Research Square

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Diagnostic accuracy of the MultNAT MTC/RIF assay for rapid detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective comparative study

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Abstract

Purpose

Tuberculosis (TB) remains a leading cause of morbidity and mortality worldwide, with diagnostic gaps most pronounced in extrapulmonary disease and resource-limited settings. Although the WHO-endorsed Xpert MTB/RIF assay revolutionized TB diagnostics, its cost, infrastructure requirements, and suboptimal sensitivity in paucibacillary specimens constrain accessibility. The MultNAT MTC/RIF assay is a newly developed dual-target, cartridge-based molecular platform designed to overcome these limitations and to support decentralized molecular testing in routine clinical laboratories.

Methods

We conducted a prospective diagnostic accuracy study among 414 patients with presumptive TB at a tertiary-care hospital serving both community and referral populations. Clinical specimens—including sputum, bronchoalveolar lavage fluid (BALF), pleural effusion, and pus—were analyzed using MultNAT MTC/RIF, Xpert MTB/RIF (reference comparator), ZEESAN, and CapitalBio assays. Diagnostic accuracy was evaluated by positive percent agreement (PPA), negative percent agreement (NPA), predictive values, overall percent agreement (OPA), and Cohen's κ, with subgroup analyses by specimen type. Rifampicin resistance detection was also assessed.

Results

Among the 414 specimens analyzed, 182 were sputum (43.9%), 182 bronchoalveolar lavage fluid (BALF, 43.9%), 27 pleural effusion (6.5%), and 23 pus (5.6%) samples. For MTC detection compared with Xpert, MultNAT achieved excellent PPA of 96.55% (95% CI 93.32–98.50) and NPA of 98.35% (95% CI 95.26–99.66), yielding an OPA of 97.34% and κ = 0.95. Critically, PPA remained high (≥ 91.7%) across all extrapulmonary samples. For rifampicin resistance, MultNAT demonstrated a PPA of 93.75% (95% CI 69.77–99.84) and an NPA of 99.50% (95% CI 97.26–99.99), surpassing CapitalBio.

Conclusion

MultNAT MTC/RIF assay combines high analytical accuracy with rapid (< 2 h) turnaround and ambient reagent stability, providing reliable detection of TB and rifampicin resistance across diverse specimen types. These features suggest strong potential for decentralized implementation in resource-constrained settings.

Introduction

Tuberculosis (TB) remains a persistent global health challenge, responsible for an estimated 10.8 million new infections and 1.25 million deaths in 2023 [1]. Despite progress in expanding diagnostic coverage, early and accurate detection continues to lag behind incidence, a gap felt most acutely in low-income and decentralized settings [2–3]. This diagnostic challenge is compounded by extrapulmonary TB, which accounts for 15–20% of cases and presents difficulties due to low bacillary loads and variable clinical manifestations [4–5]. Timely diagnosis is critical for effective patient management, for interrupting transmission, and for achieving the WHO End TB Strategy targets. Molecular nucleic acid amplification tests (NAATs) have revolutionized this landscape, enabling simultaneous detection of Mycobacterium tuberculosis complex (MTBC) and resistance to key drugs like rifampicin [6–7]. The Xpert MTB/RIF assay, endorsed by the WHO in 2010, remains the cornerstone of rapid TB detection [8]. Its widespread application in peripheral laboratories, however, is restricted by high per-test costs, reliance on stable electricity, and diminished sensitivity in paucibacillary specimens [9]. While the subsequent Xpert MTB/RIF Ultra improved analytical sensitivity using multicopy targets (IS6110 and IS1081), it has faced challenges with reduced specificity in some low-prevalence settings [10–11].

The MultNAT MTC/RIF assay is a new, fully automated, cartridge-based molecular diagnostic optimized for point-of-care use. To enhance both analytical sensitivity and operational feasibility, the platform integrates several key features: dual-target amplification (IS6110 and IS1081), ultrasonic lysis for rapid nucleic-acid release, and vitrified, ambient-stable reagents that eliminate cold-chain dependency. Here, we report a prospective comparative study evaluating MultNAT MTC/RIF assay

performance against the WHO-endorsed Xpert MTB/RIF and two widely used domestic molecular assays (ZEESAN and CapitalBio). Using both pulmonary and extrapulmonary specimens, we assessed diagnostic accuracy for MTBC detection and rifampicin resistance, using Xpert MTB/RIF as the primary comparator.

