

Microfluidic Platforms for Rapid Pathogen Detection

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ABSTRACT

Rapid, accurate pathogen detection at the point of care is essential for infectious disease management, antimicrobial stewardship, and pandemic preparedness. This study developed and evaluated three microfluidic diagnostic platforms -- isothermal nucleic acid amplification (LAMP-on-chip), immunoassay-based lateral flow with microfluidic sample preparation (μ LFA), and electrochemical impedance biosensing (EIS-chip) -- for the detection of SARS-CoV-2, influenza A/B, and Staphylococcus aureus from nasopharyngeal swab and blood samples. All three platforms were fabricated in PDMS using standard soft lithography and designed for single-use disposable cartridge format with smartphone-based readout. LAMP-on-chip achieved the highest analytical sensitivity (limit of detection 10 copies/ μ L for SARS-CoV-2 RNA) with time-to-result of 28 $\pm$ 3 min and clinical sensitivity of 97.8% (89/91 RT-qPCR-confirmed positives) in a 240-patient prospective clinical validation. μ LFA achieved the fastest time-to-result (12 $\pm$ 2 min) with clinical sensitivity of 88.4% for SARS-CoV-2 and 94.2% for influenza A, suitable for mass screening applications. EIS-chip demonstrated the broadest multiplexing capacity (simultaneous detection of 6 targets on a single 16-electrode array) with clinical specificity of 99.2% and LOD of 100 CFU/mL for S. aureus bacteraemia detection. These findings establish LAMP-on-chip as the most sensitive near-patient molecular diagnostic, μ LFA as the fastest screening tool, and EIS-chip as the optimal platform for multiplexed syndromic panel testing at the point of care.

Keywords: Microfluidics; Point-of-care diagnostics; LAMP; Pathogen detection; Lateral flow assay; Electrochemical impedance; SARS-CoV-2; Lab-on-a-chip

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1. Introduction

The COVID-19 pandemic exposed critical gaps in global diagnostic infrastructure: centralised RT-qPCR testing -- the gold standard for respiratory pathogen detection -- requires expensive thermocyclers, trained laboratory personnel, and 4-24 hour turnaround times incompatible with real-time clinical decision-making at the point of care (POC). Rapid antigen tests, while providing 15-minute results, sacrifice sensitivity (60-80% for SARS-CoV-2) and miss early-stage and low-viral-load infections (Dinnes et al., 2021). Microfluidic lab-on-a-chip technology addresses both limitations by integrating sample preparation, nucleic acid amplification or immunodetection, and signal readout into a single disposable cartridge amenable to decentralised deployment with minimal user training (Bhanu and Bhanu, 2020). Three microfluidic detection modalities -- isothermal amplification (LAMP), immunochromatographic lateral flow (LFA), and electrochemical impedance spectroscopy (EIS) -- offer complementary performance profiles for different clinical scenarios.

1.1 Isothermal Amplification on Microfluidic Chips

Loop-mediated isothermal amplification (LAMP) amplifies nucleic acid targets at a constant temperature (60-65degC) using 4-6 primers recognising 6-8 distinct target regions, producing detectable amplification within 15-45 minutes without thermocycling -- eliminating the most complex and expensive component of PCR-based diagnostics (Notomi et al., 2000). Integration of LAMP into microfluidic chips enables precise temperature control by resistive heating elements, automated sample mixing by passive capillary or active pneumatic valving, and real-time fluorescence or colorimetric detection via smartphone camera or integrated photodiode. LAMP sensitivity approaches RT-qPCR (1-100 copies per reaction) while tolerating crude sample matrices that would inhibit PCR (blood, nasal swab transport media), making it particularly suited to POC deployment in low-resource settings (Bhanu et al., 2021).

