

Lab-on-a-Chip Diagnostics for Resource-Limited Settings

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ABSTRACT

*Lab-on-a-chip (LOC) platforms integrate sample preparation, biochemical reactions, and detection onto miniaturised microfluidic devices, offering rapid, low-cost, and portable diagnostics suitable for resource-limited settings (RLS) where centralised laboratories are unavailable. Despite substantial academic progress, fewer than 12% of published LOC prototypes have reached field deployment in low- and middle-income countries (LMICs). This study presents a systematic evaluation of five LOC architectures -- paper-based microfluidic analytical devices (muPADs), centrifugal microfluidics (lab-on-a-disc), droplet microfluidics, lateral flow immunoassays (LFIA) with integrated signal amplification, and elastomeric valve-based chips -- across four clinically relevant analytes: malaria (*Plasmodium falciparum* HRP2), HIV-1 viral load, blood glucose, and C-reactive protein (CRP). Analytical performance was benchmarked against WHO ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, Deliverable). muPADs achieved the lowest per-test cost (\$0.08 +/- 0.03) and highest field-readiness score (ASSURED 5.8/7), while droplet microfluidics provided the best analytical sensitivity (LOD 1.2 pg/mL for CRP) but required external instrumentation incompatible with field deployment. The proposed integrated muPAD-LFIA hybrid platform achieved 94.6% sensitivity and 97.2% specificity for malaria HRP2 detection (n = 384 clinical samples, endemic setting), with time-to-result of 18 +/- 3 minutes and no cold chain requirement. These findings establish muPAD-LFIA hybrids as the most deployment-ready LOC technology for RLS diagnostics and provide an evidence-based framework for selecting microfluidic architectures matched to target-product-profile constraints in global health applications.*

Keywords: Lab-on-a-chip; Microfluidics; Point-of-care diagnostics; Resource-limited settings; Paper-based microfluidic devices; Lateral flow immunoassay; ASSURED criteria; Global health diagnostics

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1. Introduction

An estimated 5.4 billion people worldwide lack access to essential diagnostic testing, with the greatest burden concentrated in sub-Saharan Africa and South-East Asia, where fewer than 1 in 10 primary health centres possess functional laboratory infrastructure (Fleming et al., 2021). The absence of timely diagnostics drives empirical treatment, antimicrobial resistance, and preventable mortality: the WHO estimates that 47% of malaria treatments in endemic regions are administered without parasitological confirmation, contributing to both overtreatment and drug resistance (Urdea et al., 2006). Lab-on-a-chip (LOC) technologies -- integrating sample handling, reagent mixing, amplification, and detection onto devices smaller than a credit card -- promise to bridge this diagnostic gap by delivering laboratory-quality results at the point of care without electricity, cold chains, or trained technicians (Yager et al., 2006).

1.1 The ASSURED Framework

The WHO ASSURED criteria -- Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users -- were established in 2006 as the benchmark for evaluating point-of-care (POC) diagnostics for resource-limited settings (Peeling et al., 2006). Despite fifteen years of microfluidic innovation, a 2021 systematic review found that only 11.6% of published LOC prototypes reported field validation data from LMICs, and fewer than 3% had achieved regulatory approval in any jurisdiction (Sachdeva et al., 2021). The translation gap between academic prototypes and deployed products stems from manufacturing scalability, reagent stability at ambient temperatures (25-40 C), integration of sample preparation for complex matrices (whole blood, saliva), and the absence of standardised benchmarking across platforms.

1.2 Microfluidic Platform Diversity

Five dominant LOC architectures have emerged for RLS diagnostics. Paper-based microfluidic analytical devices (muPADs) exploit capillary wicking through patterned cellulose substrates, enabling equipment-free fluid transport at costs below \$0.10 per device (Martinez et al., 2010). Centrifugal microfluidics (lab-on-a-disc) use rotational forces from a simple motor to drive fluid handling, sedimentation, and metering with precise volume control (Gorkin et al., 2010). Droplet microfluidics compartmentalise reactions into picoliter volumes for digital quantification but

require syringe pumps and optical detection systems (Teh et al., 2008). Lateral flow immunoassays (LFIAs) -- the platform underlying rapid antigen tests -- provide sub-20-minute results for protein biomarkers but suffer from limited sensitivity for low-abundance analytes (Parolo et al., 2020). Elastomeric valve-based chips (Quake-style) offer programmable fluidic logic but depend on external pneumatic controllers (Unger et al., 2000).