Materials and Methods

Study Design and Participants

We performed a prospective diagnostic accuracy study at Xiamen Xinglin Hospital (Xiamen, China), a tertiary-care center serving both community and referral populations. Consecutive patients presenting between 1 June 2024 and 30 June 2025 with clinical or radiological suspicion of tuberculosis were enrolled. Inclusion criteria comprised age ≥ 15 years and provision of at least one diagnostic specimen suitable for molecular testing. Patients were excluded if they had received antituberculosis therapy for ≥ 7 days, had insufficient specimen volume, or provided visibly contaminated samples. Written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of Xiamen Xinglin Hospital (Approval No. [2025] KY-EC-091) and conducted in accordance with the Declaration of Helsinki.

Specimen collection and processing

Specimen types included sputum, bronchoalveolar lavage fluid (BALF), pleural effusion, and pus. Each sample was divided into aliquots for parallel testing by MultNAT MTC/RIF, Xpert MTB/RIF, ZEESAN, and CapitalBio assays.

Xpert MTB/RIF Assay

Specimens were mixed with Sample Reagent (SR) at a 1:2 ratio, vortexed, and incubated at room temperature for 15 min with intermittent agitation. Two milliliters of liquefied material were transferred into a cartridge for automated processing on the GeneXpert instrument, following the manufacturer's instructions.

MultNAT MTC/RIF Assay

Samples were liquefied with 4% NaOH at a 1:4 ratio, vortexed for ≥ 10 s, and 1 mL of the treated specimen was transferred to the Internal Control tube. Ultrasonic lysis was performed using an ultrasonic processor, followed by addition of the provided buffer. After thorough mixing, 1 mL of lysate was transferred into the MultNAT cartridge. DNA Extraction Solution was mixed until no visible brown sediment was present, then transferred to the cartridge and secured the cap. Finally, automated extraction, amplification, and detection were then executed on the MultNAT analyzer, with software generating reports indicating TB detection and rifampicin-resistance status, as shown in Fig. 1.

ZEESAN MTB Test

DNA extraction and amplification followed the manufacturer's protocol. Purified DNA was mixed with lyophilized amplification reagents and subjected to PCR under standardized cycling conditions. Results were interpreted automatically by the ZEESAN system.

CapitalBio Assay

DNA was extracted using the proprietary kit, amplified by asymmetric PCR on the E-Cycler™ 96 system, and hybridized on a microarray slide (BioMixer II). Slides were washed and dried on the SlideWasher-8, scanned with a LuxScan 10K/D scanner, and analyzed using CapitalBio's software. The entire procedure was completed in under 9 hours, allowing simultaneous processing of up to four samples for *Mycobacterium tuberculosis* identification and two samples for drug resistance profiling.

Reference Standard and Definitions

Because culture data were unavailable for all participants, Xpert MTB/RIF served as the primary comparator. Indeterminate or invalid results were repeated once; persistent indeterminate outcomes were documented but excluded from 2 × 2 accuracy calculations. Patients were clinically classified as TB, nontuberculous mycobacterial (NTM) infection, or other non-mycobacterial disease according to clinical, radiological data.

Statistical Analysis

Diagnostic performance metrics—including positive percent agreement (PPA), negative percent agreement (NPA), positive predictive value (PPV), negative predictive value (NPV), overall percent agreement (OPA), and Cohen's κ (95% confidence interval [CI])—were computed using Wilson 95% CIs. McNemar's test assessed paired differences between assays. P values < 0.05 were considered statistically significant. Subgroup analyses were conducted by specimen type (sputum, BALF, pleural effusion, pus). Statistical analyses were performed with SPSS v27 (IBM Corp., Armonk, NY, USA).

Results

Study Population

A total of 414 patients were enrolled in this study, including 112 women (27.05%) and 302 men (72.95%), with a median age of 53 years (IQR, 42–63 years). As summarized in Table 1, specimens included 182 sputum (43.96%), 182 bronchoalveolar lavage fluid (BALF, 43.96%), 27 pleural effusion (6.52%), and 23 pus (5.56%) samples.