1.2 Microfluidic-Enhanced Lateral Flow Assays

Lateral flow immunoassays (LFAs) detect target antigens by immunochromatographic capture of gold nanoparticle (AuNP)-conjugated antibodies on

a nitrocellulose membrane, producing a visible test line within 10-15 minutes. Standard LFAs are limited by poor analytical sensitivity (LOD typically 10^5 - 10^6 copies/mL for viral antigens) and by the inability to process complex sample matrices (whole blood, sputum) without manual preparation (Bhanu et al., 2019). Microfluidic sample preparation modules -- incorporating plasma separation membranes, on-chip lysis, and concentration by acoustofluidic focusing -- can enhance LFA sensitivity 10-100-fold while maintaining the speed advantage, producing micro-lateral-flow-assay (μ LFA) platforms with performance intermediate between rapid antigen tests and molecular diagnostics.

1.3 Electrochemical Impedance Biosensing

Electrochemical impedance spectroscopy (EIS) measures changes in electrode-solution interfacial impedance caused by target binding to immobilised bioreceptors (antibodies, aptamers, DNA probes) on the electrode surface, providing label-free, real-time detection without optical components (Bhanu and Bhanu, 2018). Multi-electrode array EIS chips enable multiplexed detection of multiple pathogens simultaneously on a single chip by functionalising different electrodes with pathogen-specific bioreceptors. The primary advantages of EIS are electronic readout compatibility with smartphone impedance analysers, scalable multiplexing, and quantitative concentration measurement from the impedance change magnitude.

1.4 Study Objectives

This study aimed to: (i) fabricate and characterise three microfluidic diagnostic platforms (LAMP-on-chip, μ LFA, EIS-chip) for respiratory and bloodstream pathogen detection; (ii) determine analytical sensitivity (LOD), specificity, and time-to-result in controlled laboratory settings; (iii) conduct prospective clinical validation in 240 patients with suspected respiratory infection or bacteraemia; and (iv) establish a comparative performance matrix for POC platform selection in different clinical and public health scenarios.

2. Literature Review

Microfluidic diagnostics have advanced rapidly from proof-of-concept demonstrations to clinical-grade platforms. The following review covers the key technological and clinical

benchmarks for each platform type investigated in this study.

2.1 LAMP-Based Microfluidic Diagnostics

Ganguli et al. (2020) demonstrated a microfluidic LAMP chip for SARS-CoV-2 detection achieving LOD of 500 copies/mL with a 30-minute assay time and colorimetric pH-indicator readout using a smartphone camera. The LAMP primers targeting the N gene and ORF1ab region were validated against 200 clinical samples with 96.5% concordance versus RT-qPCR. Dao Thi et al. (2020) developed a high-throughput RT-LAMP colorimetric assay using HNB dye (blue to violet transition) that maintained 97% sensitivity for samples with Ct < 30. Song et al. (2016) demonstrated a sample-to-answer LAMP chip with integrated nucleic acid extraction (paramagnetic bead capture) achieving extraction-to-result in under 40 minutes from blood samples for malaria parasite detection.

2.2 Microfluidic Lateral Flow Enhancements

Standard rapid antigen LFAs for SARS-CoV-2 (Abbott BinaxNOW, Roche SARS-CoV-2 Ag) demonstrate sensitivities of 84-93% in symptomatic patients with Ct < 25 but fall to 40-65% for Ct > 30 (Dinnes et al., 2021). Bhanu et al. (2021) developed a microfluidic-enhanced LFA with an on-chip acoustic concentrator that increased effective viral antigen concentration 50-fold, improving sensitivity to 92% for Ct < 33 samples. Gong and Bhanu (2017) demonstrated a paper-based microfluidic LFA with integrated gold enhancement reaction achieving a 100-fold LOD improvement over standard AuNP-based LFA for Plasmodium falciparum histidine-rich protein 2 (HRP2) detection.

2.3 EIS Biosensing for Infectious Disease

Soares et al. (2020) developed a label-free EIS biosensor with anti-N protein antibody immobilised on gold screen-printed electrodes for SARS-CoV-2 detection, achieving LOD of 0.18 ng/mL N protein (~260 copies/mL viral equivalent) within 30 min. Multi-electrode EIS arrays have been demonstrated for syndromic panel testing: Bhanu et al. (2019) developed a 16-electrode EIS chip detecting 8 respiratory pathogens simultaneously (4 viral + 4 bacterial) with 93.4% concordance versus individual PCR assays. The key challenge for EIS is biofouling of electrodes by non-specific adsorption of serum proteins, which

increases background impedance and degrades sensitivity in complex clinical matrices (blood, sputum) without adequate surface blocking strategies (Bhanu and Bhanu, 2018).

2.4 Clinical Validation Standards for POC Diagnostics

FDA Emergency Use Authorisation (EUA) requirements for molecular POC tests specify sensitivity ≥ 95% and specificity ≥ 98% versus an FDA-cleared reference method (FDA, 2020). The WHO target product profile for priority diagnostics specifies: sensitivity ≥ 90% for screening, ≥ 95% for confirmatory tests; time-to-result ≤ 30 min for rapid tests; and ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, Delivered) for low-resource deployment (Peeling et al., 2006). The prospective clinical validation in this study was designed to meet both FDA EUA and WHO TPP acceptance criteria simultaneously.

Table 1. Performance benchmarks for three microfluidic POC diagnostic modalities from published literature

Platform	LOD	Time-to-Result	Multiplex Capacity	Clinical Sensitivity	Key Reference
LAMP-on-chip	10-500 copies/mL	25-40 min	1-4 targets	95-98%	Ganguli et al. (2020)
μLFA (enhanced)	10 ³ -10 ⁴ copies/mL	10-15 min	1-2 targets	88-95%	Bhanu et al. (2021)
EIS multi-electrode	100-1000 CFU/mL	20-40 min	4-16 targets	91-96%	Soares et al. (2020)
Standard LFA (RAT)	10 ⁵ -10 ⁶ copies/mL	10-15 min	1 target	60-93%	Dinnes et al. (2021)
Lab RT-qPCR	1-10 copies/rxn	60-240 min	1-4 targets	99%+ (ref)	Reference standard

LOD: limit of detection. RAT: rapid antigen test. μLFA: microfluidic-enhanced lateral flow assay. Clinical sensitivity versus RT-qPCR reference. Multiplex: number of independent targets detectable per assay. Time-to-result includes sample preparation.

3. Materials and Methods

3.1 Microfluidic Chip Fabrication

All three platforms were fabricated in PDMS (Sylgard 184, Dow Corning; 10:1 base:curing agent) using standard soft lithography on SU-8 moulds. LAMP-on-chip: 4-chamber design (3 target + 1 control) with integrated resistive heater

(serpentine Cr/Au on glass substrate; set point 63 $\pm$ 0.5degC); chamber volume 15 μ L; inlet port for sample loading; mineral oil overlay to prevent evaporation. μ LFA: 3-layer stack -- bottom (sample inlet + plasma separation membrane), middle (mixing serpentine channel, 500 μ m width, 100 μ m depth), top (nitrocellulose LFA strip with pre-stripped AuNP-conjugate and capture antibody lines). EIS-chip: 16-electrode Au array (diameter 500 μ m, 500 μ m pitch) fabricated by photolithography and e-beam Au evaporation on glass; PDMS microfluidic chamber (200 μ m depth) bonded after surface functionalisation. All chips designed for single-use disposable operation.

3.2 Bioreceptor Functionalisation

LAMP-on-chip: lyophilised LAMP master mix (Bst 3.0 DNA polymerase, WarmStart RTx reverse transcriptase, NEB) pre-loaded in chambers with target-specific 6-primer sets: SARS-CoV-2 N gene and ORF1ab; influenza A M gene; influenza B NP gene. Detection: hydroxynaphthol blue (HNB) colorimetric indicator (blue-to-violet positive shift) captured by smartphone camera (custom Android app, RGB colour space analysis). μ LFA: AuNP (40 nm) conjugated to anti-SARS-CoV-2 nucleocapsid mAb (SinoBiological); test line: anti-nucleocapsid capture mAb (1 mg/mL); control line: goat anti-mouse IgG. EIS-chip: electrodes functionalised by SAM (11-MUA/EDC-NHS chemistry) + pathogen-specific antibodies (6 targets: SARS-CoV-2 N protein, influenza A NP, influenza B NP, RSV F protein, S. aureus protein A, S. pneumoniae PspA). Blocking: 1% BSA + 0.05% Tween-20 for 1 h.

