlation. The NTFGH Institutional Review Board approved the study and waived the requirement for informed consent (JHC-RNR-2026-1005).

Study population

The cohort included hospitalized adults aged ≥ 18 years who underwent evaluation for suspected pulmonary TB during an eligible admission episode. Potentially eligible episodes were identified by at least one order for sputum Xpert testing, sputum AFB smear microscopy, or sputum mycobacterial culture, or by the institution of airborne isolation for suspected pulmonary TB. These test-ordering and isolation criteria served as operational proxies for evaluation of suspected pulmonary TB. Completion of Xpert testing was not required because Xpert utilization was itself evaluated as a measure of pathway uptake. All Xpert, AFB smear, and mycobacterial culture results included in the analysis were derived from sputum specimens. Patients were included regardless of their eventual diagnosis; the cohort therefore included both patients subsequently diagnosed with active pulmonary TB and those in whom TB was ultimately excluded.

Culture-confirmed TB was defined at the admission-episode level as identification of MTB complex from at least one sputum mycobacterial culture obtained during the admission. Multiple positive cultures

from the same admission were counted as a single culture-confirmed TB admission episode

Patients were excluded if they: 1) were evaluated exclusively for extrapulmonary TB without suspected pulmonary involvement, including isolated lymph-node, pleural, bone, genitourinary, or central nervous system TB, 2) had known active TB at admission including patients already receiving anti-TB treatment or transferred from another institution with an established TB diagnosis, 3) were evaluated solely for latent TB infection or screening without clinical suspicion of active disease, or 4) were aged < 18 years because of differences in diagnostic pathways and isolation practices.

Diagnostic strategy and comparator

Before November 2024, usual care consisted of concurrent upfront sputum Xpert and AFB smear testing, with both investigations generally performed during the initial microbiological evaluation of patients with suspected pulmonary TB. Sputum Xpert testing was performed using the Xpert MTB/RIF Ultra assay on the GeneXpert platform (Cepheid). The Xpert-first diagnostic pathway was implemented on 4 November 2024, with an operational transition period from 4 through 29 November 2024. For analytic purposes, study periods were assigned according to the admission date, and all episodes with admission dates from 1 through 30 November 2024 were excluded. Excluding the entire calendar month avoided assigning episodes occurring immediately before, during, or immediately after implementation to either comparison period. The post-implementation period began on 1 December 2024. Implementation was supported by a revised institutional diagnostic policy, hospital-wide clinician education, and a laboratory reflex workflow.

Under the intended pathway, sputum Xpert was performed as the initial rapid molecular test, and AFB smear microscopy was intended to be performed only when Xpert was positive. When Xpert and AFB smear were ordered concurrently, laboratory staff manually cancelled the smear if the Xpert result was negative. This process was not automated through the electronic medical record or laboratory information system. Adherence therefore depended on appropriate test ordering and manual laboratory workflow. Sputum mycobacterial culture remained part of the diagnostic evaluation and was not replaced by the Xpert-first strategy.

Before implementation, institutional microbiological criteria for discontinuing airborne isolation required at least two negative sputum AFB smear results and two negative sputum Xpert results. After implementation, two negative sputum Xpert results met the microbiological criteria for de-isolation without requiring negative AFB smear results. De-isolation decisions were made by the treating clinical team in accordance with hospital policy. When clinical suspicion of pulmonary TB persisted despite these criteria being met, the policy permitted de-isolation at the treating team's discretion while further diagnostic evaluation, including bronchoscopy when clinically indicated, was pursued. Adherence to these de-isolation criteria could not be verified at the admission-episode level.

Analysis periods

The primary analysis compared two equivalent 12-month periods: a pre-implementation period from 1 November 2023 to 31 October 2024 and a post-implementation period from 1 December 2024 to 30 November 2025. November 2024 was excluded as the analytic transition month containing the operational transition period. Equivalent observation periods were used to reduce potential bias from unequal follow-up duration and seasonal variation.

A secondary extended follow-up analysis used the same pre-implementation period but extended the post-implementation period from 1 December 2024 through 31 March 2026. This analysis assessed whether changes in diagnostic and resource utilization observed in the primary analysis were sustained during longer-term implementation. The extended follow-up also increased the number of observations available to assess relatively infrequent safety outcomes among patients with culture-confirmed TB.

Outcomes

The primary outcome was AFB smear utilization, defined as the total number of sputum AFB smears divided by the number of eligible admission episodes and expressed per 100 episodes. Secondary diagnostic and laboratory outcomes included the proportion of admission episodes in which a sputum AFB smear was performed; sputum Xpert and mycobacterial culture utilization; sputum Xpert, AFB smear, and mycobacterial culture positivity; and Xpert and AFB smear turnaround times.

Microbiological results were classified separately at the admission-episode level for Xpert, AFB smear, and mycobacterial culture. For each test type, an episode was classified as positive if at least one corresponding result during the admission was positive. It was classified as negative if all valid corresponding results were negative and no positive result was recorded. An episode was classified as invalid or indeterminate only when no valid positive or negative result was available and at least one corresponding invalid or indeterminate result was recorded. Episodes without a test of that type were classified as not tested and were not treated as negative. For episode-level result classification, multiple tests of the same type during one admission were counted once; all AFB smears remained included in the count-based primary outcome.