1.3 Study Objectives

This study aimed to: (i) systematically benchmark five LOC architectures against WHO ASSURED criteria using standardised analytical panels; (ii) quantify cost, sensitivity, specificity, and time-to-result for four clinically relevant analytes in RLS contexts; (iii) evaluate a novel muPAD-LFIA hybrid platform integrating paper-based sample preparation with signal-amplified lateral flow detection; (iv) conduct field validation of the hybrid platform in a malaria-endemic setting; and (v) develop an evidence-based decision framework for matching LOC architecture to target product profiles in global health diagnostics.

2. Literature Review

The field of microfluidic diagnostics for global health has expanded rapidly since the early demonstrations of paper-based devices in 2007. This review covers the key advances in each platform category and identifies the critical gaps motivating the present benchmarking study.

2.1 Paper-Based Microfluidic Devices

Martinez et al. (2007) first demonstrated photolithographically patterned muPADs for multiplexed glucose and protein detection, establishing paper as a viable substrate for microfluidic assays. Subsequent work introduced wax printing (Carrilho et al., 2009) and inkjet-deposited reagents (Abe et al., 2010) for scalable manufacturing. Three-dimensional muPADs achieved multi-step assay sequences through stacked paper layers with tape-sealed fluidic vias (Martinez et al., 2010). A major limitation remains the narrow dynamic range of colorimetric readout, prompting integration of electrochemical (Dungchai et al., 2009) and fluorescent (Noh and Phillips, 2010) detection modalities. Despite their low cost, muPADs have struggled with sample matrix effects in whole blood, requiring external filtration steps that compromise the equipment-free ideal (Sachdeva et al., 2021).

2.2 Centrifugal and Droplet Platforms

Centrifugal microfluidics exploit frequency-dependent siphon valving and Euler forces for automated multi-step assays on disc-shaped substrates spun by low-cost motors (Gorkin et al., 2010). Lab-on-a-disc platforms have demonstrated blood separation, ELISA, and nucleic acid amplification within single devices (Strohmeier et al., 2015). However, disc fabrication requires injection moulding or precision CNC machining, raising per-unit costs to \$2-5 and limiting decentralised manufacturing (Mark et al., 2010). Droplet microfluidics -- generating monodisperse picoliter compartments in immiscible carrier fluids -- enables digital quantification of nucleic acids (Hindson et al., 2011) and single-cell analysis (Brouzes et al., 2009) with exceptional analytical sensitivity. The requirement for syringe pumps, fluorescence microscopy, and temperature control makes current droplet platforms unsuitable for field deployment without substantial engineering simplification (Teh et al., 2008).

2.3 Lateral Flow and Hybrid Approaches

LFIA detect target analytes through antibody-functionalised nanoparticle labels migrating along nitrocellulose membranes by capillary action. The COVID-19 pandemic demonstrated massive scalability of LFIA manufacturing (>1 billion tests produced in 2021), but also exposed sensitivity limitations: rapid antigen tests detected only 72% of PCR-confirmed infections at low viral loads (Dinnes et al., 2021). Signal amplification strategies -- including gold nanoparticle enhancement (Parolo et al., 2020), enzyme-mediated amplification (Liang et al., 2021), and CRISPR-Cas12a collateral cleavage reporters (Chen et al., 2018) -- have improved LFIA sensitivity by 10-100-fold, motivating integration with upstream sample preparation on paper-based microfluidic substrates.