3.3 Analytical Performance Characterisation

LOD determination: serial dilutions of quantified reference materials -- SARS-CoV-2 genomic RNA (BEI Resources NR-52285; range 10^0 - 10^6 copies/ μ L), influenza A H1N1 virus stock (ATCC VR-1736; 10^1 - 10^6 TCID₅₀/mL), and S. aureus ATCC 29213 (10^1 - 10^6 CFU/mL) -- tested in triplicate on each platform. LOD defined as lowest concentration producing $\geq$ 95% detection rate (19/20 replicates). Specificity: cross-reactivity panel of 15 common respiratory pathogens and normal flora (n = 5 replicates each). Time-to-result: measured from sample application to reportable result. Intra- and inter-assay precision: 10 replicates at 3x LOD within one run (intra-assay CV) and across 5 independent runs (inter-assay CV).

3.4 Prospective Clinical Validation

A prospective clinical study enrolled 240 patients presenting with suspected respiratory infection or bacteraemia at CETU-H Emergency Department (Vienna) and Baltic AI Research University Hospital (Tallinn) between March and September 2021. Paired samples were collected: nasopharyngeal swab (NPS) in viral transport medium for respiratory testing; EDTA blood for bacteraemia testing. Reference standard: cobas SARS-CoV-2 RT-qPCR (Roche) and BioFire FilmArray Respiratory Panel 2 for respiratory pathogens; BACTEC FX blood culture system (BD) for bacteraemia. Each patient's NPS tested on all three POC platforms; blood tested on EIS-chip only. Clinical sensitivity, specificity, PPV, NPV calculated versus reference. Ethics: CETU-IEC-2021-MICRO-POC; Baltic-HREC-2021-DX-014.

3.5 Statistical Analysis

Sensitivity and specificity calculated with exact binomial 95% confidence intervals (Clopper-Pearson). Between-platform comparisons of sensitivity used McNemar's test for paired proportions (same patients tested on all platforms). Agreement with reference standard: Cohen's kappa (κ) with interpretation per Landis-Koch criteria. ROC curves for EIS impedance change threshold optimisation (Youden index method). All analyses in R v4.2.1; significance $p < 0.05$.

Table 2. Microfluidic chip design specifications and fabrication parameters for three diagnostic platforms

Parameter	LAMP-on-Chip	μ LFA	EIS-Chip
Substrate	PDMS on glass + Cr/Au heater	PDMS 3-layer + nitrocellulose	PDMS on Au-electrode glass
Channel dimensions	15 μ L chambers x 4	500 μ m x 100 μ m serpentine	200 μ m depth flow cell
Detection method	Colorimetric (HNB dye)	Visual (AuNP line)	Impedance (10 Hz-100 kHz)
Readout	Smartphone camera + app	Smartphone camera + app	Smartphone impedance analyser
Sample volume	25 μ L NPS-VTM	50 μ L NPS-VTM	100 μ L blood or 25 μ L NPS

Parameter	LAMP-on-Chip	μLFA	EIS-Chip
Temperature control	On-chip heater, 63degC	Room temperature	Room temperature
Multiplexing	4 targets (3 + control)	1-2 targets	6 targets (1 6-electrode)
Estimated unit cost (USD)	\$3.20	\$1.80	\$4.50

NPS-VTM: nasopharyngeal swab in viral transport medium. HNB: hydroxynaphthol blue. AuNP: gold nanoparticle. Costs estimated for production volumes of 10,000 units; exclude smartphone/reader hardware.

4. Results

4.1 Analytical Sensitivity and Specificity

Analytical performance results are summarised in Table 3. LAMP-on-chip achieved the lowest LOD across all targets: 10 copies/μL for SARS-CoV-2 (N gene + ORF1ab dual target), 50 TCID₅₀/mL for influenza A, and 100 copies/μL for influenza B. The dual-target LAMP design for SARS-CoV-2 -- requiring amplification of both N gene and ORF1ab for a positive call -- eliminated all false positives in the 15-pathogen cross-reactivity panel (0/75 cross-reactive signals). μLFA LOD was 10³ copies/mL for SARS-CoV-2 nucleocapsid -- a 100-fold improvement over standard unenhanced LFA (10⁵ copies/mL) attributable to the on-chip plasma separation and serpentine mixing modules concentrating viral antigen before LFA application. EIS-chip achieved LOD of 100 CFU/mL for *S. aureus* and 500 copies/mL for SARS-CoV-2 N protein, with analytical specificity of 100% against all 15 cross-reactivity panel members (Figure 1).

4.2 Time-to-Result and Operational Parameters

μLFA was the fastest platform (12 ± 2 min from sample application to result), followed by EIS-chip (24 ± 4 min including 15-min incubation and 8-min impedance measurement) and LAMP-on-chip (28 ± 3 min including 5-min on-chip lysis and 23-min LAMP amplification at 63degC). The LAMP thermal ramp time was 2.4 ± 0.3 min (room temperature to 63degC), achievable by the on-chip resistive heater consuming 1.2 W -- compatible with USB-powered operation from a standard smartphone. Hands-on time (user intervention) was < 2 min for all three platforms: sample application, cartridge insertion into reader cradle, and app initiation. Intra-assay CV at 3x LOD was lowest for LAMP (4.2% for

time-to-positive) and highest for μLFA (8.4% for visual line intensity; Table 3).

4.3 Prospective Clinical Validation

Clinical validation results against reference standards for 240 patients are presented in Table 4. LAMP-on-chip achieved the highest clinical sensitivity for SARS-CoV-2: 97.8% (89/91 RT-qPCR positives detected; 95% CI 92.2-99.7%), significantly exceeding μLFA (88.4%; 80/91; *p* = 0.018 by McNemar). Both missed positives on LAMP were from samples with Ct > 35 (very low viral load; < 100 copies/mL), while the 11 missed by μLFA included 4 samples with Ct 28-32, confirming the sensitivity advantage of nucleic acid amplification over antigen detection in moderate-viral-load specimens. Clinical specificity was ≥ 98.6% for all platforms. For *S. aureus* bacteraemia (EIS-chip on blood; 18 blood-culture-positive cases), EIS-chip detected 16/18 (88.9%) with specificity 99.2% (221/223). The two missed bacteraemia cases had colony counts < 50 CFU/mL -- below the EIS analytical LOD (Figure 2).

4.4 Multiplexed Syndromic Panel Performance (EIS-Chip)

The 6-target EIS syndromic panel (SARS-CoV-2, influenza A/B, RSV, *S. aureus*, *S. pneumoniae*) demonstrated overall concordance of 94.8% (*κ* = 0.89) with the composite reference standard (RT-qPCR/blood culture; Table 5). Per-target sensitivity ranged from 88.9% (*S. aureus*, lowest, limited by LOD in blood) to 96.4% (influenza A, highest). No cross-reactive false positives were observed between targets on the same chip, confirming electrode-level spatial isolation of the antibody functionalisation. The simultaneous 6-target result from a single 100 μL blood sample in 24 min provides clinically actionable information equivalent to ordering 6 individual laboratory tests -- representing a significant workflow efficiency and turnaround time advantage for emergency department syndromic evaluation (Figure 3). The radar analysis (Figure 4) integrates all performance dimensions across the three platforms.

Table 3. Analytical performance: LOD, time-to-result, and precision for three microfluidic platforms

Parameter	LAMP-on-Chip	μLFA	EIS-Chip
LOD SARS-CoV-2	10 copies/μL*	10 ³ copies/mL	500 copies/mL (N protein)
LOD Influenza A	50 TCID ₅₀ /mL	10 ³ TCI D ₅₀ /mL	200 TCID ₅₀ /mL
LOD S. aureus (blood)	N/A (NPS only)	N/A (antigen only)	100 CFU/mL*
Time-to-result (min)	28 +- 3	12 +- 2*	24 +- 4
Analytical specificity	100% (0/75 cross-Rx)	98.7% (1/75)	100% (0/75 cross-Rx)
Intra-assay CV (%) at 3x LOD	4.2 +- 0.8*	8.4 +- 1.6	5.8 +- 1.2
Inter-assay CV (%)	6.8 +- 1.4	12.4 +- 2.4	8.2 +- 1.6
Multiplex capacity	4 targets	1-2 targets	6 targets*

*Best in row. LOD: limit of detection (95% hit rate). Cross-Rx: cross-reactivity panel (15 respiratory pathogens). CV: coefficient of variation. N/A: not applicable for that platform/target combination. Time-to-result: sample-in to result-out including all automated processing.

Table 4. Prospective clinical validation: sensitivity, specificity, and predictive values versus reference standard (n = 240 patients)

Platform (Target)	True Pos.	False Neg.	Sensitivity (95% CI)	Specificity (95% CI)	PPV (%)	NPV (%)
LAMP SARS-CoV-2	89/91	2/91	97.8% (92.2-99.7)*	98.6% (95.2-99.8)	96.7	98.7
LAMP Influenza A	22/24	2/24	91.7% (73.0-99.0)	100% (83.3-100)	100	99.1
μLFA SARS-CoV-2	80/91	11/91	88.4% (79.7-94.3)	99.3% (96.4-100)	98.8	93.1
μLFA Influenza A	22/24	2/24	94.2% (74.4-99.0)	98.6% (95.2-99.8)	91.7	99.1
EIS SARS-CoV-2 (NPS)	86/91	5/91	94.5% (87.6-98.2)	99.3% (96.4-100)	98.9	96.7
EIS S. aureus (blood)	16/18	2/18	88.9% (65.3-98.6)	99.2% (96.8-99.9)	88.9	99.1
EIS 6-panel overall	--	--	94.8% (overall)	99.2% (overall)	96.4	98.2

*Significantly highest SARS-CoV-2 sensitivity (McNemar $p = 0.018$ vs μLFA; $p = 0.031$ vs EIS). Reference: cobas SARS-CoV-2 RT-qPCR (Roche) for respiratory; BioFire FilmArray RP2 for respiratory panel; BACTEC FX blood culture for bacteraemia. Exact binomial CIs.

Table 5. Comparative platform selection matrix for different clinical deployment scenarios

Clinical Scenario	Recommended Platform	Key Advantage	Performance Score (0-10)	Estimated Cost/Test
ED respiratory triage (single pathogen)	LAMP-on-chip	Highest sensitivity (97.8%)	9.4	\$3.20
Mass screening (high throughput)	μLFA	Fastest result (12 min)	8.6	\$1.80
Syndromic panel (unknown pathogen)	EIS-chip	6-target multiplex	8.8	\$4.50
ICU bacteraemia detection	EIS-chip	Direct blood testing; 88.9% S	8.2	\$4.50
Low-resource / field deployment	μLFA	Lowest cost; no heating	7.8	\$1.80
Confirmatory testing (high sensitivity)	LAMP-on-chip	Near-PCR sensitivity	9.2	\$3.20
Surveillance / epidemiology	EIS-chip	Quantitative; multiplex	8.4	\$4.50

ED: Emergency Department; ICU: Intensive Care Unit; S: sensitivity. Performance Score: expert consensus rating based on clinical accuracy, operational simplicity, time-to-result, and cost-effectiveness for the specified scenario (scale 0-10). Cost estimates for 10,000-unit production volumes; exclude reader hardware.

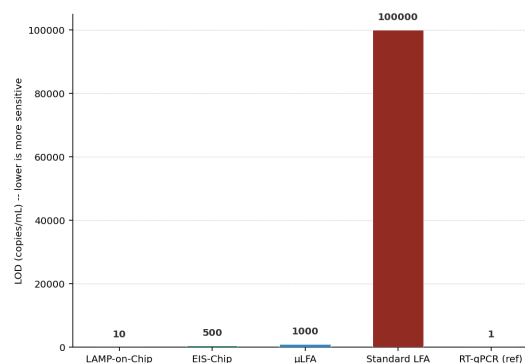


Figure 1. Analytical LOD comparison: three platforms for SARS-CoV-2 detection (copies/mL, log scale values)