Health-system outcomes included the institution of airborne isolation, total active airborne-isolation time, discontinuation of the first isolation period within 24 hours, a first isolation period lasting > 48 hours, hospital LOS, and intensive care unit (ICU) admission. Total active airborne-isolation time was defined as the sum of all active isolation intervals during an admission and intervening periods without active isolation were excluded. Overlapping or contiguous isolation orders were merged and counted as a single interval. If the final isolation interval remained active at discharge or death, the corresponding discharge or death timestamp was used as the endpoint. Outcomes involving discontinuation within 24 hours and duration > 48 hours were based on the first isolation period and therefore reflected the initial de-isolation episode rather than total active isolation burden. Isolation-duration analyses were restricted

to admission episodes in which airborne isolation was instituted. Episodes with missing required timestamps were excluded from the corresponding analyses.

Safety outcomes were evaluated among sputum culture-confirmed TB admission episodes and included Xpert-positive culture-confirmed TB, AFB smear-positive culture-confirmed TB, Xpert-negative but culture-confirmed TB, culture-confirmed TB episodes in which no sputum AFB smear was performed, ICU admission, and hospital LOS.

Statistical analysis

Continuous variables were summarized as medians and interquartile ranges (IQRs), and categorical variables as counts (N) and percentages. The hospital admission episode was the primary unit of analysis. Because patients could contribute more than one eligible episode, including episodes in both study periods, analyses accounted for within-patient correlation but were not adjusted for baseline demographic or clinical covariates. The individual laboratory order was the unit of analysis for laboratory turnaround-time outcomes.

Baseline balance between the pre- and post-implementation periods was assessed using absolute standardized differences (ASDs), with values ≥ 0.10 considered potentially meaningful. Although continuous variables were presented as medians and IQRs, their ASDs were calculated as the difference in means divided by the pooled standard deviation. For categorical variables, ASDs were calculated using standardized differences in proportions; for variables with more than two categories, the largest absolute category-specific ASD was reported. Missing body mass index (BMI) values were not imputed, and BMI summaries were based on available observations.

Binary outcomes were analyzed using Poisson generalized estimating equations with a log link, an independence working correlation structure, and robust standard errors clustered by patient. Effect estimates were reported as post-implementation versus pre-implementation risk ratios (RRs) with 95% confidence intervals (CIs). The number of sputum AFB smears was aggregated at the admission-episode level and analyzed using the same Poisson framework. AFB smear utilization was expressed as the number of smears per 100 eligible admission episodes, with the intervention effect reported as a rate ratio.

Test-utilization percentages were calculated using all eligible admission episodes as the denominator. In the primary and extended diagnostic analyses, test-positivity percentages were calculated among admission episodes in which the corresponding test was performed. In the safety analyses, percentages were calculated using all culture-confirmed TB admission episodes as the denominator, irrespective of whether Xpert or AFB smear testing was performed. Isolation-duration threshold outcomes were calculated among admission episodes in which airborne isolation was instituted.

Continuous outcomes were compared using median differences, calculated as post-implementation minus pre-implementation. To account for repeated observations within patients, 95% CIs were

estimated using patient-cluster bootstrap resampling, in which patients were resampled and all eligible observations from each selected patient were retained.

Laboratory order-to-result time was calculated for each unique laboratory order as the interval from order placement to final result release. The post-implementation AFB smear analysis included both the original smear requests and additional reflex smear orders created after a positive Xpert result. For original requests, the original order-placement timestamp was used. For newly created reflex smear orders, timing began when the smear order was placed after Xpert positivity and therefore excluded the preceding Xpert-testing interval. Orders with missing timestamps or negative intervals were excluded.

No adjustment for multiple comparisons was applied to secondary outcomes, which were considered exploratory. All statistical tests were two-sided, with $P < 0.05$ considered statistically significant. The secondary extended follow-up analysis repeated selected diagnostic-testing, resource-utilization, isolation, and safety analyses using post-implementation follow-up through 31 March 2026. Statistical analyses were performed using SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Study population

The primary analysis included 3,408 eligible admission episodes: 1,580 pre-implementation episodes and 1,828 post-implementation episodes contributed by 3,172 unique patients (Fig. 1). There were 1,500 unique patients in the pre-implementation period and 1,727 in the post-implementation period; 55 patients contributed episodes in both periods (Table 1). Median age was 66.0 years (IQR 51.0–76.0) before implementation and 69.0 years (IQR 55.0–77.0) after implementation. Most baseline characteristics were well balanced between periods. Variables with an ASD ≥ 0.10 were age (ASD 0.123), Charlson Comorbidity Index (ASD 0.150), and diabetes mellitus (ASD 0.116). ASDs for sex, ethnicity, BMI, smoking status, chronic obstructive pulmonary disease, bronchiectasis, and end-stage renal disease requiring hemodialysis or chronic kidney disease stage 5 were < 0.10.