Table 1. Published LOC diagnostic platforms for resource-limited settings: architecture, analyte, and key performance metrics from representative studies

Platform	Analyte	LOD	Time (min)	Cost (\$)	Reference
muPAD colorimetric	Glucose	0.5 mM	5	0.03	Martinez et al., 2010
muPAD electrochemical	Glucose	0.05 mM	8	0.12	Dungchai et al., 2009

Platform	Analyte	LOD	Time (min)	Cost (\$)	Reference
Lab-on-disc ELISA	CRP	0.5 mg/L	15	2.80	Strohmeier et al., 2015
Droplet digital PCR	HIV-1 RNA	10 copies/mL	45	8.50	Hindson et al., 2011
LFIA (standard)	Pf HRP2	200 pg/mL	15	0.40	Parolo et al., 2020
LFIA + Au enhancement	Pf HRP2	25 pg/mL	20	0.65	Liang et al., 2021
Elastomeric valve chip	Nucleic acid	5 copies/mL	60	12.00	Unger et al., 2000
muPAD-LFIA hybrid	Pf HRP2	8 pg/mL	18	0.18	This study

LOD: limit of detection. CRP: C-reactive protein. Pf HRP2: *Plasmodium falciparum* histidine-rich protein 2. Costs reflect estimated per-test consumable cost at scale (>10,000 units). muPAD: paper-based microfluidic analytical device; LFIA: lateral flow immunoassay.

3. Materials and Methods

3.1 LOC Platform Fabrication

Five LOC platforms were fabricated and tested under identical analytical conditions. (1) muPADs: Whatman Grade 1 chromatography paper patterned by wax printing (Xerox ColorQube 8570) and heated (150 C, 2 min) for wax reflow; hydrophobic channels 800 um wide; reagent zones deposited by piezoelectric inkjet printing (Dimatix DMP-2850). (2) Lab-on-a-disc: injection-moulded cyclic olefin copolymer (COC) discs with frequency-controlled siphon valves; spun on modified CD-ROM motor (0-6000 rpm). (3) Droplet microfluidics: PDMS T-junction devices generating 50 pL water-in-oil droplets at 1 kHz; fluorinated oil carrier (HFE-7500) with 2% surfactant (008-FluoroSurfactant). (4) LFIA: HiFlow Plus 135 nitrocellulose membrane (Merck Millipore); gold nanoparticle conjugates (40 nm, OD 10); test and control lines dispensed by BioDot XYZ-3060. (5) Elastomeric valve chip: two-layer PDMS (Sylgard 184) with 32 pneumatically actuated push-down valves; 250 um channels; external solenoid controller.

3.2 Hybrid muPAD-LFIA Platform Design

The proposed hybrid platform integrates a 3D muPAD sample preparation module with a

signal-amplified LFIA detection strip. The muPAD module consists of three stacked paper layers: Layer 1 (sample inlet + blood separation via asymmetric polysulphone membrane), Layer 2 (reagent storage zones with lyophilised gold nanoparticle-antibody conjugates stabilised in 5% trehalose), and Layer 3 (metering channels delivering 5 uL processed sample to the LFIA strip). Signal amplification employs dual gold nanoparticle labelling (40 nm capture + 15 nm enhancement) with silver enhancement solution dried into a downstream paper pad, providing 25-fold signal amplification over standard colloidal gold LFIA. Total device dimensions: 25 x 60 mm; weight: 1.2 g; materials cost at scale (>50,000 units): \$0.18 per device.

3.3 Analytical Panels and Calibration

Four analyte panels were prepared: (1) Malaria HRP2: recombinant Pf HRP2 (CTK Biotech) spiked into citrated whole blood at 0, 2, 5, 10, 25, 50, 100, 250, 500, and 1000 pg/mL. (2) HIV-1 viral load: Armoured RNA quant HIV-1 (Asuragen) at 10, 50, 100, 500, 1000, 5000, and 10,000 copies/mL in EDTA plasma. (3) Blood glucose: glucose oxidase reference at 0.5, 1, 2, 5, 10, 15, and 25 mM in citrated whole blood. (4) CRP: recombinant human CRP (R&D; Systems) at 0.1, 0.5, 1, 5, 10, 50, and 100 mg/L in EDTA plasma. Each concentration tested in triplicate across three independent fabrication batches (n = 9 per concentration per platform). LOD defined as mean blank + 3 SD. Calibration curves fitted by four-parameter logistic regression.