Table 1 Demographic and clinical characteristics of enrolled participants and collected specimens (N = 414)

Variable	n (%)
Sex	
Female	112 (27.05)
Male	302 (72.95)
Age (years)	
Median (IQR)	53 (42–63)
≤24	38 (9.17)
25–44	85 (20.53)
45–64	172 (41.55)
≥65	119 (28.74)
Specimen type	
Sputum	182 (43.96)
BALF	182 (43.96)
Pleural effusion	27 (6.52)
Pus	23 (5.56)

Abbreviations: BALF bronchoalveolar lavage fluid; NTM non-tuberculous mycobacteria; TB tuberculosis.

Overall diagnostic performance

When compared with the Xpert MTB/RIF assay, the MultNAT MTC/RIF assay demonstrated the highest overall diagnostic accuracy (Table 2). The MultNAT assay achieved a PPA of 96.55% (95% CI 93.32—98.50) and a NPA of 98.35% (95% CI 95.26–

99.66), yielding an overall percent agreement of 97.34% (95% CI 95.30–98.67) and κ = 0.95 (95% CI 0.91–0.98), indicating almost perfect concordance. In contrast, ZEESAN and CapitalBio yielded substantially lower PPA—80.17% and 74.14%, respectively—though their NPA remained high (96.70% and 98.35%). McNemar's test revealed no significant difference between MultNAT and Xpert (P >0.05), while both ZEESAN and CapitalBio differed significantly (P <0.05).

Table 2 Overall diagnostic performance of three molecular assays compared with Xpert MTB/RIF assay

Assay	TP	FN	FP	TN	PPA % (95% CI)	NPA % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	OPA % (95% CI)	κ (95% CI)	*P*-value
MultNAT	224	8	3	179	96.55 (93.32–98.50)	98.35 (95.26–99.66)	98.68 (96.19–99.73)	95.72 (91.75–98.14)	97.34 (95.30–98.67)	0.95 (0.91–0.98)	ns
ZEESAN	186	46	6	176	80.17 (74.45–85.10)	96.70 (92.96–98.78)	96.88 (93.32–98.84)	79.28 (73.35–84.41)	87.44 (83.86–90.48)	0.75 (0.69–0.81)	<0.05
CapitalBio	172	60	3	179	74.14 (68.00–79.65)	98.35 (95.26–99.66)	98.29 (95.07–99.64)	74.90 (68.90–80.26)	84.78 (80.95–88.10)	0.70 (0.64–0.77)	<0.05

Abbreviations: PPA, positive percent agreement; NPA, negative percent agreement; TP, true positive; FN, false negative; FP, false positive; TN, true negative; PPV, positive predictive value; NPV, negative predictive value; OPA, overall percent agreement; κ, Cohen’s kappa; CI, confidence interval; ns, not significant (P >0.05).

P values were calculated using McNemar's test for paired comparisons with Xpert MTB/RIF as reference; 95% CIs were calculated by the Wilson method.

Performance by specimen type

We further performed a stratified analysis to evaluate the diagnostic performance of the three assays across the four primary specimen types as shown in Table 3. The MultNAT assay maintained consistently high accuracy, with PPA ranging from 91.67% to 97.46% and NPA from 95.31% to 100.00%. None of the subgroup comparisons differed significantly from Xpert MTB/RIF (all P >0.05). By contrast, ZEESAN and CapitalBio displayed marked specimen-dependent variability, with substantial PPA loss in extrapulmonary specimens. In pleural effusion, PPA declined to 58.33% and 33.33%, respectively, and to 58.33% for both assays in pus samples. Despite retaining NPA >90%, κ values fell below 0.57, indicating only moderate agreement.

Table 3 Diagnostic performance of molecular assays stratified by specimen type compared with Xpert MTB/RIF assay