Primary effectiveness analysis

Diagnostic testing and laboratory resource-utilization outcomes are summarized in Table 2. Implementation of the sputum Xpert-first strategy was associated with a marked reduction in sputum AFB smear utilization. The proportion of admission episodes in which a sputum AFB smear was performed decreased from 92.1% (1,455/1,580) before implementation to 50.7% (927/1,828) after implementation (RR 0.55, 95% CI 0.52–0.58; $P < 0.001$). The number of sputum AFB smears decreased from 121.5 to 59.8 per 100 eligible admission episodes, corresponding to a rate ratio of 0.49 (95% CI 0.46–0.52; $P < 0.001$).

Table 2
Diagnostic testing and laboratory resource utilization

Outcome	All eligible admission episodes (N = 3,408)	Pre-implementation (November 2023 to October 2024) (N = 1,580)	Post-implementation (December 2024 to November 2025) (N = 1,828)	Effect estimate (95% CI)	P-value
Test Utilization					
AFB smears per 100 eligible admission episodes	88.4	121.5	59.8	Rate ratio 0.49 (0.46–0.52)	< 0.001*
Sputum Xpert performed	2,717 (79.7%)	1,256 (79.5%)	1,461 (79.9%)	RR 1.01 (0.97–1.04)	0.758
Sputum AFB smear performed	2,382 (69.9%)	1,455 (92.1%)	927 (50.7%)	RR 0.55 (0.52–0.58)	< 0.001*
Sputum mycobacterial culture performed	3,272 (96.0%)	1,525 (96.5%)	1,747 (95.6%)	RR 0.99 (0.98-1.00)	0.153
Test positivity among tested episodes					
Sputum Xpert positive	160 (5.9%)	75 (6.0%)	85 (5.8%)	RR 0.97 (0.72–1.32)	0.867
Sputum AFB smear positive	89 (3.7%)	47 (3.2%)	42 (4.5%)	RR 1.40 (0.93–2.11)	0.104
Sputum culture-confirmed TB among cultured episodes	144 (4.4%)	68 (4.5%)	76 (4.4%)	RR 0.98 (0.71–1.35)	0.881
Post-implementation testing pattern					
Minimum AFB smear-tested episodes with Xpert testing but no positive Xpert result recorded	-	-	475 (51.2%)	-	-

Outcome	All eligible admission episodes (N = 3,408)	Pre-implementation (November 2023 to October 2024) (N = 1,580)	Post-implementation (December 2024 to November 2025) (N = 1,828)	Effect estimate (95% CI)	P-value
Order-to-result time					
Sputum Xpert order-to-result time, hours	6.3 (1.9–21.4)	6.5 (2.2–22.2)	6.0 (1.7–20.8)	MD -0.5 (-1.5 to 0.2)	0.225
Sputum AFB smear order-to-result time, hours	4.8 (0.9–21.0)	6.2 (1.5–22.5)	2.4 (0.3–14.4)	MD -3.8 (-4.6 to -3.0)	< 0.001*

Data are presented as N (%) or median (IQR), unless otherwise indicated. Test-utilization percentages use all eligible admission episodes, whereas test-positivity percentages use episodes in which the corresponding test was performed; culture-confirmed TB percentages are calculated among episodes undergoing sputum mycobacterial culture. AFB smear utilization is expressed as the total number of smears per 100 eligible episodes. The post-implementation testing-pattern measure is a conservative lower-bound estimate derived from aggregate counts (927 − 85 − 367 = 475); 51.2% uses the 927 post-implementation smear-tested episodes as the denominator. This estimate assumes that all Xpert-positive and Xpert-untested episodes underwent smear testing. Order-to-result times are based on unique laboratory orders with valid, non-negative intervals; column N values refer to admission episodes. Effect estimates compare post-implementation with pre-implementation. Risk ratios are reported for binary outcomes, the rate ratio for AFB smear counts per episode, and median differences as post-implementation minus pre-implementation. Confidence intervals and P values account for patient clustering. *P < 0.05.

Abbreviations: AFB, acid-fast bacilli; CI, confidence interval; IQR, interquartile range; MD, median difference; RR, risk ratio; TB, tuberculosis. Xpert refers to the Xpert MTB/RIF Ultra assay for detecting Mycobacterium tuberculosis complex and rifampicin resistance.

Sputum Xpert utilization was similar before and after implementation (79.5% vs 79.9%; RR 1.01, 95% CI 0.97–1.04; P = 0.758), as was sputum mycobacterial culture utilization (96.5% vs 95.6%; RR 0.99, 95% CI 0.98–1.00; P = 0.153). Sputum Xpert positivity was 6.0% before implementation and 5.8% after implementation (RR 0.97, 95% CI 0.72–1.32; P = 0.867). Culture-confirmed TB detection among cultured episodes was also similar (4.5% vs 4.4%; RR 0.98, 95% CI 0.71–1.35; P = 0.881), as was sputum AFB smear positivity (3.2% vs 4.5%; RR 1.40, 95% CI 0.93–2.11; P = 0.104).