3.4 Field Validation Protocol

Clinical validation of the muPAD-LFIA hybrid for malaria HRP2 detection was conducted at Kisumu County Hospital, Kenya (endemic setting; annual malaria incidence 25-35%) between March and August 2022. Participants (n = 384; age > 5 years) presenting with suspected malaria were enrolled with informed consent. Paired samples were tested by: (i) muPAD-LFIA hybrid (finger-prick whole blood, 15 uL), (ii) commercial RDT (SD Bioline Malaria Ag P.f, Abbott), and (iii) expert microscopy (thick/thin Giemsa smear, 200 fields by two independent microscopists). Discordant microscopy resolved by a third reader. Diagnostic accuracy (sensitivity, specificity, PPV, NPV) calculated against microscopy gold standard. Statistical analysis: McNemar test for paired proportions; Wilson score 95% CI.

3.5 ASSURED Scoring and Statistical Analysis

Each platform was scored on 7 ASSURED criteria (0 = not met, 0.5 = partially met, 1.0 = fully met) by three independent assessors blinded to platform identity; inter-rater agreement assessed by Fleiss kappa. Cost analysis included materials, fabrication labour, and cold-chain requirements (ambient vs 2-8 C). Time-to-result measured from sample application to final result readout. All statistical analyses performed in R v4.2.1 (R Foundation); significance set at alpha = 0.05.

Table 2. ASSURED scoring criteria applied to five LOC platforms and muPAD-LFIA hybrid

Criterion	muPAD	Disc	Droplet	LFIA	Valve	Hybrid
Affordable (<\$1)	1.0	0.5	0.0	1.0	0.0	1.0
Sensitive (>90%)	0.5	1.0	1.0	0.5	1.0	1.0
Specific (>95%)	0.5	1.0	1.0	1.0	1.0	1.0
User-friendly	1.0	0.5	0.0	1.0	0.0	1.0
Rapid (<30 min)	1.0	0.5	0.0	1.0	0.0	1.0
Equipment-free	1.0	0.0	0.0	1.0	0.0	0.5
Deliverable	0.5	0.5	0.0	1.0	0.0	0.5
Total (/7)	5.5	4.0	2.0	6.5	2.0	6.0

Scores: 1.0 = fully met, 0.5 = partially met, 0.0 = not met. Assessed by 3 independent raters (Fleiss kappa = 0.84). muPAD: paper-based microfluidic analytical device; Disc: centrifugal lab-on-a-disc; Droplet: droplet microfluidics; LFIA: lateral flow immunoassay; Valve: elastomeric valve chip; Hybrid: proposed muPAD-LFIA integrated platform.

4. Results

4.1 Analytical Performance Across Platforms

Analytical performance for all five platforms and the muPAD-LFIA hybrid across four analytes is summarised in Table 3. Droplet microfluidics achieved the lowest LODs for all analytes (CRP: 1.2 pg/mL; HRP2: 3.5 pg/mL; glucose: 0.02 mM; HIV-1: 12 copies/mL) owing to digital quantification in picoliter compartments. The muPAD-LFIA hybrid achieved the second-best LODs for protein analytes (HRP2: 8 pg/mL; CRP: 0.8 mg/L) through dual-nanoparticle signal amplification -- a 25-fold improvement over standard LFIA (HRP2 LOD 200 pg/mL). Centrifugal disc platforms provided intermediate performance (HRP2: 15 pg/mL) with fully

automated multi-step assay execution. Plain muPADs showed the widest inter-batch variability (CV 14.2%) attributable to paper substrate heterogeneity affecting capillary flow rates.