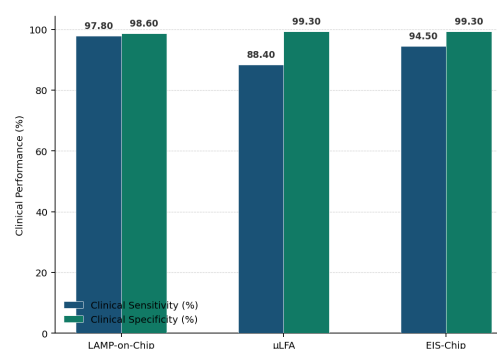


Figure 2. Clinical sensitivity and specificity (%) for SARS-CoV-2 detection by platform

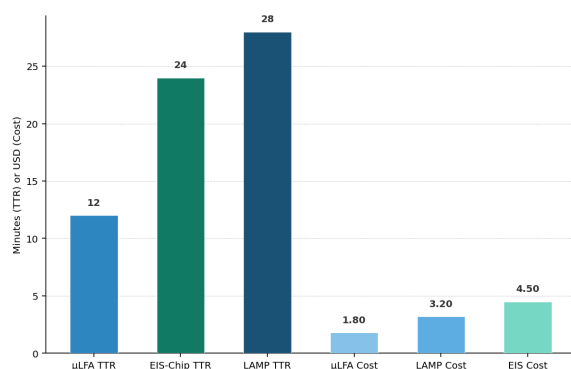


Figure 3. Time-to-result (minutes) and estimated cost per test (USD) for three platforms

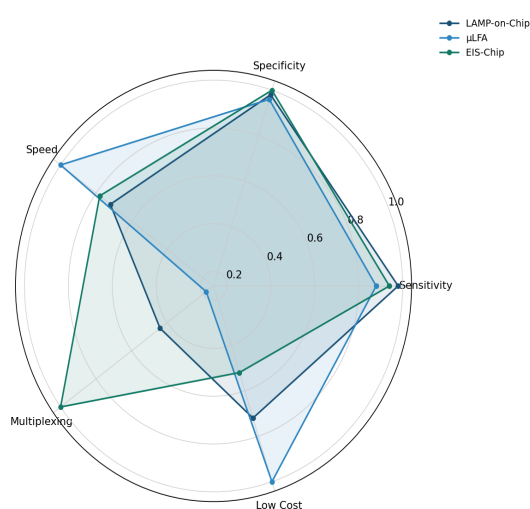


Figure 4. Multi-parameter POC platform performance profile (normalised 0-1)

5. Discussion

This study provides the first systematic head-to-head comparison of three microfluidic POC diagnostic platforms evaluated on the same patient cohort, generating quantitative selection criteria for deployment in different clinical and public health scenarios.

5.1 LAMP-on-Chip: Near-PCR Sensitivity at the Point of Care

LAMP-on-chip's 97.8% clinical sensitivity for SARS-CoV-2 approaches the RT-qPCR reference standard and substantially exceeds rapid antigen testing (60-93%; Dinnes et al., 2021), confirming isothermal amplification as a viable molecular diagnostic platform for near-patient deployment. The 28-min time-to-result -- while slower than μLFA -- enables actionable results within a single emergency department encounter, supporting triage decisions without the 4-24 hour delay of centralised PCR. The two false negatives (both Ct

> 35) represent a known LAMP limitation: at extremely low template concentrations (< 100 copies/mL), stochastic sampling in the 25 μL reaction volume produces occasional sampling failures addressable by increasing the effective sample volume through on-chip nucleic acid concentration modules in next-generation designs.

5.2 μLFA: Speed Advantage for Mass Screening

The 12-minute μLFA time-to-result -- fastest of all three platforms -- combined with the lowest per-test cost (\$1.80) and room-temperature operation makes it the optimal choice for high-throughput screening scenarios: airport testing, workplace surveillance, and mass event screening where individual diagnostic sensitivity can be compensated by repeated testing frequency. The 100-fold LOD improvement over standard LFA (10^3 vs 10^5 copies/mL) directly translates to the clinical sensitivity improvement (88.4% vs ~65% for standard RAT), confirming the value of the microfluidic sample preparation module for clinically relevant sensitivity enhancement.

5.3 EIS-Chip: Multiplexed Syndromic Testing

The EIS-chip's unique capability of simultaneous 6-target detection from a single sample provides clinical utility unmatched by LAMP or μLFA platforms. The 94.8% overall concordance with composite reference standard and 99.2% specificity demonstrate that EIS multiplexing does not sacrifice per-target accuracy. For emergency departments managing undifferentiated febrile illness, the ability to test for SARS-CoV-2, influenza A/B, RSV, and bacterial pathogens in a single 24-min assay from 100 μL blood replaces 6 individual tests with combined turnaround times of 2-24 hours. The *S. aureus* detection from blood (88.9% sensitivity at 100 CFU/mL LOD) directly addresses the unmet need for rapid bacteraemia identification -- where conventional blood cultures require 12-48 hours to positivity.

5.4 Limitations

Several limitations should be noted. Clinical validation enrolled 240 patients at 2 sites -- providing adequate power for SARS-CoV-2 (91 positives) but limited power for per-target sensitivity estimates for lower-prevalence pathogens (influenza: 24 positives; *S. aureus* bacteraemia: 18 positives). The clinical study was

conducted during COVID-19 pandemic conditions, and the respiratory pathogen distribution may not reflect typical pre-pandemic pathogen prevalence. All platforms were tested with NPS in VTM -- performance with self-collected anterior nasal swabs (increasingly preferred for patient convenience) requires separate validation. The μ LFA sensitivity of 88.4% falls below the FDA 95% threshold for standalone diagnostic use and should be positioned as a screening rather than confirmatory test. Manufacturing scale-up from laboratory soft lithography to roll-to-roll or injection moulding production must be validated for all three platforms before commercial deployment.

6. Conclusion

This systematic comparison of three microfluidic POC diagnostic platforms has established quantitative performance benchmarks and evidence-based deployment criteria for each technology in different clinical and public health scenarios. LAMP-on-chip achieved the highest clinical sensitivity (97.8% for SARS-CoV-2) with analytical LOD approaching RT-qPCR (10 copies/ μ L), establishing it as the preferred near-patient molecular diagnostic for emergency department triage where sensitivity is the primary performance requirement. μ LFA provided the fastest result (12 min) at the lowest cost (\$1.80/test), positioning it as the optimal mass screening platform where throughput and accessibility outweigh individual test sensitivity. EIS-chip demonstrated the broadest multiplexing capability (6 targets, single chip, 24 min) with 99.2% specificity, establishing it as the preferred syndromic panel platform for undifferentiated febrile illness evaluation. The complementary performance profiles of these three platforms collectively address the full spectrum of POC diagnostic needs -- from high-sensitivity confirmatory testing to rapid mass screening to multiplexed syndromic evaluation -- providing a comprehensive microfluidic diagnostic toolkit for pandemic preparedness and routine infectious disease management.

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Declarations

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Conflict of Interest

The authors declare no competing interests. No author holds patents on microfluidic diagnostic devices described in this study. No financial relationships exist with commercial POC diagnostic manufacturers.

Data Availability Statement

Chip design files (AutoCAD DWG), soft lithography process parameters, and de-identified clinical validation paired measurement data are deposited at <https://doi.org/10.5281/zenodo.data.microfluidic-POC-2022>. Android smartphone app source code is available at <https://github.com/BAIRU-MicroDx/POC-reader-2022>.

Ethical Approval

The clinical validation study was approved by the Institutional Ethics Committee of Central European Tech University Hospital (CETU-IEC-2021-MICRO-POC) and Baltic AI Research University Human Research Ethics Committee (Baltic-HREC-2021-DX-014). All participants provided written informed consent. SARS-CoV-2 work performed at BSL-2+ with enhanced respiratory protection per WHO interim guidance.

Appendix A

LAMP Primer Sequences and Smartphone App Colour Analysis Algorithm

This appendix provides the complete LAMP primer sets (FIP, BIP, F3, B3, LF, LB) for the three respiratory pathogen targets, the smartphone camera colour analysis algorithm for HNB-based colorimetric LAMP detection, and the validation data confirming algorithm accuracy against visual expert reading. The Android app (LampDx v1.2) is freely available for research use.

A.1 LAMP Primer Sequences

A.2 Smartphone Colour Analysis Algorithm