Post-implementation, 927 of 1,828 admission episodes underwent AFB smear testing, whereas only 85 of 1,461 Xpert-tested episodes were Xpert-positive; 367 episodes did not undergo Xpert testing. Even assuming that every Xpert-positive episode and every episode without Xpert testing underwent AFB smear testing, at least 475 of the 927 smear-tested episodes (51.2%) occurred among episodes with Xpert testing but no positive Xpert result recorded during the admission.

Median sputum Xpert order-to-result time was similar between periods (6.5 vs 6.0 hours; median difference − 0.5 hours, 95% CI − 1.5 to 0.2; P = 0.225). In contrast, median sputum AFB smear order-to-

result time decreased from 6.2 to 2.4 hours, corresponding to a median difference of − 3.8 hours (95% CI − 4.6 to − 3.0; P < 0.001).

Isolation and health-system outcomes

Isolation and health-system outcomes are shown in Table 3. The proportion of admission episodes in which airborne isolation was instituted was similar before and after implementation (51.8% vs 51.7%; RR 1.00, 95% CI 0.93–1.06; P = 0.935). Among isolated episodes, median total active airborne-isolation time decreased from 54.6 hours (IQR 35.7–91.0) before implementation to 43.3 hours (IQR 26.9–70.4) after implementation, corresponding to a median difference of − 11.2 hours (95% CI − 16.6 to − 6.9; P < 0.001).

Table 3
Airborne-isolation and hospital outcomes

Outcome	All eligible admission episodes (N = 3,408)	Pre-implementation (November 2023 to October 2024) (N = 1,580)	Post-implementation (December 2024 to November 2025) (N = 1,828)	Effect estimate (95% CI)	P-value
Airborne isolation instituted	1,764 (51.8%)	819 (51.8%)	945 (51.7%)	RR 1.00 (0.93–1.06)	0.935
Duration among isolated admission episodes					
Isolation duration, hours	47.1 (29.1–76.4)	54.6 (35.7–91.0)	43.3 (26.9–70.4)	MD -11.2 (-16.6 to -6.9)	< 0.001*
Airborne isolation discontinued within 24 hours	282 (16.0%)	109 (13.3%)	173 (18.3%)	RR 1.38 (1.10–1.72)	0.005*
Isolation duration > 48 hours	853 (48.4%)	465 (56.8%)	388 (41.1%)	RR 0.72 (0.66–0.80)	< 0.001*
Hospital outcomes					
Hospital LOS, days	7.9 (4.2–14.6)	7.9 (4.3–14.6)	7.8 (4.1–14.8)	MD -0.1 (-0.7 to 0.5)	0.841
ICU admission	426 (12.5%)	199 (12.6%)	227 (12.4%)	RR 0.99 (0.82–1.18)	0.877

Data are presented as N (%) or median (IQR). Percentages for airborne isolation and ICU admission and hospital length-of-stay summaries use all eligible admission episodes. Total active airborne-isolation time is the sum of all active intervals during an admission, excluding intervening periods without isolation. The ≤ 24-hour and > 48-hour outcomes refer to the first isolation period. Isolation-duration outcomes are restricted to episodes in which airborne isolation was instituted (overall, N = 1,764; pre-implementation, N = 819; post-implementation, N = 945); episodes with missing required timestamps were excluded. Effect estimates compare post- with pre-implementation. RRs are reported for binary outcomes and MDs as post-implementation minus pre-implementation. CIs and P values account for patient clustering. *P < 0.05.

Abbreviations: CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; LOS, length of stay; MD, median difference; RR, risk ratio.

The proportion of isolated episodes in which isolation was discontinued within 24 hours increased from 13.3% to 18.3% (RR 1.38, 95% CI 1.10–1.72; P = 0.005), whereas the proportion remaining isolated for > 48 hours decreased from 56.8% to 41.1% (RR 0.72, 95% CI 0.66–0.80; P < 0.001). Median hospital LOS was similar between periods (7.9 vs 7.8 days; median difference − 0.1 days, 95% CI − 0.7 to 0.5; P = 0.841), as was ICU admission (12.6% vs 12.4%; RR 0.99, 95% CI 0.82–1.18; P = 0.877).

Exploratory microbiological, process, and clinical outcomes among culture-confirmed TB episodes

Safety outcomes among sputum culture-confirmed TB admission episodes are summarized in Table 4. The primary-period safety analysis included 144 culture-confirmed TB episodes: 68 before implementation and 76 after implementation. Sputum Xpert was positive in 86.8% of episodes before implementation and 85.5% after implementation (RR 0.99, 95% CI 0.87–1.12; P = 0.827), whereas sputum AFB smear positivity was 58.8% and 48.7%, respectively (RR 0.83, 95% CI 0.61–1.12; P = 0.224).