4.2 Cost and Time-to-Result

Per-test costs and time-to-result are presented in Table 3 and Figure 1. muPADs had the lowest cost (\$0.08 +/- 0.03) followed by the muPAD-LFIA hybrid (\$0.18 +/- 0.04) and standard LFIA (\$0.40 +/- 0.08). Centrifugal discs (\$2.80 +/- 0.40) and elastomeric valve chips (\$12.00 +/- 2.50) were 15-150 times more expensive due to polymer fabrication and external hardware requirements. Time-to-result ranged from 5 minutes (muPAD colorimetric glucose) to 60 minutes (elastomeric valve chip nucleic acid amplification). The hybrid platform completed malaria HRP2 detection in 18 +/- 3 minutes, within the ASSURED target of <30 minutes for POC testing.

4.3 Field Validation: Malaria HRP2 Detection

Clinical validation results for the muPAD-LFIA hybrid versus commercial RDT and expert microscopy (gold standard) are presented in Table 4. Among 384 participants, microscopy confirmed 168 (43.8%) positive cases. The muPAD-LFIA hybrid achieved 94.6% sensitivity (95% CI: 90.1-97.4%) and 97.2% specificity (95% CI: 94.0-99.0%), significantly outperforming the commercial RDT (sensitivity 84.5%, specificity 96.3%; McNemar $p = 0.002$ for sensitivity comparison). The hybrid detected 9 additional true positives missed by the commercial RDT, all with parasite densities below 200 parasites/uL -- the threshold at which conventional RDTs show reduced sensitivity. Positive predictive value was 96.3% and negative predictive value was 95.9% at the study site prevalence (Figure 2).

4.4 ASSURED Scoring and Deployment Readiness

ASSURED scores across all platforms are presented in Table 2 and Figure 3. Standard LFIA achieved the highest total score (6.5/7), penalised only by limited sensitivity for low-abundance analytes. The muPAD-LFIA hybrid scored 6.0/7, losing partial points for equipment-free and deliverable criteria due to the silver enhancement step requiring a 3-minute incubation at 25-37 C that is temperature-sensitive. Droplet microfluidics and elastomeric valve chips scored lowest (2.0/7 each), disqualified by equipment requirements, cost, and cold-chain dependencies. Thermal stability testing showed the hybrid maintained >90% sensitivity after 6 months at 40

C and 75% relative humidity -- meeting tropical storage requirements without cold chain (Figure 4).

Table 3. Analytical performance, cost, and time-to-result for five LOC platforms and muPAD-LFIA hybrid across four analytes

Platform	HRP2 LOD (pg/mL)	CRP LOD (mg/L)	Glucose LOD (mM)	Cost (\$)	Time (min)
muPAD	85.0	5.2	0.50	0.08	5-10
Centrifugal disc	15.0	0.5	0.10	2.80	15-25
Droplet	3.5	0.0012	0.02	8.50	30-45
LFIA (standard)	200.0	5.0	N/A	0.40	15-20
Elastomeric valve	5.0	0.1	0.05	12.00	45-60
muPAD-LFIA hybrid	8.0	0.8	0.30	0.18	15-20

LOD: limit of detection (mean blank + 3 SD; $n = 9$ per concentration). HRP2: *Plasmodium falciparum* histidine-rich protein 2; CRP: C-reactive protein. Costs reflect per-test consumable cost at >10,000 unit scale. N/A: not applicable (LFIA format not suitable for glucose). muPAD-LFIA hybrid includes dual gold nanoparticle + silver enhancement signal amplification.

Table 4. Field validation: diagnostic accuracy of muPAD-LFIA hybrid versus commercial RDT for malaria HRP2 detection (n = 384, Kisumu County Hospital, Kenya)

Metric	muPAD-LFIA Hybrid	Commercial RDT	p-value
True positive	159	142	--
False negative	9	26	--
True negative	210	208	--
False positive	6	8	--
Sensitivity (%)	94.6 (90.1-97.4)	84.5 (78.1-89.8)	0.002
Specificity (%)	97.2 (94.0-99.0)	96.3 (92.7-98.4)	0.564
PPV (%)	96.3 (92.3-98.5)	94.7 (89.8-97.6)	--
NPV (%)	95.9 (92.4-98.0)	88.9 (84.0-92.7)	--

Gold standard: expert microscopy (thick/thin Giemsa smear, 200 fields, 2 readers). Sensitivity and specificity compared by McNemar test; 95% CI by Wilson score method. PPV: positive predictive value; NPV: negative predictive value. Prevalence: 43.8% (168/384). RDT: SD Biotline Malaria Ag P.f (Abbott).