Specimen type	Assay	TP	FN	FP	TN	PPA % (95% CI)	NPA % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	OPA % (95% CI)	κ (95% CI)	*P*-value
Sputum	MultNAT	115	3	3	61	97.46 (92.75–99.47)	95.31 (86.91–99.02)	97.46 (92.75–99.47)	95.31 (86.91–99.02)	96.70 (92.96–98.78)	0.93 (0.87–0.98)	ns
	ZEESAN	102	16	4	60	86.44 (78.92–92.05)	93.75 (84.76–98.27)	96.23 (90.62–98.96)	78.95 (68.08–87.46)	89.01 (83.54–93.16)	0.77 (0.67–0.86)	<0.05
	CapitalBio	100	18	2	62	84.75 (76.97–90.70)	96.88 (89.16–99.62)	98.04 (93.10–99.76)	77.50 (66.79–86.09)	89.01 (83.54–93.16)	0.77 (0.68–0.86)	<0.05
BALF	MultNAT	87	3	0	92	96.67 (90.57–99.31)	100.00 (96.07–100.00)	100.00 (95.85–100.00)	96.84 (91.05–99.34)	98.35 (95.26–99.66)	0.97 (0.93–1.00)	ns
	ZEESAN	70	20	1	91	77.78 (67.79–85.87)	98.91 (94.09–99.97)	98.59 (92.40–99.96)	81.98 (73.55–88.63)	88.46 (82.90–92.71)	0.77 (0.68–0.86)	<0.05
	CapitalBio	61	29	1	91	67.78 (57.10–77.25)	98.91 (94.09–99.97)	98.39 (91.34–99.96)	75.83 (67.17–83.18)	83.52 (77.31–88.59)	0.67 (0.57–0.77)	<0.05
Pleural effusion	MultNAT	11	1	0	15	91.67 (61.52–99.79)	100.00 (78.20–100.00)	100.00 (71.51–100.00)	93.75 (69.77–99.84)	96.30 (81.03–99.91)	0.92 (0.78–1.00)	ns
	ZEESAN	7	5	0	15	58.33 (27.67–84.83)	100.00 (78.20–100.00)	100.00 (59.04–100.00)	75.00 (50.90–91.34)	81.48 (61.92–93.70)	0.61 (0.32–0.89)	<0.05
	CapitalBio	4	8	0	15	33.33 (9.92–65.11)	100.00 (78.20–100.00)	100.00 (39.76–100.00)	65.22 (42.73–83.62)	70.37 (49.82–86.25)	0.36 (0.07–0.64)	<0.05
Pus	MultNAT	11	1	0	11	91.67 (61.52–99.79)	100.00 (71.51–100.00)	100.00 (71.51–100.00)	91.67 (61.52–99.79)	95.65 (78.05–99.89)	0.91 (0.75–1.00)	ns
	ZEESAN	7	5	1	10	58.33 (27.67–84.83)	90.91 (58.72–99.77)	87.50 (47.35–99.68)	66.67 (38.38–88.18)	73.91 (51.59–89.77)	0.49 (0.20–0.77)	<0.05
	CapitalBio	7	5	0	11	58.33 (27.67–84.83)	100.00 (71.51–100.00)	100.00 (59.04–100.00)	68.75 (41.34–88.98)	78.26 (56.30–92.54)	0.57 (0.27–0.87)	<0.05

Abbreviations: PPA, positive percent agreement; NPA, negative percent agreement; TP true positive; FN false negative; FP false positive; TN true negative; PPV positive predictive value; NPV negative predictive value; OPA overall percent agreement; κ Cohen's kappa; CI confidence interval; ns, not significant (P >0.05).

P values were calculated using McNemar's test for paired comparisons with Xpert MTB/RIF as reference.

Note: Results for pleural-effusion (n = 27) and pus (n = 23) specimens are exploratory due to small subgroup sizes (n <30).

Detection of Rifampicin Resistance

The performance of MultNAT and CapitalBio for rifampicin (RIF) resistance detection was evaluated using Xpert MTB/RIF as the reference. Indeterminate results from either assay were excluded. Among 217 evaluable MTB-positive specimens, MultNAT achieved a PPA of 93.75% (95% CI 69.77—99.84) and NPA of 99.50% (95% CI 97.26—99.99), corresponding to κ = 0.93 (95% CI 0.84—1.00), indicating almost perfect agreement (Table 4). In contrast, CapitalBio, evaluated in 168 samples, yielded a lower PPA of 69.23% (95%CI 38.57—90.91) and NPA of 98.71%, with κ = 0.73 (95% CI 0.53—0.94), reflecting only substantial agreement. Although McNemar's test revealed no significant difference (P >0.05), this likely reflected the small number of resistant isolates (n = 13). The full cross-tabulations, including indeterminate results, are shown in Table 5 and Table 6.

Table 4 Diagnostic performance of MultNAT and CapitalBio assays for rifampicin resistance detection in MTB-positive samples compared with Xpert MTB/RIF assay

Assay	n	PPA % (95% CI)	NPA % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	OPA % (95% CI)	κ (95% CI)	*P*-value
MultNAT	217	93.75 (69.77–99.84)	99.50 (97.26–99.99)	93.75 (69.77–99.84)	99.50 (97.26–99.99)	99.08 (96.71–99.89)	0.93 (0.84–1.00)	ns
CapitalBio	168	69.23 (38.57–90.91)	98.71 (95.42–99.84)	81.82 (48.22–97.72)	97.45 (93.61–99.30)	96.43 (92.39–98.68)	0.73 (0.53–0.94)	ns

Abbreviations: n, number of evaluable samples (indeterminate results from either assay were excluded); PPA, positive percent agreement; NPA, negative percent agreement; PPV, positive predictive value; NPV, negative predictive value; OPA, overall percent agreement; κ, Cohen's kappa; CI, confidence interval; ns, not significant (P >0.05).

P values were calculated using McNemar's test for paired comparisons with Xpert MTB/RIF as reference; 95% CIs were calculated by the Wilson method.

Table 5 Contingency tables for rifampicin resistance detection by MultNAT compared with Xpert MTB/RIF assay

Xpert MTB/RIF	R	S	I	Total
MultNAT R	15	1	0	16
MultNAT S	1	200	3	204
MultNAT I	0	3	1	4
Total	16	204	4	224

Abbreviations: R, resistant; S, susceptible; I, indeterminate.

Table 6 Contingency tables for rifampicin resistance detection by CapitalBio compared with Xpert MTB/RIF assay

Xpert MTB/RIF	R	S	I	Total
CapitalBio R	9	2	0	11
CapitalBio S	4	153	2	159
CapitalBio I	0	2	0	2
Total	13	157	2	172

Abbreviations: R, resistant; S, susceptible; I, indeterminate.

Discussion

In this prospective diagnostic accuracy study, we evaluated the MultNAT MTC/RIF assay, a cartridge-based molecular point-of-care test integrating dual-target amplification and automated sample processing. The assay demonstrated diagnostic performance comparable to the WHO-endorsed Xpert MTB/RIF across both pulmonary and extrapulmonary specimens and maintained high sensitivity in pleural effusion and pus samples—specimen types in which molecular diagnostics often show reduced yield [12–14].

The MultNAT platform was designed to address several operational and analytical challenges encountered in routine tuberculosis (TB) diagnostics. Its fully enclosed "sample-to-answer" cartridge integrates ultrasonic lysis, magnetic-bead nucleic acid purification, and dual-target real-time PCR (IS6110 and IS1081) within a single sealed system. This configuration minimizes contamination, reduces operator variability, and shortens the total assay time to <2 h. Unlike the Xpert family, MultNAT employs vitrified, ambient-stable reagents, thereby removing cold-chain requirements and facilitating implementation in laboratories with limited infrastructure. The use of dual multicopy targets is conceptually aligned with strategies adopted in other high-sensitivity assays, including Xpert MTB/RIF Ultra, to improve detection in paucibacillary disease [15–16]. Consistent with this approach, MultNAT achieved comparable sensitivity gains without sacrificing specificity, likely reflecting balanced amplification of IS6110 and IS1081 and mitigating false negatives in isolates with low IS6110 copy numbers [17–18].

For rifampicin-resistance detection, MultNAT demonstrated almost perfect agreement with Xpert (κ = 0.93) and higher PPA compared with CapitalBio (69.2%). The platform's real-time PCR coupled with melting-curve analysis allows precise discrimination of *rpoB* mutations through characteristic melting temperatures, affording higher single-nucleotide resolution than hybridization-based assays [19]. Although current functionality is limited to rifampicin resistance, expansion to include isoniazid and fluoroquinolones would markedly enhance its clinical value.

Beyond analytical accuracy, MultNAT provides operational advantages consistent with the WHO target product profiles for next-generation TB diagnostics [20]. Ambient-temperature reagent stability, minimal biosafety requirements, and rapid turnaround support deployment in decentralized and intermediate-resource laboratories where continuous electricity or refrigeration may be unavailable [21]. Compared with other domestic assays that require prolonged processing times, the shorter time to result may facilitate earlier clinical decision-making and more timely initiation of appropriate therapy.

Several limitations of this study should be acknowledged. First, analyses of extrapulmonary specimens were based on relatively small subgroup sizes, and sensitivity estimates should therefore be interpreted with caution. Second, mycobacterial culture or sequencing was not used as an independent reference standard for discrepant results, which may have influenced specificity estimates. Third, the low prevalence of rifampicin-resistant isolates limited statistical power for resistance-detection analyses. Nevertheless, the prospective study design and inclusion of multiple specimen types reflect routine clinical practice and enhance the generalizability of the findings.

Importantly, tuberculosis remains a global disease with persistent diagnostic gaps extending beyond individual regions. The MultNAT MTC/RIF assay addresses challenges common to many high-burden and resource-constrained settings, including delayed diagnosis, limited laboratory infrastructure, and reduced sensitivity in extrapulmonary disease. By enabling rapid molecular detection and rifampicin-resistance identification at the point of care, the assay may support earlier clinical decision-making across diverse healthcare systems.

In summary, the MultNAT MTC/RIF assay represents a clinically relevant addition to the molecular diagnostic toolbox for tuberculosis. By combining high diagnostic accuracy with simplified, field-adapted operation, it has the potential to improve case detection and timely treatment initiation where diagnostic gaps persist. Larger prospective studies and expanded resistance panels will be required to further define its role in global TB control.

Declarations

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Xiamen Xinglin Hospital (Approval No. [2025] KY-EC-091). Written informed consent was obtained from all participants prior to enrollment.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' Contributions

Y.Y. and Y.C. designed the study, performed the experiments, and wrote the main manuscript text. These authors contributed equally to this work and share first authorship.

C.D., Y.H., and W.W. analyzed the data and prepared the figures.

R.H. and Q.S. conceptualized the study, supervised the project, and critically revised the manuscript.

All authors reviewed and approved the final manuscript.

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Figures

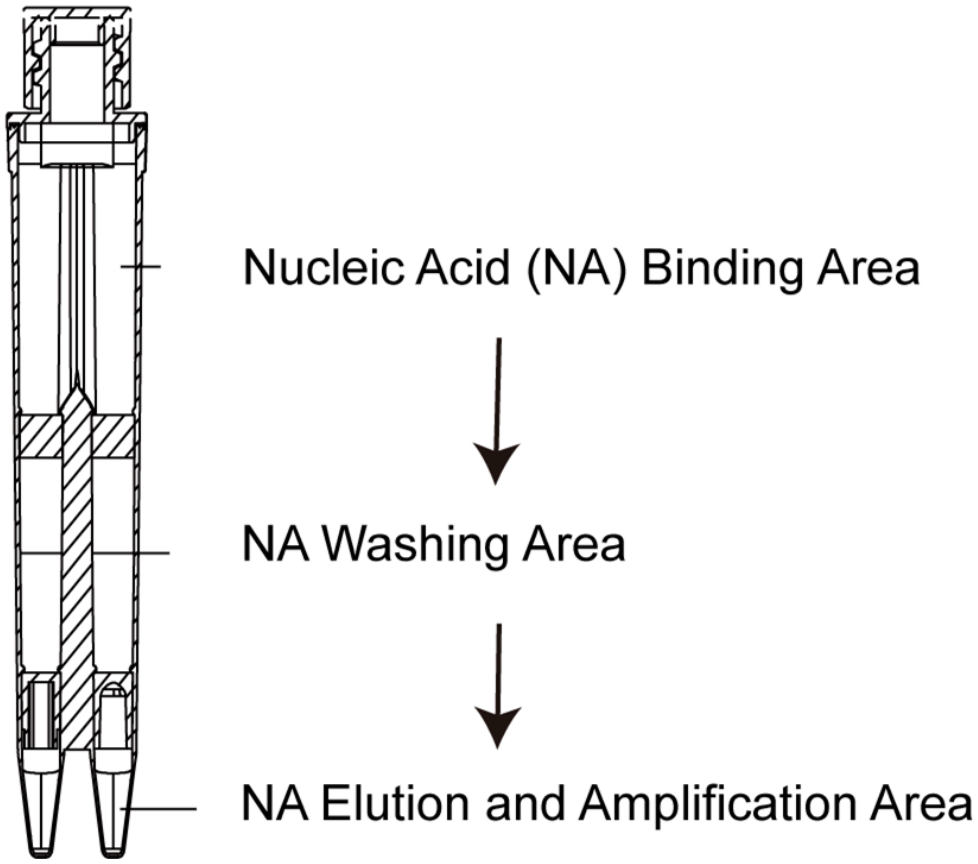


Figure 1

Integrated Cartridge Structure. Schematic representation of the cartridge architecture showing three functional zones: the nucleic acid (NA) binding area, washing area, and elution/PCR amplification area. Arrows indicate the directional movement of nucleic acids within the cartridge.