Table 4

Microbiological, process, and clinical outcomes among culture-confirmed TB admission episodes

Outcome	All culture-confirmed TB episodes (N = 144)	Pre-implementation (November 2023 to October 2024) (N = 68)	Post-implementation (December 2024 to November 2025) (N = 76)	Effect estimate (95% CI)	P-value
Microbiological outcomes					
Sputum Xpert positive	124 (86.1%)	59 (86.8%)	65 (85.5%)	RR 0.99 (0.87–1.12)	0.827
Sputum AFB smear positive	77 (53.5%)	40 (58.8%)	37 (48.7%)	RR 0.83 (0.61–1.12)	0.224
Sputum Xpert negative	16 (11.1%)	7 (10.3%)	9 (11.8%)	RR 1.15 (0.45–2.93)	0.769
Process outcome					
No sputum AFB smear performed	9 (6.3%)	1 (1.5%)	8 (10.5%)	RR 7.16 (0.92–55.81)	0.060
Clinical outcomes					
ICU admission	12 (8.3%)	7 (10.3%)	5 (6.6%)	RR 0.64 (0.21–1.90)	0.422
Hospital LOS, days	7.9 (4.3–16.4)	7.3 (4.7–16.3)	7.9 (4.1–16.4)	MD 0.6 (-2.7 to 7.3)	0.800

Data are presented as N (%) or median (IQR). Percentages use all culture-confirmed TB admission episodes in each column, irrespective of whether sputum Xpert or AFB smear testing was performed. Culture-confirmed TB was defined as identification of *Mycobacterium tuberculosis* complex in at least one sputum culture during the admission; multiple positive cultures within the same admission were counted as one episode. Effect estimates compare post-implementation with pre-implementation. Risk ratios are reported for binary outcomes, and median differences as post-implementation minus pre-implementation. Confidence intervals and P values account for patient clustering.

Abbreviations: AFB, acid-fast bacilli; CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; LOS, length of stay; MD, median difference; RR, risk ratio; TB, tuberculosis. Xpert refers to the Xpert MTB/RIF Ultra assay.

Xpert-negative but culture-confirmed TB occurred in 7 of 68 episodes (10.3%) before implementation and 9 of 76 episodes (11.8%) after implementation (RR 1.15, 95% CI 0.45–2.93; P = 0.769). The

proportion of culture-confirmed TB episodes without a sputum AFB smear was 1.5% (1/68) before implementation and 10.5% (8/76) after implementation (RR 7.16, 95% CI 0.92–55.81; P = 0.060). ICU admission occurred in 7 of 68 episodes (10.3%) before implementation and 5 of 76 episodes (6.6%) after implementation (RR 0.64, 95% CI 0.21–1.90; P = 0.422). Median hospital LOS was 7.3 versus 7.9 days (median difference 0.6 days, 95% CI − 2.7 to 7.3; P = 0.800).

Extended follow-up analysis

The secondary extended follow-up analysis retained the same pre-implementation period and extended post-implementation follow-up through 31 March 2026. Selected diagnostic, resource-utilization, and isolation outcomes are presented in Supplementary Table S1. The reduction in sputum AFB smear utilization persisted during extended follow-up. The proportion of admission episodes in which a sputum AFB smear was performed decreased from 92.1% before implementation to 48.1% during extended post-implementation follow-up (RR 0.52, 95% CI 0.50–0.55; P < 0.001). The number of sputum AFB smears decreased from 121.5 to 56.5 per 100 eligible admission episodes, corresponding to a rate ratio of 0.47 (95% CI 0.44–0.49; P < 0.001).

Sputum Xpert utilization remained similar between periods (79.5% vs 80.1%; RR 1.01, 95% CI 0.98–1.04; P = 0.654), as did sputum mycobacterial culture utilization (96.5% vs 95.6%; RR 0.99, 95% CI 0.98–1.00; P = 0.145) and culture-confirmed TB detection among cultured episodes (4.5% vs 4.2%; RR 0.95, 95% CI 0.70–1.29; P = 0.748). The use of airborne isolation also remained similar during extended follow-up (51.8% vs 52.0%; RR 1.00, 95% CI 0.94–1.07; P = 0.935). However, median isolation duration remained shorter after implementation, decreasing from 54.6 to 42.6 hours (median difference − 12.0 hours, 95% CI − 17.3 to − 7.7; P < 0.001).

Extended safety analysis

Extended safety outcomes through March 2026 are presented in Supplementary Table S2. This analysis included 169 culture-confirmed TB admission episodes: 68 before implementation and 101 after implementation. Sputum Xpert positivity remained similar between periods (86.8% vs 86.1%; RR 0.99, 95% CI 0.88–1.12; P = 0.906), as did sputum AFB smear positivity (58.8% vs 48.5%; RR 0.82, 95% CI 0.62–1.10; P = 0.184). Xpert-negative but culture-confirmed TB occurred in 7 of 68 episodes (10.3%) before implementation and 12 of 101 episodes (11.9%) after implementation (RR 1.15, 95% CI 0.48–2.79; P = 0.750).