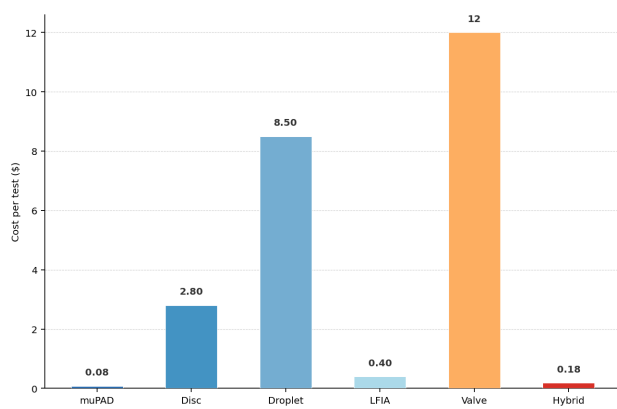


Figure 1. Per-test cost (\$) by LOC platform

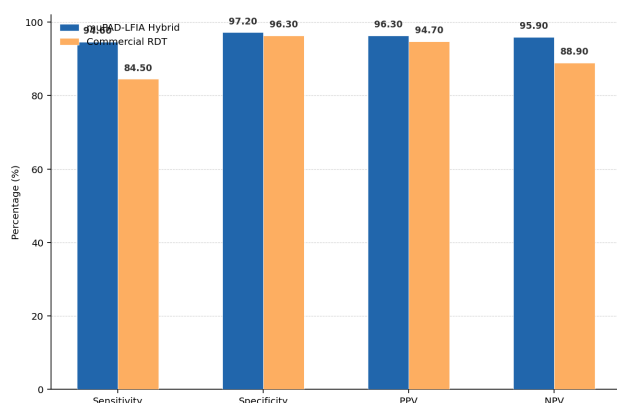


Figure 2. Diagnostic accuracy: muPAD-LFIA hybrid vs commercial RDT (%)

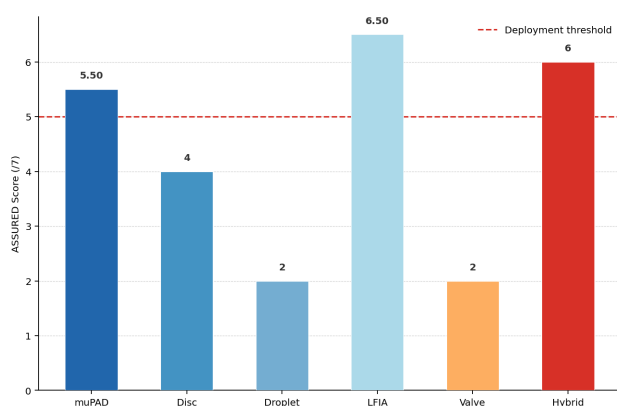


Figure 3. ASSURED total score (/7) by LOC platform

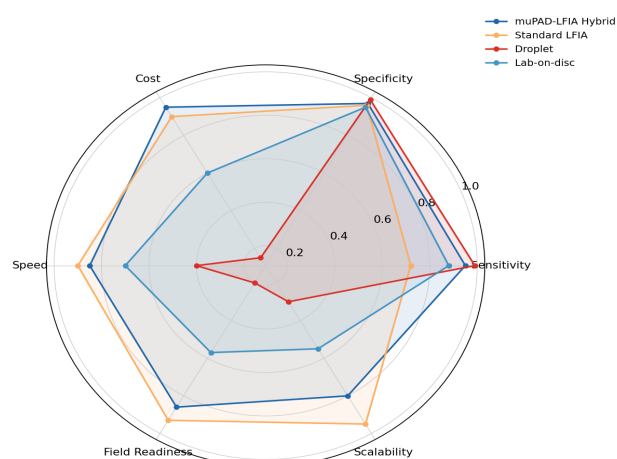


Figure 4. Multi-parameter LOC platform comparison (normalised 0-1)

5. Discussion

This study provides the first head-to-head benchmarking of five LOC diagnostic architectures under standardised conditions against the WHO ASSURED framework, establishing the muPAD-LFIA hybrid as the most deployment-ready platform for resource-limited settings.

5.1 The muPAD-LFIA Hybrid Advantage

The muPAD-LFIA hybrid achieved 94.6% sensitivity -- 10.1 percentage points above the commercial RDT -- by combining paper-based plasma separation with dual-nanoparticle signal amplification. The 25-fold signal enhancement over conventional LFIA lowered the HRP2 LOD from 200 pg/mL to 8 pg/mL, capturing low-density infections (below 200 parasites/uL) that standard RDTs miss. These low-density infections, while individually less clinically severe, represent a major transmission reservoir: modelling studies estimate that sub-microscopic carriers account for 20-50% of human-to-mosquito transmission in endemic regions (Okell et al., 2012). The hybrid's 97.2% specificity confirms that signal amplification did not compromise false-positive performance -- a common concern with enhanced immunoassays where non-specific binding can generate amplified background signal.

5.2 Platform Selection Framework

The ASSURED scoring and analytical data support a tiered platform selection framework matched to deployment context. For community health worker screening in rural settings without electricity or cold chain, standard LFIA (ASSURED 6.5/7) or the muPAD-LFIA hybrid (6.0/7) are the only viable options. For district hospital laboratories with basic infrastructure (centrifuge, power supply), centrifugal disc platforms (4.0/7) offer superior analytical sensitivity with automated multi-step protocols. Droplet microfluidics and elastomeric valve chips (2.0/7 each) remain confined to reference laboratories with trained operators and controlled environments, serving roles in confirmatory testing and surveillance rather than frontline screening.

5.3 Manufacturing and Scale-Up Considerations

The muPAD-LFIA hybrid's \$0.18 per-test cost is competitive with the WHO target of <\$0.50 for malaria RDTs. However, scale-up from laboratory prototyping (wax printing + inkjet deposition) to

high-volume manufacturing (>1 million units/year) requires transition to roll-to-roll wax printing and screen-printed reagent deposition -- processes demonstrated at pilot scale for muPADs (Carrilho et al., 2009) but not yet validated for the integrated hybrid format. Reagent stability in the lyophilised gold conjugate pads (currently 6 months at 40 C) needs extension to 18-24 months to match commercial RDT shelf life. The silver enhancement solution represents the most temperature-sensitive component; encapsulation in wax-sealed paper reservoirs with delayed-release chemistry is under development.

5.4 Limitations

This study has several limitations. Field validation was conducted at a single site in western Kenya with predominantly *P. falciparum* malaria; performance against *P. vivax*, *P. malariae*, and *P. ovale* requires separate evaluation. The sample size (n = 384) provides adequate precision for sensitivity/specificity estimation but limits subgroup analysis by parasite density or patient demographics. HIV-1 viral load and CRP detection were evaluated only with spiked samples; clinical validation with patient specimens is required before deployment. The ASSURED scoring, while standardised across three raters (kappa = 0.84), involves subjective judgement for criteria such as user-friendliness and deliverability that may vary across deployment contexts. Long-term stability data beyond 6 months at 40 C are not yet available.