The proportion of culture-confirmed TB episodes without a sputum AFB smear was 1.5% (1/68) before implementation and 10.9% (11/101) after implementation (RR 7.41, 95% CI 0.98–56.08; P = 0.053). ICU admission occurred in 10.3% of episodes before implementation and 6.9% after implementation (RR 0.67, 95% CI 0.25–1.83; P = 0.437). Median hospital LOS was 7.3 versus 8.8 days (median difference 1.5 days, 95% CI − 2.4 to 5.6; P = 0.435).

Discussion

In this single-centre retrospective before-and-after diagnostic-stewardship study, implementation of a sputum Xpert-first strategy with reflex AFB smear testing was associated with a substantial reduction in AFB smear utilization. In the primary analysis comparing two 12-month periods, AFB smear use decreased from 121.5 to 59.8 smears per 100 eligible admission episodes, an approximately 51% relative reduction. This reduction remained evident when post-implementation follow-up was extended through March 2026. No statistically significant differences were observed in Xpert utilization, mycobacterial culture utilization, or culture-confirmed TB detection among cultured episodes. The proportion of episodes in which airborne isolation was instituted also remained similar. Among isolated episodes, median isolation duration decreased by 11.2 hours, and the proportion lasting > 48 hours decreased from 56.8% to 41.1%. Hospital LOS and ICU admission did not differ significantly.

Our findings are consistent with previous Xpert-based evaluations and extend the evidence to diagnostic stewardship of AFB smear use.[6, 8, 11] Whereas earlier studies focused primarily on diagnostic accuracy, TB exclusion, or de-isolation, we evaluated actual AFB smear utilization as the primary outcome after implementation of an Xpert-first/reflex-smear pathway in a setting where Xpert testing was already widely used. The reduction in isolation duration was directionally consistent with that reported by Chaisson et al.[11] Campbell et al.'s meta-analysis reported a pooled reduction in median airborne-isolation duration of 38.8 hours (95% CI 20.2–57.3) with Xpert testing of a single unconcentrated respiratory specimen compared with serial AFB smears.[12] Differences in baseline testing strategies and diagnostic workflows limit direct comparison of effect sizes. Continued collection of mycobacterial cultures remains important for detecting Xpert-negative TB and obtaining isolates for phenotypic drug-susceptibility testing.[5]

In our study, Xpert utilization remained approximately 80%, and median Xpert order-to-result time changed little (6.5 vs 6.0 hours) (Table 2). Nevertheless, the revised policy allowed de-isolation after two negative Xpert results without waiting for two negative AFB smears. Laboratory turnaround time did not capture the intervals from initiation of airborne isolation to specimen collection, completion of serial testing, or the clinical de-isolation decision. Shorter isolation may therefore reflect fewer results being awaited and changes in test sequencing or clinician practice rather than faster Xpert reporting (Table 3). Isolation duration was not stratified by Xpert result, so the mechanism underlying the shorter isolation duration remains uncertain.

The discrepancy between the intended reflex workflow and observed test utilization suggests partial or variable pathway uptake (Table 2). Possible explanations include the collection or processing of AFB smears before Xpert results became available, failure or delay in manually cancelling concurrent smear orders, and clinician-directed testing outside the intended pathway. The aggregate data could not distinguish among these explanations. Hospital-wide clinician education may have influenced ordering and de-isolation decisions, while the laboratory reflex workflow and manual cancellation of smears after negative Xpert results may have reduced unnecessary microscopy. Their individual contributions could not be evaluated.

The shorter AFB smear order-to-result time also requires cautious interpretation because the post-implementation analysis included reflex orders placed after Xpert positivity (Table 2). For these reflex orders, the measured interval excluded the preceding Xpert-testing interval and therefore did not represent the time required to complete the full diagnostic pathway. Operationally, shorter isolation could make negative-pressure rooms available sooner and reduce the burden of isolation for patients and staff. However, shorter isolation was not accompanied by shorter hospital LOS, and the study did not quantify changes in bed availability, staff workload, or costs.

The safety findings also require cautious interpretation. Among culture-confirmed TB episodes, the proportion without an AFB smear was higher after implementation in both the primary analysis (1/68 [1.5%] vs 8/76 [10.5%]; RR 7.16, 95% CI 0.92–55.81) and the extended analysis (1/68 [1.5%] vs 11/101 [10.9%]; RR 7.41, 95% CI 0.98–56.08) (Table 4). Both confidence intervals were wide and included 1, reflecting sparse events, particularly the single pre-implementation episode without a smear. Smear omission is a process finding and does not itself demonstrate clinical harm or pathway non-adherence. Under the intended pathway, omission could be appropriate after a negative Xpert result, even if culture subsequently confirmed TB. The aggregate data could not distinguish these episodes from those in which an indicated reflex smear was omitted despite a positive Xpert result.

No statistically significant differences were observed in Xpert-negative culture-confirmed TB, ICU admission, or hospital LOS, but these findings do not establish safety equivalence (Table 4). Gandhi et al.'s meta-analysis reported pooled sensitivities of 86% and 92% for one- and two-specimen Xpert strategies, respectively, supporting consideration of repeat molecular testing when clinical suspicion persists after an initial negative result.[13] The continuing role of smear status in infectiousness assessment and contact-tracing decisions supports retaining reflex AFB smear testing after a positive Xpert result.[2, 3] Continued monitoring of pathway adherence and clinical outcomes, together with larger multi-centre evaluations, is warranted.

This study has several strengths. First, it evaluates an implemented clinical pathway rather than a hypothetical diagnostic strategy. Second, the primary analysis used equivalent 12-month pre- and post-implementation periods, reducing differences arising from unequal observation duration and seasonal variation. Third, the extended follow-up enabled assessment of sustainability beyond the primary post-implementation period. Fourth, repeated admission episodes were accounted for using patient-clustered statistical methods. Finally, safety was evaluated specifically among culture-confirmed TB admission episodes rather than inferred solely from overall laboratory utilization.

Several limitations should be considered. First, this was a retrospective single-centre before-and-after study without a contemporaneous control hospital, limiting causal inference. Secular trends, changes in clinical practice, and other time-varying factors could not be fully separated from the intervention's effects. Second, although the pre- and post-implementation periods each comprised 12 months, baseline differences remained in age, Charlson Comorbidity Index, and DM (ASD ≥ 0.10; Table 1). Patient-clustered methods accounted for repeated admissions but did not address these differences because

comparisons were not adjusted for baseline covariates and residual confounding may therefore have contributed to the observed associations. Third, despite the exclusion criteria, the broad evaluation cohort may have included admission episodes in which mycobacterial testing was undertaken for indications other than suspected active pulmonary TB, including possible nontuberculous mycobacterial disease. Routinely extracted data may not fully capture the clinical indication for testing or the reasons for deviation from the intended pathway.

Fourth, implementation fidelity could not be measured directly. Although aggregate counts imply that at least 475 post-implementation AFB smear-tested episodes had no positive Xpert result recorded during the admission (Table 2), the data could not establish whether these smears were obtained before Xpert results became available, reflected failures in manual cancellation, or represented clinically directed deviations from the intended pathway. Consequently, this study evaluates real-world implementation of an Xpert-first policy rather than complete adherence to a reflex-only protocol. Fifth, safety analyses were restricted to culture-confirmed TB admission episodes (Table 4) and therefore could not assess TB that remained undiagnosed, delayed treatment initiation, inappropriate de-isolation, TB diagnosed only after discharge, or onward transmission. The absence of statistically significant differences in the measured outcomes should not be interpreted as evidence of safety equivalence or the absence of clinically important harm. Finally, the extended analysis was cumulative and included the primary post-implementation period (Supplementary tables 1 and 2). It was not an independent replication and could not establish stable month-to-month pathway uptake. Changes in bed availability, staff workload, and costs were not quantified, leaving the broader operational effects uncertain.

Conclusions

Implementation of an Xpert-first strategy with reflex AFB smear testing was associated with reductions in AFB smear utilization and shorter airborne-isolation duration. Xpert utilization, mycobacterial culture utilization, and culture-confirmed TB detection remained broadly similar in the primary and extended analyses. These findings suggest that the pathway may reduce routine microscopy use and support more efficient use of isolation resources in routine practice. The proportion of culture-confirmed TB episodes without an AFB smear was higher after implementation. However, the estimates were imprecise due to the limited number of events and the inability to distinguish between appropriate omission after a negative Xpert result and missed reflex testing after a positive result. The study therefore does not establish safety equivalence. Continued episode-level monitoring of reflex-smear adherence and clinical outcomes, together with larger multi-centre evaluations, is warranted.

Abbreviations

AFB, acid-fast bacilli

ASD, absolute standardized difference

ATS, American Thoracic Society

BMI, body mass index

CDC, Centers for Disease Control and Prevention

CI, confidence interval

CKD, chronic kidney disease

COPD, chronic obstructive pulmonary disease

DM, diabetes mellitus

EMR, electronic medical record

ESRD, end-stage renal disease

GEE, generalized estimating equation

HD, hemodialysis

ICU, intensive care unit

IDSA, Infectious Diseases Society of America

IQR, interquartile range

LOS, length of stay

MD, median difference

MTB, *Mycobacterium tuberculosis*

NAAT, nucleic acid amplification test

NTFGH, Ng Teng Fong General Hospital

PCR, polymerase chain reaction

RR, risk ratio

TB, tuberculosis

TB-PCR, tuberculosis polymerase chain reaction

WHO, World Health Organization

Xpert MTB/RIF Ultra, Xpert *Mycobacterium tuberculosis*/rifampicin assay

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Ng Teng Fong General Hospital, National University Health System, Singapore (reference: JHC-RNR-2026–1005). The requirement for informed consent was waived by the Institutional Review Board because the study involved retrospective analysis of de-identified electronic medical records.

Consent for publication

Not applicable. No identifiable individual participant information is presented.