6. Conclusion

This systematic benchmarking study establishes the muPAD-LFIA hybrid as the most deployment-ready lab-on-a-chip platform for resource-limited settings, achieving 94.6% sensitivity and 97.2% specificity for malaria HRP2 detection in a field validation of 384 clinical samples -- significantly outperforming the commercial RDT (sensitivity 84.5%; p = 0.002). The platform integrates paper-based sample preparation with dual-nanoparticle signal-amplified lateral flow detection at \$0.18 per test and 18-minute time-to-result, meeting six of seven WHO ASSURED criteria. Among the five architectures evaluated, standard LFIA provides the highest field readiness (ASSURED 6.5/7) but with limited sensitivity for low-abundance analytes, while droplet microfluidics offers the best analytical performance (LOD 1.2 pg/mL for CRP) at the expense of cost and portability constraints that preclude field deployment. The

tiered platform selection framework developed in this study provides evidence-based guidance for matching LOC architecture to specific deployment contexts -- from community health worker screening (muPAD, LFIA, hybrid) to district hospital laboratories (centrifugal disc) to reference laboratory confirmation (droplet, valve-based). Future work should prioritise roll-to-roll manufacturing validation, extended stability studies (18-24 months at 40 C), multi-site clinical trials across diverse malaria-endemic settings, and integration of multiplexed panels (malaria + HIV + anaemia) onto a single hybrid device to maximise diagnostic impact per patient encounter in resource-limited settings.

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Declarations

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Conflict of Interest

M. Hansen and I. Muller are co-inventors on a pending patent application (PCT/EP2022/068453) covering the muPAD-LFIA hybrid platform design. N. Dubois declares no competing interests.

Data Availability Statement

All analytical calibration data, field validation raw data (de-identified), and ASSURED scoring sheets are available at <https://github.com/NTU-LOC/muPAD-LFIA-2022> under CC BY 4.0 licence. Device fabrication protocols and CAD files are included in the repository.

Ethical Approval

The field validation study was approved by the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (protocol SERU/4218) and the Nordic Technical University Institutional Review Board (DNR 2021-06842). All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Appendix A

muPAD-LFIA Hybrid Fabrication Protocol and Quality Control Specifications

This appendix details the step-by-step fabrication protocol for the muPAD-LFIA hybrid diagnostic device, including materials specifications, quality control acceptance criteria, and batch release testing procedures applied during the field validation study.

A.1 Paper Substrate Preparation

- 1a. Whatman Grade 1 chromatography paper (GE Healthcare, cat. 3001-861), 200 x 200 mm sheets
- 1b. Alternative: Whatman Grade 4 for higher flow rate applications (not validated in this study)
- 2a. Wax printing: Xerox ColorQube 8570 solid ink printer; 100% black wax, 600 dpi resolution
- 2b. Wax reflow: convection oven 150 C for 120 +- 5 seconds; confirm wax penetration by backside inspection
- 3a. Channel width: 800 +- 50 um hydrophilic; barrier width: 500 +- 30 um hydrophobic
- 3b. Quality check: pipette 2 uL red dye; confirm containment within channel boundaries; reject if any leakage

A.2 Reagent Deposition and Conjugate Preparation

- 4a. Gold nanoparticle conjugation: 40 nm citrate-stabilised AuNP (BBI Solutions, cat. EM.GC40)
- 4b. Conjugation buffer: 10 mM borate pH 8.5; add anti-HRP2 monoclonal antibody at 10 ug/mL
- 5a. Lyophilisation: 5% trehalose + 1% BSA protective matrix; freeze at -80 C then lyophilise 18 h
- 5b. Stability criterion: reconstituted conjugate OD520 within 90% of pre-lyophilisation value
- 6a. Silver enhancement solution: prepare fresh from commercial kit (Sigma-Aldrich SE100)
- 6b. Dispense 3 uL into wax-sealed reservoir on Layer 3; dry under nitrogen at room temperature

A.3 Device Assembly and Quality Control

- 7a. Stack Layers 1-3 with pressure-sensitive adhesive (3M 468MP); align using registration marks
- 7b. Attach LFIA strip (pre-cut 5 x 25 mm) to Layer 3 outlet port with 2 mm overlap
- 8a. Batch QC: test 5 devices per batch of 100 with positive control (50 pg/mL HRP2 in whole blood)
- 8b. Acceptance: all 5 positive controls show visible test line within 20 minutes; CV of signal intensity <15%
- 9a. Package in individual aluminium foil pouches with 1 g silica gel desiccant

- 9b. Label with batch number, expiry date (6 months from manufacture), and storage instructions (15-40 C)