Competing interests

The authors declare that they have no competing interests.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contribution

WHKC: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, and writing—original draft.DA and WJDO: Conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, and writing—original draft.NCAL and RVST: Conceptualization, investigation, validation, visualization, and writing—review and editing.NSWTT: Conceptualization, project administration, supervision, and writing—review and editing.SGDC: Conceptualization, data curation, project administration, resources, supervision, validation, visualization, and writing—review and editing.All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Availability of data and materials

The datasets analysed during this study are not publicly available because of institutional data-governance and patient-privacy requirements governing electronic medical records within the National University Health System, Singapore. De-identified data may be available from the corresponding author upon reasonable request, subject to Institutional Review Board approval and institutional data-governance policies.

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Figures

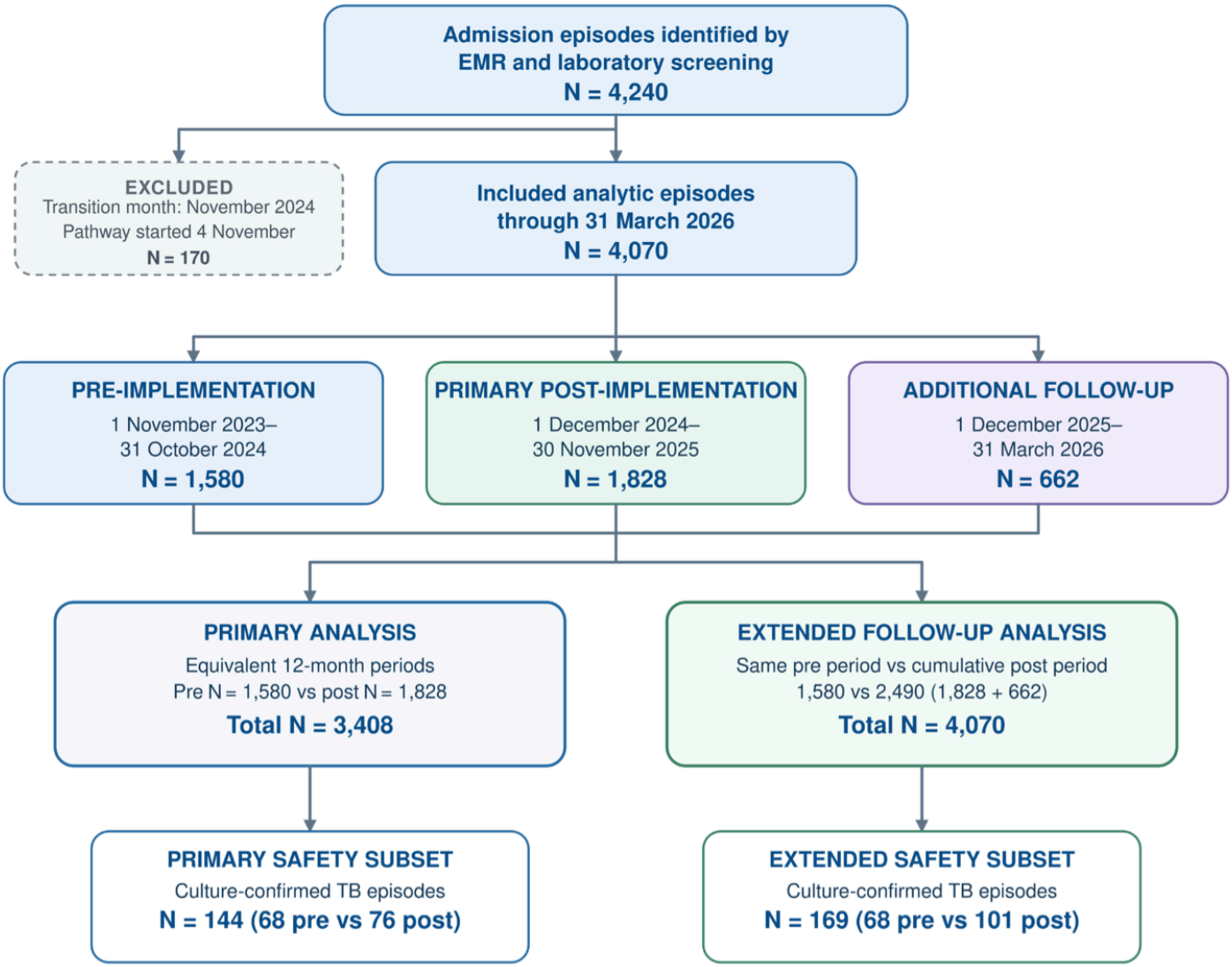


Figure 1

Of 4,240 identified admission episodes, 170 with admission dates during the analytically excluded month of November 2024 were excluded, leaving 4,070 episodes. The pathway was implemented on 4 November 2024, with operational transition continuing through 29 November 2024. The primary analysis compared 1,580 pre-implementation with 1,828 post-implementation episodes (N=3,408). Extended follow-up added 662 episodes, resulting in 2,490 cumulative post-implementation episodes and 4,070 episodes overall. The primary and extended safety subsets included 144 and 169 culture-confirmed TB episodes, respectively. Patients could contribute multiple admission episodes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx