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Translational opportunities in aptamer and nanobody lateral flow assays within the WHO REASSURED framework

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The World Health Organization's REASSURED framework underscores the role of lateral flow assays (LFAs) in addressing diagnostic access gaps in low-resource regions affected by emerging and re-emerging pathogens, neglected tropical diseases, and diseases of unknown etiology. While antibody-based LFAs dominate clinical use, aptamers and nanobodies have emerged as alternative recognition elements with favorable stability, reproducibility, and manufacturing characteristics. This review examines the analytical performance of aptamer- and nanobody-based LFAs across infectious and immune-mediated disease contexts and situates these systems within the diagnostic development landscape. Across disease settings, analytically robust performance comparable to antibody-based formats is routinely demonstrated under controlled conditions. However, comparative analysis reveals a persistent disconnect between analytical feasibility and progression into standardized clinical validation and deployment. Translational outcomes are more often constrained by specimen access, comparator definition, manufacturing readiness, and regulatory precedent than by limitations in binder affinity or assay chemistry. These findings indicate that for researchers developing next-generation LFAs, analytical optimization alone is insufficient; successful translation increasingly depends on alignment with validation, manufacturing, and deployment frameworks that govern diagnostic evaluation.

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1. Introduction

Access to timely and reliable diagnostic testing varies substantially across global health systems. In high-resource settings, laboratory-supported diagnostic workflows are widely available, whereas in many low- and middle-income regions, access to essential diagnostic tools remains limited. It is estimated that a substantial proportion of the global population lacks reliable access to basic diagnostics, particularly in settings with constrained laboratory infrastructure.[1,2]

Infectious diseases account for a significant fraction of global mortality and disability-adjusted life years (DALYs), with disproportionate impact in regions where diagnostic access is limited.[1,2] In these contexts, delays in diagnosis can affect patient management, disease surveillance, and outbreak control. The COVID-19 pandemic highlighted both the strengths of centralized laboratory diagnostics under optimal conditions and their vulnerability to disruptions in supply chains, power availability, and trained personnel.[1]

These challenges extend across diverse disease classes. The World Health Organization (WHO) categorizes infectious diseases based on mortality burden, chronic morbidity, and epidemic potential, all of which depend on timely diagnosis for effective disease management.[3] The importance of timely diagnostic access is particularly evident for neglected tropical diseases such as Chagas disease, African trypanosomiasis, and dengue, which remain concentrated in resource-limited settings.[4] The same diagnostic constraints also affect conditions with unresolved etiology, including Kawasaki disease, multisystem inflammatory syndrome in children (MIS-C), and chronic kidney disease of unknown origin (CKDu), where delayed or inconsistent diagnostic access complicates clinical management and etiological investigation.[3–5]

Currently, established laboratory methods including enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), next-generation sequencing (NGS), Western blotting, microbial culture, and microscopy remain the reference standards for detecting infectious diseases and immune responses globally, but are largely concentrated within resource-rich and centralized laboratory settings.[2] These platforms offer high analytical sensitivity and specificity in ideal controlled laboratory conditions, but, they depend on stable power, specialized equipment, and trained personnel. When any of these requirements are disrupted, testing capacity is rapidly constrained. The COVID-19 pandemic made these infrastructure dependencies explicit

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and exposed their vulnerabilities at scale. At the same time, it demonstrated how lateral flow assays (LFAs) could partially offset these limitations by enabling decentralized testing. As paper-based diagnostics, LFAs expanded access by bringing screen capabilities into homes, clinics and community settings beyond the reach of centralized laboratories.[6]

To improve global health, the WHO developed the REASSURED (Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly, Rapid/Robust, Equipment-free, and Deliverable) framework. The framework describes the requirements for the development of equitable disease diagnostics in resource limited settings. The goal being to inform disease control strategies, strengthen health systems and improve patient outcomes. The REASSURED framework provides a lens for evaluating diagnostic platforms designed for use beyond centralized laboratory settings. While LFAs already meet many of these goals, gaps in sensitivity and stability remain.[6]

This review evaluates LFAs for infectious and immune-mediated disease diagnostics in relation to the WHO REASSURED framework, with specific attention to the performance and translational context of emerging recognition elements, including aptamers and nanobodies, relative to conventional antibody-based systems. We examine evidence demonstrating that these alternative binders can achieve analytical performance comparable to antibodies, while also encountering distinct challenges during validation and clinical translation within regulatory and evaluation pathways originally established for protein-based reagents. By identifying where existing validation, regulatory, and deployment systems support or constrain these technologies, this review highlights structural considerations that shape the development and implementation of next-generation LFAs for infectious, neglected, and emerging diseases.

2. Diagnostic landscape: LFAs and laboratory reference methods

Access to essential diagnostic testing remains limited for a substantial fraction of the global population, particularly in regions where laboratory infrastructure, trained personnel, and reliable supply chains are constrained.[1,7] This access gap persists despite a global *in vitro* diagnostics market exceeding 100 billion USD annually, indicating that market growth has not translated into equitable deployment of diagnostic capacity.[1,8,9] Diagnostic platforms must therefore be evaluated not only by analytical performance, but by the operational, logistical, and infrastructural requirements that determine whether testing can be delivered reliably outside centralized laboratory environments.

Established laboratory reference methods for infectious disease diagnostics include nucleic acid amplification tests and immunoassays, which provide high analytical sensitivity and specificity under controlled conditions and benefit from mature regulatory validation pathways.[2] However, their dependence on stable electrical power, calibrated instrumentation, cold chain reagent storage, and trained personnel fundamentally differentiates them from decentralized testing formats. Disruption of any of these components can sharply reduce testing capacity and reliability, a vulnerability made explicit during the COVID-19 pandemic through consumable shortages, logistics interruptions, and personnel constraints that limited laboratory throughput despite analytically mature assays.[1]

In contrast, lateral flow assays are designed as self-contained devices that integrate sample conditioning, reagent release, analyte capture, and signal development into a single membrane strip, enabling capillary driven transport without external instrumentation.

While LFAs typically exhibit reduced analytical sensitivity relative to amplification based or enzyme amplified laboratory assays, particularly for low abundance targets or complex clinical matrices, they remain central to decentralized screening and surveillance workflows where short time to result, accessibility, and deliverability are prioritized over maximal analytical sensitivity. Within the WHO REASSURED framework, LFAs frequently satisfy criteria related to affordability, user friendliness, rapidity, and equipment free operation, while sensitivity, selectivity, and long-term stability remain context dependent limitations.[10]

As illustrated in Fig. 1, PCR, ELISA, and LFA platforms exhibit distinct trade-offs in analytical sensitivity, turnaround time, cost, and infrastructure dependence.[6]

Meaningful comparison across diagnostic platforms requires distinguishing between analytical and clinical performance, as these metrics capture fundamentally different aspects of assay behavior and translational readiness.

Performance is evaluated at two levels:

- Analytical performance is defined by figures of merit such as limit of detection (LOD), limit of quantitation (LOQ), analytical specificity, precision, dynamic range, and

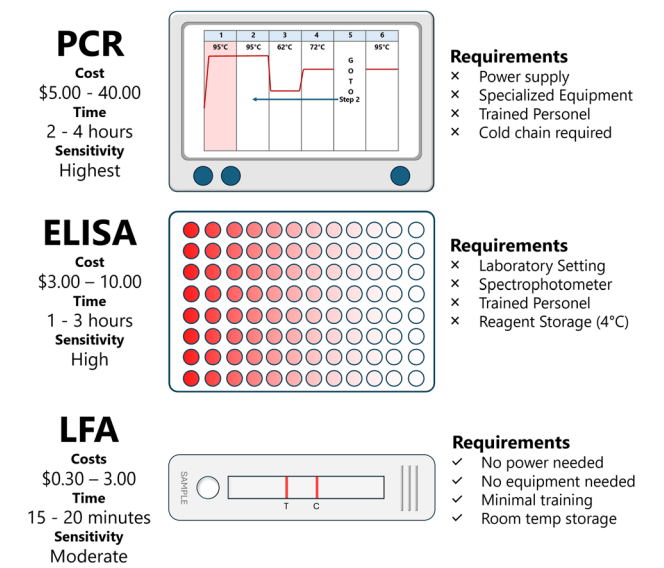

Fig. 1 Diagnostic performance characteristics of PCR, ELISA, and LFAs, showing relative cost, turnaround time, and analytical sensitivity.

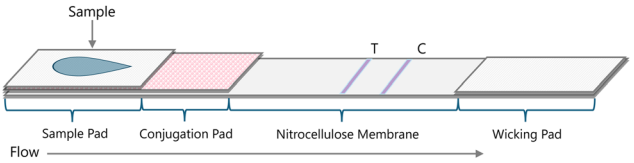

Fig. 2 Structure of a lateral flow assay strip, showing sample pad, conjugate pad, nitrocellulose membrane with test (T) and control (C) lines, and wicking pad.

susceptibility to interference, as measured under controlled laboratory conditions.[11,12]

- Clinical performance is defined by clinical sensitivity and specificity, predictive values, and likelihood ratios measured in intended-use populations, matrices, and workflows.[12,13]

Where reported, limits of detection and quantitation are distinguished explicitly, as many LFAs provide qualitative or semi-quantitative output without external readers, whereas PCR and ELISA typically support quantitative measurement across defined dynamic ranges.[14,15]

The review also notes whether analytical performance is derived from artificial matrices or contrived samples, as matrix effects and pre-analytical variables can substantially alter observed performance at the point of care.

The physical architecture of LFAs has a central role in governing assay robustness, flow kinetics, and sensitivity. The structure of a typical lateral flow assay strip is shown in Fig. 2, highlighting the modular components responsible for sample conditioning, reagent storage and release, analyte capture, and capillary driven transport.

Materials, functional roles, and translational considerations associated with each strip component are summarized in Table 1. These design parameters collectively influence assay time, analytical sensitivity, reproducibility, and long-term stability under decentralized use conditions.

Comparative analytical performance trade-offs, workflow requirements, infrastructure needs, and alignment with WHO REASSURED criteria across PCR, ELISA, and LFA formats are summarized in Table 2. PCR provides unmatched molecular sensitivity through target amplification but remains constrained to centralized laboratory infrastructure. ELISA offers robust quantitative protein detection with strong reproducibility under controlled workflows. LFAs trade analytical depth for speed, accessibility, and decentralization, enabling rapid screening and field deployment in settings where laboratory methods are impractical.

Head-to-head analytical performance data for representative infectious diseases are summarized in Table 3, with assays matched by target class to enable meaningful platform comparison. Across diseases, ELISA typically achieves one to two orders of magnitude lower limits of detection than LFAs due to enzymatic signal amplification and controlled workflows. LFAs consistently demonstrate high specificity, often exceeding 95 percent, with turnaround times under 20-minutes, supporting decentralized screening despite reduced analytical sensitivity relative to laboratory assays.

Low cost, stable diagnostics enable a shift from centralized laboratories to patient facing settings, particularly in regions where electrical power, cold chain infrastructure, and laboratory capacity remain limited. A cryptococcal antigen LFA implemented in Uganda achieved an incremental cost effectiveness ratio of approximately 6 USD per disability adjusted life year averted, illustrating the public health impact of deployable diagnostics in low resource settings.[28] Despite such efficiencies, access remains uneven. During the COVID-19 pandemic, more than three billion diagnostic tests were performed globally, yet fewer than one percent reached low income regions, reflecting supply chain and logistics constraints rather than fundamental technical limitations.[29,30] In parallel, many inflammatory and environmentally associated diseases lack definitive molecular biomarkers, relying instead on retrospective clinical criteria or exclusion based diagnoses.[31,32] LFAs address both access inequities and biomarker uncertainty through room temperature stability, minimal infrastructure requirements, and compatibility with decentralized manufacturing and distribution workflows, positioning them as critical components of equitable diagnostic strategies aligned with WHO REASSURED where centralized laboratory methods cannot be reliably deployed.

Table 1 Key components of LFA strips: materials, functions and considerations

Component	Typical materials	Function	Key considerations
Sample pad	Glass fiber, cellulose	Introduces and conditions sample	Minimize nonspecific binding; allows buffer/detergent pretreatment
Conjugation pad	Polyester, glass fiber, cellulose	Stores and releases recognition elements	Stabilize reagents for storage; optimized for rapid release during assay
Nitrocellulose membrane	Nitrocellulose (varied pore sizes)	Analytical surface with printed test & control lines	Pore size, binding capacity and flow rate dictate sensitivity and reproducibility
Wicking pad	Cellulose high absorbency fibers	Sustains capillary flow and collects excess fluid	Must draw entire sample through membrane
Protective overlay	Transparent polymer film	Protects printed membranes from abrasion, dust and moisture	Optically clear to allow visual or instrument detection. Adhesive compatibility. Can affect assay kinetics
Backing card	Polyvinyl chloride, polyester, composite laminate	Structural support	Alignment of strip components
Plastic cassette	Polystyrene, ABS plastic	Housing, protection, user interface	Defines sample port, protects strip, enhances handling

Table 2 Analytical and practical comparison of PCR, ELISA, and LFA formats

Category	PCR	ELISA	LFA
Analytical precision *vs.* accessibility	Highest sensitivity for nucleic acid detection through target amplification	Quantitative detection of diverse analyte classes, including proteins, antibodies, small molecules, and haptens, using adaptable assay formats with strong reproducibility	Rapid, low-cost detection trading analytical depth for speed and accessibility
Infrastructure and expertise	Requires stable power, calibrated instrumentation, and trained personnel	Requires controlled laboratory conditions and trained operators	Functions in decentralized or resource limited environments
Time and throughput	Multi-hour workflow	Multi-hour workflow	Results available within minutes
Cost and scalability	High per test cost and instrument expense	Moderate cost, suited to high throughput microplates	Lowest cost, easily mass produced and distributed
Quantitative output	Fully quantitative using amplification curves	Quantitative or semi quantitative using standard curves	Largely qualitative or semi-quantitative, improving with digital readers
Complementarity	Provides confirmatory nucleic acid-based diagnosis	Provides quantitative detection of antigenic, serological, or small-molecule targets for confirmatory analysis	Enables rapid screening and field surveillance
Equity and alignment with WHO REASSURED	Limited to centralized laboratories	Limited by infrastructure needs	Aligns with affordability, portability, and user-friendliness for equitable access

Table 3 Analytical performance of ELISA and LFAs across representative infectious diseases. PCR is excluded from this comparison as it detects nucleic acids rather than proteins; head-to-head comparison with ELISA and LFA formats is therefore not meaningful for the protein-based targets presented here

Disease	Assay format	Target analyte	Sensitivity (%)	Specificity (%)	Limit of detection	Assay time	Ref.
COVID-19	ELISA (IgG)	Anti-SARS-CoV-2	94.0–100	98.0–100	1–10 ng mL^{-1}	2–4 h	16, 17
COVID-19	LFA (IgG)	Anti-SARS-CoV-2	43.0–78.0	90.5–100	50–200 ng mL^{-1}	15–20 min	16, 18
COVID-19	ELISA (IgM)	Anti-SARS-CoV-2	88.0–94.0	98.0–100	Variable	2–4 h	17
COVID-19	LFA (IgM)	Anti-SARS-CoV-2	19.4–64.5	84.3–100	Variable: >100 ng mL^{-1}	15–20 min	18, 19
Malaria	ELISA (HRP2)	HRP2 antigen	85–95	95–98	<0.025 ng mL^{-1}	2–4 h	20, 21
Malaria	LFA (HRP2)	HRP2 antigen	55.7–71.0	98.2–100	Analytical: 0.8–1.6 ng mL^{-1} Field: ~0.5–12 ng mL^{-1}	15–20 min	12, 22
Malaria	ELISA (pLDH)	pLDH enzyme	80–90	95–98	5–50 ng mL^{-1}	2–4 h	12
Malaria	LFA (pLDH)	pLDH enzyme	49.9–64.0	90.4–99.5	49–200 parasites per μL	15–20 min	12, 13
HIV	4th Gen ELISA	Anti-HIV-1/2 + p24 antigen	99.5–100	99.5–100	Antibodies: sub-ng mL^{-1} p24: 0.5–10 pg mL^{-1}	2–4 h	23
HIV	ELISA	HIV-1 p24 antigen	Research	Research	p24: 0.003–0.035 pg mL^{-1}	3–5 h	24, 25
HIV	LFA (4th Gen)	Antibodies + p24	97.0–99.5	98.5–99.5	p24: >50–100 pg mL^{-1}	15–20 min	26, 27
HIV	3rd Gen ELISA	Anti-HIV-1/2	99.0–99.9	99.0–99.9	1–10 ng mL^{-1}	2–4 h	26
HIV	LFA (antibody)	Anti-HIV-1/2	98.0–100	99.0–100	Estimated: >50 ng mL^{-1}	15–20 min	26
HIV	OraQuick oral fluid	Anti-HIV-1/2	91.7–98.0	99.7–100	N/A	4 min	26, 27

3. Scientific innovations in LFA technology

LFAs have progressed from purely qualitative visual tests toward platforms capable of semi-quantitative and quantitative measurement through advances in molecular recognition, signal generation, and materials engineering. For a comprehensive primer on the variety of LFA formats the reader is directed to Conrad and DeRosa (2025).[33] Antibodies remain the dominant and most clinically established recognition elements, while aptamers and nanobodies have emerged as alternatives with physicochemical properties that influence assay stability, manufacturability, and storage under decentralized conditions. In parallel, platform level innovations in reporter chemistry, digital interpretation, and strip architecture have expanded LFA capability without increasing infrastructure requirements.

3.1 Molecular recognition strategies for LFAs

Molecular recognition elements govern not only analytical sensitivity and specificity, but also manufacturability, batch reproducibility, environmental robustness, and regulatory translation. These attributes directly influence LFA performance under decentralized deployment conditions. Antibodies, aptamers, and nanobodies achieve comparable binding affinities, yet differ substantially in production workflows, stability profiles, and regulatory familiarity. Translationally relevant properties across these recognition

Table 4 Properties of LFA recognition elements

Property	Antibodies[34]	Aptamers[35,36]	Nanobodies[37]
Origin	Biological (animal/cell)	Chemical (SELEX)	Recombinant (camelid VHH)
Size	150 kDa	6–30 kDa	15 kDa
Affinity (K_D)	nM–pM	nM–pM	nM–pM
Stability	Moderate (4 °C storage)	Excellent (ambient)	Excellent (ambient)
Production cost	High	Low	Moderate
Batch reproducibility	Polyclonal: Variable Monoclonal: Excellent	Excellent	Excellent
Clinical examples	All commercial LFAs	OTA, SARS-CoV-2	Emerging (COVID, malaria)

strategies are summarized in Table 4, emphasizing features such as cold chain dependence, synthetic accessibility, and clinical precedent rather than equilibrium dissociation constants alone.

Antibodies, most commonly immunoglobulin G (IgG), remain the reference recognition elements for LFAs due to extensive clinical precedent and regulatory familiarity. Standard immobilization strategies enable reliable capture in lateral flow formats, but assay performance is sensitive to orientation, surface density, and drying conditions, with suboptimal immobilization reducing capture efficiency and increasing signal variability. Dependence on refrigerated storage, susceptibility to thermal and chemical denaturation, and variability inherent to biological production workflows influence shelf life and robustness during storage and use in decentralized settings. The canonical IgG structure underlying these considerations is shown schematically in Fig. 3.[38–42]

Aptamers are single stranded nucleic acids generated through *in vitro* selection and chemical synthesis, with binding properties encoded by nucleotide sequence and structure.[35,43] Selection is performed using systematic evolution of ligands by exponential enrichment (SELEX), illustrated in Fig. 4.[44] While unmodified aptamers are susceptible to nuclease degradation in biological matrices, post selection chemical modifications can substantially improve stability and enable conjugation for LFA use. Immobilization strategies include thiol gold interactions, biotin streptavidin coupling, and covalent attachment to nitrocellulose. Despite favourable stability, low production cost, and excellent batch reproducibility, regulatory adoption of aptamer-based diagnostics remains limited, increasing evidentiary requirements during clinical translation.[35,43,45–48]

Nanobodies are single domain antigen binding fragments derived from heavy chain only antibodies found in camelids. Their small size and recombinant production enable high yield, batch consistency, and enhanced thermal and chemical stability relative to full length immunoglobulins. These properties support reduced cold chain dependence and improved robustness under variable storage and handling conditions. The heavy chain only antibody architecture and isolated nanobody domain are depicted in Fig. 5.[49–53]

3.2 Platform innovations enabling quantitative and decentralized LFA deployment

Advances in reporter chemistry and portable readers have extended LFAs beyond qualitative formats toward semi quantitative and quantitative diagnostics compatible with decentralized deployment. While colloidal gold remains widely used due to reliability and low cost, alternative labels including fluorescent dyes, enzymes, and upconverting nanoparticles improve sensitivity and dynamic range when paired with appropriate reader systems.[54–58]

Digital interpretation has become a key enabler of quantitative LFA performance. Smartphone based and dedicated readers standardize test line quantification, reduce user dependent variability, and enable data storage and transmission for remote oversight. Machine learning assisted

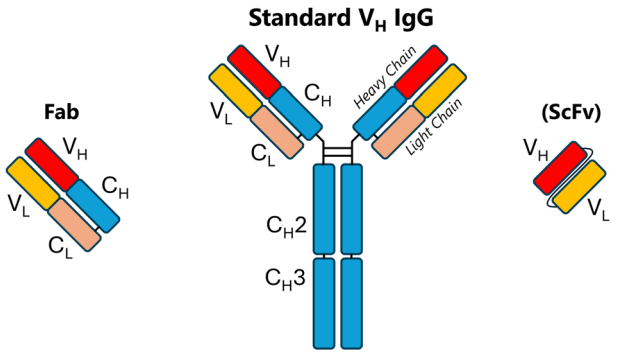

Fig. 3 Typical Y shaped antibody – heavy chains and light chains.

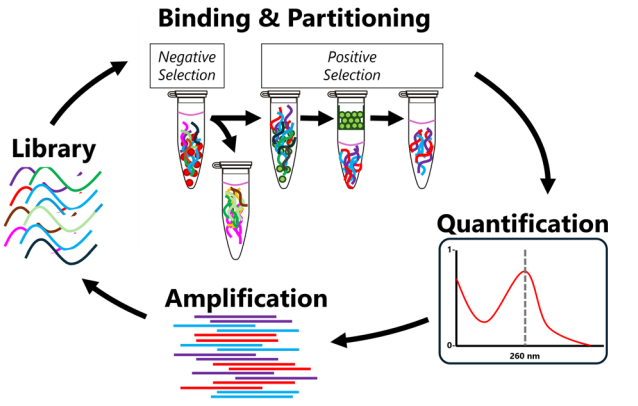

Fig. 4 SELEX-based selection and enrichment of nucleic acid aptamers for molecular recognition applications.

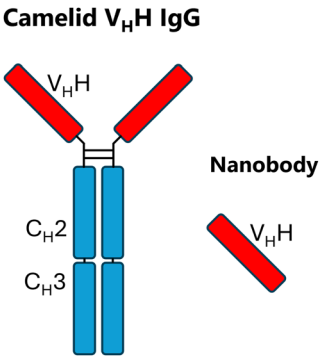

Fig. 5 Camelid IgG architecture showing the heavy-chain-only format and the isolated VHH nanobody derived from it.

analysis further improves robustness by correcting for lighting variation, weak signal intensity, and strip-to-strip heterogeneity.[59] These features directly support REASSURED criteria related to real time connectivity, user friendliness, and robustness.

Multiplexed strip designs increase informational yield by enabling simultaneous detection of pathogen derived and host response markers without increasing procedural complexity. Materials engineering advances, including optimized nitrocellulose membranes and improved pad architectures, enhance flow uniformity and reduce coefficients of variation, supporting lot to lot consistency in high volume or locally manufactured assays.[60]

Together, these platform level innovations expand LFA capability while preserving the low infrastructure requirements essential for equitable deployment.

4. Translational challenges and validation bottlenecks

Analytical validation establishes what an assay can measure under controlled conditions, but translation into deployed LFAs requires that performance persist across real-world samples, users, and operating environments. For LFAs, this transition is governed by three coupled domains: clinical validation, regulatory evaluation, and manufacturing consistency. Innovation in molecular recognition expands feasible assay chemistries, but it also increases evidentiary requirements related to comparability, stability, and process control. This section examines how mismatches between analytical capability and translational infrastructure shape deployment timelines for LFAs intended for infectious and immune mediated disease diagnostics.

4.1 Analytical *versus* clinical validation in LFA deployment

Analytical and clinical validation address distinct dimensions of diagnostic performance. Analytical validation establishes detection capability under standardized laboratory conditions, whereas clinical validation determines whether that capability translates into accurate classification in intended-use populations and workflows. This distinction is especially important for LFAs deployed outside centralized laboratory settings, where biological heterogeneity and operational variability are unavoidable. These differences are formally recognized in diagnostic evaluation guidelines and regulatory frameworks.[61,62]

Analytical validation typically relies on well-characterized samples evaluated under controlled conditions, with performance assessed using metrics such as limits of detection, analytical specificity, precision, and interference testing.[61,63,64] These measurements are often performed in buffer systems, contrived matrices, or banked specimens in which pre-analytical variables are minimized. Clinical validation, by contrast, evaluates performance under real-world conditions, incorporating biological variability, differences in sample quality, and user-dependent operational factors.[65–67]

The divergence between analytical and clinical performance is illustrated by both nucleic acid amplification tests and immunoassays. Under controlled conditions, PCR achieves very low LODs, yet clinical sensitivity depends strongly on specimen type, collection timing, and sample handling.[62,68] Similarly, antibody-based immunoassays, including ELISA-derived formats, report host immune responses that vary with infection stage and prior exposure, influencing performance across population contexts.[65,69]

For antibody-based LFAs, the relationship between analytical performance and clinical utility is supported by decades of use across multiple disease areas.[16–19,21–27] In contrast, LFAs incorporating alternative recognition elements such as aptamers or nanobodies have far less clinical precedent and fewer intended-use validation datasets. As a result, analytically promising assays may still require additional bridging between laboratory performance and real-world use, for example matrix-specific nuclease-stability studies for aptamers or expanded lot-to-lot comparability and aggregation analyses for recombinant nanobodies. The practical consequences of this limited precedent are discussed further in section 4.2.[70,71]

4.2 Regulatory precedent and manufacturing consistency as translational constraints

Regulatory pathways for *in vitro* diagnostics define the requirements for translating analytically validated LFAs into authorized and manufacturable products. ISO 13485 establishes quality-management system requirements governing design control, supplier qualification, process validation, traceability, and batch consistency, independent of assay chemistry.[72] Within this broader framework, ISO 23640 provides a stability-evaluation framework for demonstrating that an *in vitro* diagnostic (IVD) maintains its performance characteristics across claimed shelf life, transport, and in-use conditions.[73] Analytical performance expectations are commonly established using Clinical and Laboratory Standards Institute guidelines such as EP17, which define standardized approaches for detection-capability claims, while EP25-style study designs and

WHO technical guidance define real-time and accelerated stability, in-use stability, and transport or challenge studies that support end-of-shelf-life claims.[74–77]

In practice, these frameworks require manufacturers to define acceptance criteria *a priori* and demonstrate that assay performance remains within specification across real-time, accelerated, and in-use conditions, with stability claims tied directly to functional readouts such as signal intensity or detection limits rather than solely reagent integrity, implicitly assuming degradation pathways and stability behaviours consistent with protein-based systems.

Clinical equivalence and market authorization are then assessed through mechanisms including FDA 510(k) clearance and WHO Prequalification, the latter emphasizing performance and usability under decentralized conditions.[78,79]

Although these frameworks are nominally chemistry-agnostic in design, most regulatory precedent was developed around antibody-based assays. As a result, validation expectations often reflect antibody-specific degradation pathways, immobilization strategies, and stability behaviours. Diagnostics incorporating aptamers or nanobodies may therefore require additional evidence to demonstrate equivalence, even when analytical metrics align with established tests.[65,66] Such evidence can include extended stability studies, alternative stress-testing protocols, or supplemental comparability analyses designed to show that performance is preserved across shelf-life, in-use, and transport conditions.[80,81]

Aptamers present distinct challenges because they are nucleic-acid-based reagents subject to enzymatic degradation and structure-dependent folding rather than classical protein denaturation.[45,46] In practical terms, reduced signal after storage or stress can reflect irreversible degradation, altered secondary structure, changes in surface immobilization, or buffer- and ionic-strength-dependent folding effects, which are not explicitly distinguished in standard stability templates. Stability assessments commonly applied to antibodies may therefore not capture the dominant failure modes of aptamer LFAs. As a result, aptamer-based systems may require more tailored validation approaches, including matrix-dependent nuclease-stability studies, explicit justification of whether shelf-life claims are anchored to sequence integrity, binding affinity, or final dried-strip performance, and cautious interpretation of accelerated studies in which heat-induced unfolding may be partly reversible.[74,82–84]

Nanobodies face related but narrower constraints due to their small size, lack of an Fc domain, and frequent microbial expression, which limit direct transfer of antibody-optimized validation strategies.[37,69,83,84] Under ISO 23640, loss of function in nanobody-based systems can usually be interpreted using familiar protein-centric concepts such as denaturation, aggregation, and moisture sensitivity. However, developers may still be asked for expanded lot-to-lot comparability, aggregation or charge-variant analyses, and bridging to IgG-based predicates because historical acceptance criteria and comparator expectations remain calibrated to conventional monoclonal antibodies.[37,69,83,84]

These regulatory constraints reflect limited precedent rather than resistance to new recognition strategies. Each approved diagnostic incorporating non-antibody binders contributes precedent that incrementally reduces uncertainty and evidentiary burden for subsequent submissions.[47,69,70,85]

Within this landscape, manufacturing consistency becomes a key determinant of whether analytically validated aptamer- and nanobody-based LFAs can satisfy ISO 13485 and ISO 23640 expectations at scale.[72,73] Small variations in conjugation chemistry, drying conditions, or membrane properties can have disproportionate effects on structure-dependent nucleic acids and recombinant single-domain binders, increasing the burden on process control and in-process testing relative to conventional antibody LFAs.

Table 5 provides a conceptual synthesis of the relative translational maturity of recognition-element platforms, based on trends observed across the cited studies.

4.3 Manufacturing and quality systems as performance preservation

Following regulatory authorization, diagnostic translation depends on preserving analytical performance during manufacturing, storage, transport, and routine use. In LFAs, this preservation depends primarily on process reproducibility and environmental control rather than recognition chemistry alone. Manufacturing therefore constitutes a performance critical stage of translation.[66,88]

Small variations in manufacturing parameters, including membrane pore size, pad overlap, drying time, humidity, adhesive application, and assembly order, directly influence capillary flow, reagent release kinetics, and signal intensity. These variables are well characterized for antibody based LFAs through decades of commercial production.

Assays incorporating aptamers or nanobodies introduce additional manufacturing sensitivities that are less

Table 5 Conceptual comparison of recognition-element platforms based on synthesis of analytical performance, regulatory precedent, and translational outcomes discussed in the literature

Platform or context	Analytical readiness	Regulatory precedent	Infrastructure dependence	Translational status	Ref.
Aptamer LFAs	High	Low	Low	Prototype and preclinical	47, 70
Nanobody LFAs	High	Low	Low	Clinical pilot and early approval	37, 86, 87
Antibody LFAs	High	Very high	Moderate	Fully commercialized	

Illustrative examples of aptamer and nanobody LFAs and their regulatory status are discussed in sections 4.1–4.3 and case studies in section 5.0.

standardized. Aptamer performance can be influenced by terminal functionalization efficiency, lyophilization conditions, and buffer composition that alter secondary structure despite intact sequence integrity.[45,55] Nanobody performance depends on expression host selection, folding efficiency, purification conditions, and conjugation strategy, as approaches optimized for IgG do not reliably translate to single domain binders.[6,89]

Nanobody-based assays present a different manufacturing profile. Recombinant expression in microbial systems enables high yield and scalability, but expression host selection, folding efficiency, and purification conditions can influence product heterogeneity.[37,55,69,90]

Quality systems mitigate these risks through documented process controls, in process testing, and batch release criteria.[66,88] However, the absence of standardized reference materials and functional benchmarks for non-antibody recognition elements requires many developers to establish internal validation workflows independently, increasing development timelines and limiting cross site comparability.[91,92] These constraints reflect ecosystem maturity rather than fundamental technical limitations.[74,88,90]

4.4 Validation challenges for rare and neglected diseases

Clinical validation depends on access to well characterized biological specimens collected under conditions that reflect intended use. For rare and neglected diseases, this requirement represents a major translational bottleneck independent of assay design or analytical performance.[5,93]

Diseases such as Kawasaki disease and multisystem inflammatory syndrome in children (MIS-C) lack definitive molecular biomarkers and rely primarily on clinical criteria, resulting in inconsistent specimen availability and geographically dispersed sampling. Biological heterogeneity introduced by disease stage, prior treatment, and coexisting inflammatory conditions further complicates validation.[94] Similar constraints apply across neglected tropical diseases, where outbreak driven sampling, limited infrastructure, and pre analytical variability obscure biological signal and confound diagnostic accuracy.[6,70,89]

Ethical and logistical requirements, including biosafety approvals, export permits, consent procedures, and material transfer agreements, further restrict validation timelines.[70,85] As a result, early-stage diagnostic development frequently relies on banked specimens or contrived samples that are sufficient for analytical characterization but inadequate for establishing clinical performance.[85,95]

5. Case studies and translational patterns

Selected case studies across emerging diseases, neglected diseases, and conditions of uncertain etiology are examined to assess whether analytically successful aptamer- and nanobody-based binders advance into deployable lateral flow assays. Non-lateral flow platforms (*e.g.*, ELISA-derived formats, electrochemical sensors, and interferometric systems) are referenced only when they represent the primary evidence base for binder performance during early development. Across disease settings and recognition element classes, analytical feasibility is often established under controlled conditions, yet progression into standardized clinical validation and deployable LFA formats remain limited. Where translation stalls, the dominant constraints are recurrently practical rather than biochemical, including specimen access, comparator definition, manufacturing readiness, and regulatory precedent.

5.1 Analytical properties in formats relevant to LFA development

Analytical evaluation of antibody-, aptamer-, and nanobody-based assays is commonly reported using sensitivity, specificity, limit of detection, and reproducibility under controlled conditions. In this section, these properties are treated as developmental indicators that inform feasibility within lateral flow architectures rather than as predictors of deployable performance. Characterization is typically performed using defined analytes in buffered systems, contrived matrices, or limited clinical specimens, enabling controlled benchmarking while limiting inference about real-world use. Accordingly, the case studies below emphasize analytical features that map directly onto lateral flow behaviour, including target recognition in complex matrices, signal generation at a defined capture zone, and reproducibility across replicate tests, with

Table 6 Representative aptamer- and nanobody-based assays incorporating lateral flow configurations

Disease	Target	Recognition element	Reported performance	LFA configuration	Ref.
COVID-19	SARS-CoV-2 S1	Nanobody-anti-S1	LOD ≈ 0.4×10^4 viral copies per mL	Dual-gold LFA (Au–Nb detector/ACE2 capture)	86
COVID-19	Nucleocapsid protein	Nanobody	LOD ≈ 3.15 PFU mL^{-1}	Nanobody-gold LFIA	87
Zika virus	NS1	Nanobody NbZV-2	0.9–1.1 nM; LOD ≈ 1 ng mL^{-1}	LFA	96
Dengue virus	NS1 protein from dengue virus (DENV-2)	Aptamer-antibody	LOD 5.2 ng mL^{-1} (buffer); 6.1 ng mL^{-1} (serum)	Hybrid aptamer-antibody LFA	97
African trypanosomiasis (AAT)	T. congolense pyruvate kinase (TcoPYK)	Nanobody pair (Nb44/Nb42)	Visual LOD ~880 ng mL^{-1} (PBS); ~14 ng mL^{-1} (plasma)	Sandwich Nanobody LFA	37

Abbreviations: ELASA, enzyme-linked aptamer sorbent assay; LFIA, lateral flow immunoassay.

representative examples of assays implemented in lateral flow formats summarized in Table 6. Binder discovery methods and affinity optimization are addressed earlier and are not revisited here.

Maher *et al.* (2023) developed a signal-enhanced lateral flow assay for detection of the SARS-CoV-2 spike S1 antigen in saliva using gold-conjugated nanobodies as detector probes and ACE2 as the capture ligand. In a clinical evaluation of 320 saliva samples, the nanobody-based strip achieved 97.14% sensitivity and 98.57% specificity for $C_t \leq 30$, outperforming a monoclonal antibody-based dual-gold format. The reported limit of detection of 0.4×10^4 viral copies per mL represented a four-fold improvement over the nanobody-independent comparator, demonstrating clinically relevant performance in a non-invasive sample matrix.[86]

Jeong *et al.* (2025) engineered a nanobody-gold lateral flow immunoassay targeting the SARS-CoV-2 nucleocapsid protein using high-affinity VHH–AuNP conjugates. The optimized strip achieved a limit of detection of 3.15 PFU mL^{-1} with a wide dynamic range and maintained a 15-minute time to result. Clinical testing showed improved signal–titer correlation and fewer false negatives relative to a commercial antibody-based rapid test, illustrating how nanobody-AuNP conjugation can enhance LFIA sensitivity without added amplification steps.[87]

Beyond SARS-CoV-2, Peng *et al.* (2025) reported a fully implemented nanobody-based lateral flow assay for detection of Zika virus NS1 antigen, achieving visible signal generation within minutes and a reported limit of detection of approximately 1 ng mL^{-1} in buffer and urine matrices. Importantly, the nanobody reagents retained binding activity under ambient storage conditions, supporting suitability for decentralized deployment.[96]

Aptamer integration into LFA formats is less frequently reported but is exemplified by Jadidi *et al.* (2025), who developed a proof-of-concept aptamer–antibody lateral flow assay for dengue virus NS1 detection. The assay demonstrated low-ng mL^{-1} sensitivity in buffer and spiked serum with minimal cross-reactivity, establishing feasibility of aptamer use within hybrid LFA architectures.[97]

Table 7 Representative aptamer-based assays targeting emerging and reemerging infectious diseases across diagnostic formats

Disease	Target	Recognition element	Reported performance	Intended diagnostic format	Ref.
Buruli ulcer	Mycolactone (lipid toxin from *Mycobacterium ulcerans*)	RNA aptamer	Micromolar	ELONA	98
Chagas disease	*Trypanosoma cruzi* trypomastigotes	RNA aptamers	8–25 nM	Magnetic-bead capture assay	99
Chikungunya virus	Envelope E2 protein	DNA aptamers	30–177 nM	Sandwich ELASA	100
Chikungunya virus	Envelope E2 glycoprotein	Multivalent VHH constructs	0.2–1.5 nM	ELISA; diagnostic-ready binding reagents	101
COVID-19 (SARS-CoV-2)	Nucleocapsid (N) protein	ssDNA aptamer (tNSP3)	0.6–3.5 nM (BLI)	Label-free biolayer interferometry	102
COVID-19 (SARS-CoV-2)	Nucleocapsid RNA-binding domain (N-NTD)	Compact stem-loop ssDNA	Sub-nanomolar (estimated)	Structural probe/chemical sensor	103
COVID-19 (SARS-CoV-2)	Nucleocapsid protein	Nanobody panel (Nb4–Nb12)	0.28–0.44 nM	Two-site ELISA; NanoLuc chimera	104
Pan-coronavirus (SARS-CoV-2, SARS-CoV-1, MERS-CoV)	Nucleocapsid protein	ssDNA panel (6 sequences)	1.3–135 nM (SPR)	Electrochemical sensor; therapeutic exploration	105
Middle East respiratory syndrome (MERS)	Spike RBD	Nanobody NbMS10 (±Fc fusion)	0.35–0.87 nM	ELISA; SPR; neutralization assay	106
Crimean-Congo hemorrhagic fever	Nucleoprotein (NP)	ssDNA aptamer (Apt33)	Low-nM (SPR)	Aptamer–antibody ELASA	107
Dengue virus	NS1 protein (DENV-2)	RNA aptamers	37–41 nM	ELASA	108
Ebola virus disease	Soluble glycoprotein (sGP) and GP1,2	ssDNA aptamers (6011, 6012) and 2′-fluoropyrimidine RNA	≈2 nM (binding isotherms)	Electrochemical aptasensor	109
Ebola virus disease	Nucleoprotein (NP)	Nanobodies (R14, IC13)	Sub-nanomolar	Sandwich ELISA; antigen test	110
Human African trypanosomiasis	*Trypanosoma brucei* surface proteins	RNA aptamers	60–70 nM	Binding and inhibition assay	111
African animal trypanosomiasis(AAT)	Glycosomal fructose-1,6-bisphosphate aldolase (TcoALD)	Nanobody (capture) + monoclonal antibody (detector)	ELISA LOD: 78.13 ng (≈2× negative control)	Hybrid sandwich ELISA; dot blot (LFA feasibility)	112
Rift Valley fever	Nucleocapsid (N) protein	RNA aptamers	High affinity (qualitative)	Fluorescence-based biosensor	113
Soil-transmitted helminthiasis (toxocariasis)	*Toxocara canis* excretory/secretory antigens (TES)	Nanobody Nb-TES1 on magnetic nanoparticles	Detection limit ≈0.1 ng mL^{-1}	Electrochemical magnetosensor	114

Abbreviations: ELONA, enzyme-linked oligonucleotide (aptamer) assay; ELASA, enzyme-linked aptamer sandwich assay.

In neglected disease contexts, Pinto Torres *et al.* (2018) demonstrated a nanobody-based LFA for active Trypanosoma congolense infection using a VHH pair targeting pyruvate kinase. Optimization improved performance in spiked plasma, and an initial cattle evaluation highlighted herd-level screening potential, positioning the assay as a candidate field-deployable nanobody LFA pending broader validation.

While the studies summarized in Table 6 represent cases in which aptamer- and nanobody-based binders were explicitly integrated into lateral flow architectures, they constitute a small subset of the broader diagnostic literature. In parallel, a substantially larger body of work establishes analytically robust binders and detection strategies using non-lateral flow formats, including ELISA-based, electrochemical, optical, and structural platforms. These studies frequently demonstrate high-affinity target recognition, signal generation in biologically relevant matrices, and reproducible analytical performance, yet stop short of implementation in strip-based assays. Representative examples of such non-LFA evaluations are summarized in Table 7 to contextualize the translational gap between analytical feasibility and lateral flow deployment.

5.2 Strategies for diseases of unknown etiology

Across the case studies presented above, aptamer- and nanobody-based assays are characterized using similar analytical metrics and reach comparable endpoints during early evaluation. In multiple disease contexts, analytical performance is established under controlled conditions, while progression into standardized diagnostic validation is limited.

Differences between aptamers and nanobodies persist primarily at the level of regulatory precedent and production infrastructure. Aptamer-based assays are evaluated within pathways less commonly applied to nucleic acid reagents, while nanobody-based assays align more closely with protein-oriented manufacturing systems. In both cases, diagnostic assessment proceeds within validation frameworks developed for antibody-based assays.

5.2.1 Integrative discovery & validation framework. For conditions such as CKDu, MIS-C, and Kawasaki-like inflammatory syndromes, diagnostic development often proceeds in the absence of a resolved disease mechanism or definitive molecular etiology. In these settings, biomarker discovery and diagnostic validation are frequently treated as sequential processes, delaying assessment of assay robustness while biological understanding continues to evolve.

An integrative discovery and validation framework advances biomarker identification and assay evaluation concurrently. Within this framework, analytical reagents are introduced early and applied iteratively as candidate biomarkers emerge. Aptamers and nanobodies are particularly well suited to this approach because their selection, optimization, and functional modification can be performed on timescales compatible with exploratory clinical studies and limited sample availability.

We propose the following workflow sequence:

- Identification of molecular features that distinguish cases from controls using untargeted or targeted proteomic, metabolomic, or transcriptomic datasets.
- Prioritization of candidate molecular targets, such as proteins, peptides, metabolites, or pathogen derived components, based on abundance, stability, disease association, and feasibility of assay development.
- Parallel generation of aptamers and nanobodies directed toward prioritized targets.
- Early-stage assay testing using available patient samples to assess signal reproducibility, specificity, and robustness across clinical heterogeneity.
- Iterative refinement of both target selection and binder design as additional biological and analytical data become available, prior to scale up or formal clinical validation.

5.2.2 Illustrative examples. In CKDu, proteomic surveys report recurrent alterations in proteins associated with tubular injury and oxidative stress, including KIM1, IGFBP1, and GSTM1.[115–117] In MIS-C, cytokines and acute-phase reactants such as IL1Ra, IL6, and specific CRP isoforms differentiate MIS-C from other pediatric inflammatory conditions.[118–120] Kawasaki-like inflammatory syndromes exhibit reported alterations in serum amyloid A fragments and complement components reflecting systemic inflammation and vascular involvement.[121,122] In each case, diagnostic utility arises from reproducible molecular patterns evaluated across heterogeneous populations rather than single-molecule specificity.

5.2.3 Collaborative frameworks in biomarker discovery. Several collaborative initiatives provide infrastructure that supports closer integration of biomarker discovery and diagnostic evaluation.

DIAMONDS project (Europe and UK). This program applies multi-omic profiling and digital diagnostics to distinguish infectious and inflammatory diseases in children. Building on the PERFORM consortium, it provides clinically annotated cohorts for Kawasaki disease, MIS-C, and related febrile illnesses.[123–125]

Pediatric inflammatory disease programs (United States). Clinical and proteomic studies at major centers identifying candidate biomarkers for MIS-C and Kawasaki disease using mass spectrometry, SOMAscan, and immune signature analysis. Reported targets include serum amyloid A, complement factors, and cytokine clusters.[118–122]

CKDu Networks (Global South). Regional collaborations in Central America, South Asia, and East Africa are standardizing sample collection and proteomic workflows to support cross cohort comparison of proposed CKDu biomarkers, including KIM1, IGFBP1, and GSTM1.[115–117]

Global data repositories. Repositories including PRIDE, PeptideAtlas, ProteomeXchange, MassIVE, and CPTAC link discovery datasets with assay development by providing validated spectral libraries, quantitative data, and associated clinical metadata.[126,127] These resources enable analytical reagents to be evaluated against population level datasets rather than isolated samples.

5.3 Comparative synthesis

Across the case studies presented in this section, aptamer- and nanobody-based assays demonstrate analytical performance consistent with early diagnostic development benchmarks under controlled laboratory conditions. No systematic analytical limitations are attributable to recognition element class alone.

In contrast, progression beyond proof of concept varies substantially by disease setting. Assays targeting conditions with well-defined biomarkers, established comparators, and accessible clinical specimens are more likely to advance toward translational evaluation. Assays developed for neglected diseases or conditions of unresolved etiology frequently remain confined to analytical validation, reflecting constraints in specimen availability, comparator definition, and validation environment rather than deficiencies in assay chemistry.

Table 8 summarizes representative examples illustrating how disease context, target definition, and recognition element selection interact to shape diagnostic development pathways in settings where biological understanding remains incomplete.

6. Discussion and outlook

The studies surveyed in this review report a broad range of analytical evaluations for aptamer- and nanobody-based LFAs across multiple disease contexts. These evaluations commonly include sensitivity, specificity, binding affinity, and assay reproducibility measured under controlled experimental conditions. However, validation depth varies substantially among reports, particularly with respect to comparator selection, matrix complexity, and progression beyond proof-of-concept testing.

When considered collectively, this literature reveals recurring translational themes that extend beyond binder performance. Regulatory precedent, manufacturing practices, and access to standardized reference materials repeatedly influence whether analytically characterized assays progress toward clinical validation and deployable LFA formats. The sections that follow synthesize these themes in relation to technological integration, regulatory evolution, manufacturing at scale, and infrastructure gaps that shape discovery-to-deployment timelines.

6.1 Technological integration in LFAs

Several implementations described in the literature integrate aptamers and nanobodies into composite assay architectures that include digital readout, multiplexing, or automated fluid handling. In these configurations, alternative recognition elements function within systems where interpretation and workflow control are increasingly engineered rather than left to manual execution.[55,95,96]

Digital readout strategies are commonly reported in which test-line intensity is captured using external imaging and converted into numerical values through standardized processing. These approaches preserve conventional strip designs while shifting interpretation from visual inspection toward algorithmic quantification.[70,128,129]

Multiplexed formats combine multiple recognition elements on a single strip to enable simultaneous detection of pathogen-derived targets and host-associated markers. Hybrid configurations that mix aptamers, nanobodies, and antibodies expand informational output without altering the underlying flow architecture.[95,130–132]

Automated handling strategies, including paper-polymer microfluidic channels and cartridge-based formats, aim to standardize sample processing and reagent delivery prior to or during detection. In these systems, molecular recognition is coupled to predefined fluidic workflows rather than user-dependent operation.

Taken together, these developments indicate convergence in execution, readout, and data handling, while leaving the molecular recognition chemistry largely unchanged.

6.2 Regulatory pathway evolution

Regulatory modernization is a recurring theme across international guidance documents and policy analyses. Pathways administered by WHO, FDA, and IVDR authorities increasingly emphasize performance-based evaluation, yet harmonization across jurisdictions remains incomplete. Continued incorporation of alternative recognition elements, including aptamers and nanobodies, into ISO and CLSI-aligned evaluation practices is commonly proposed as a means of reducing ambiguity without introducing chemistry-specific carve-outs.[133]

The United States FDA Breakthrough Devices Program, introduced in 2015, provides an expedited development and

Table 8 Representative examples illustrating how disease context, target definition, and recognition element selection correspond to different diagnostic development pathways in conditions with unresolved or emerging etiology

Disease example	Candidate biomarker	Detection rationale	Aptamer strategy	Nanobody strategy	Parallel validation path
CKDu	KIM1, IGFBP1, GSTM1	Tubular injury, proteomic signature	SELEX in urine-mimetic conditions	Phage display for stable binders	SOMAscan + clinical cohort comparison
MIS-C	IL1Ra, IL6, CRP isoforms	Inflammatory cytokine profile	Multiplex aptamer LFA	Nanobody panel for cytokines	Transcriptomic/proteomic validation
Kawasaki-like syndrome	Serum amyloid A, complement fragments	Acute phase reactants	Aptamer selection for SAA	Nanobody for complement	Prospective patient sample testing

review pathway for devices intended to address life-threatening or irreversibly debilitating conditions, contingent on demonstrated clinical relevance and performance rather than reagent class.[134] The WHO Prequalification Program evaluates *in vitro* diagnostics using standardized analytical, clinical, and manufacturing criteria aligned with international regulatory practice.[135]

Analytical validation standards referenced within these pathways include CLSI EP05 for precision evaluation, CLSI EP07 for interference testing, and CLSI EP17 for determination of detection capability.[75,136,137] These standards define performance assessments that are intended to be recognition-element agnostic. Compliance with ISO 13485 quality management systems, required for WHO prequalification, further establishes process controls that extend across diagnostic technologies, including synthetic and recombinant reagents.[79]

In parallel, regional harmonization initiatives seek to reduce duplication and improve mutual recognition of quality systems. The African Medical Devices Forum (AMDF), established in 2012, supports harmonization of medical device and diagnostic regulation across African jurisdictions using WHO model frameworks. The Pan-American Network for Drug Regulatory Harmonization (PANDRH) promotes regulatory convergence across the Americas. Both initiatives align with broader goals articulated through the International Medical Device Regulators Forum (IMDRF).

6.3 Manufacturing at scale

Manufacturing considerations remain central to the transition from prototype assays to broad deployment. During the COVID-19 pandemic, distributed manufacturing models were implemented in which regional facilities operated under shared quality management and technology-transfer frameworks. Initiatives such as India's Bioeconomy Mission, South Africa's Biovac program, and Brazil's Fiocruz network maintained diagnostic production using WHO Technology Transfer protocols, providing examples of decentralized manufacturing under emergency conditions.[138,139]

Published cost and yield data indicate that aptamers and nanobodies can be produced using established, scalable processes. Solid-phase phosphoramidite synthesis can yield reproducible purity profiles and low per-assay reagent costs once sequence designs stabilize.[43] Nanobody expression in microbial systems such as *Escherichia coli* or Pichia pastoris has been reported at approximately 30 mg L^{-1}, with production costs on the order of USD 1 per milligram.[37] These figures are frequently cited to support manufacturing feasibility for alternative recognition elements.[45,140–142]

Quality assurance across geographically distributed sites relies on standardized documentation, in-process monitoring, and post-market surveillance. Digital batch records, in-process analytical controls, and stability testing are addressed within standards including ISO 13485 and ISO 23640. Application of these frameworks to synthetic and recombinant recognition elements introduces additional considerations, such as sequence verification, secondary-structure confirmation, and thermal and storage stability assessment, which are reported inconsistently across current implementations.[143,144]

6.4 Priority research and infrastructure gaps

Gaps remain in the coverage of alternative recognition elements across high-priority pathogen lists. Published diagnostic studies using aptamers or nanobodies are limited or absent for several WHO priority pathogens, including Nipah virus, Lassa virus, and Marburg virus. Development for these targets often requires BSL-3 or BSL-4 facilities, constraining the scale and frequency of SELEX and phage-display campaigns reported in the literature.[145,146]

Access to well-characterized clinical specimens is an additional bottleneck. Biobanking efforts for neglected and emerging diseases are distributed across regional networks, often with variable metadata standards and limited availability of cross-platform reference materials. Fragmentation of specimen repositories complicates comparative validation and restricts benchmarking across laboratories and diagnostic formats. Harmonized specimen collections coordinated through initiatives such as the Global Health Network or the WHO Specimen Bank Initiative are frequently cited as mechanisms to support reproducible validation across sites.[147–149]

Integrated discovery and validation approaches are increasingly described in large collaborative programs, but formalized performance benchmarks remain inconsistently defined. Initiatives including DIAMONDS (Europe/UK), FIND's Digital Diagnostics Accelerator, and the Global Health Network link omics discovery with early assay development, yet analytical readiness is variably reported, and metrics such as signal-to-noise ratios, reproducibility measures, and sample diversity are not uniformly embedded within shared pipelines. Greater consistency in how such metrics are defined and reported would improve comparability between discovery-driven studies and downstream diagnostic development.[123–125]

6.5 Implementation outlook (2026–2030)

The coming five-year period is frequently identified as a critical window for changes in how diagnostic technologies are evaluated, manufactured, and deployed. As analytical methods for LFAs mature, emphasis increasingly shifts toward the infrastructure that supports translation from laboratory characterization to routine clinical use. Within this context, several priorities recur.

First, infrastructure integration is widely discussed as a limiting factor. Validation and performance benchmarking remain concentrated within individual laboratories or institutions, while distributed production and evaluation networks are less consistently established. Expansion of regionalized systems capable of producing, testing, and distributing diagnostic reagents under harmonized digital quality management is often proposed as a means of improving comparability and resilience.[1,150]

Second, implementation equity remains closely tied to manufacturing geography and procurement structures. Local and regional production capacity in low- and middle-income regions is frequently identified as a determinant of diagnostic access and supply-chain robustness. Distributed synthesis and microbial expression systems are often positioned as technically feasible components of such strategies, contingent on regulatory recognition, technology transfer, and coordinated procurement mechanisms.[1]

Overall, these trends suggest that future diagnostic credibility may depend less on precedent associated with specific reagent classes and more on the capacity of regulatory, manufacturing, and data infrastructures to accommodate diverse molecular recognition modalities within consistent evaluation frameworks. Whether analytically characterized assays achieve broad clinical impact will likely reflect how effectively these systems evolve alongside ongoing innovation.

7. Conclusion

Across the studies examined in this review, LFAs incorporating aptamers and nanobodies consistently achieve analytical performance metrics compatible with diagnostic use when evaluated under controlled conditions. Sensitivity, specificity, stability, and reproducibility comparable to antibody-based formats are routinely reported, indicating that limitations in molecular recognition modality are not the primary constraint on assay function.

Despite this analytical maturity, progression from laboratory characterization to clinically deployed diagnostics remains inconsistent. Differences in validation depth, comparator selection, regulatory precedent, specimen access, and manufacturing context strongly influence whether analytically sound assays advance beyond proof of concept. These factors operate largely independently of binder affinity or assay format and reflect the structure of existing diagnostic development pathways rather than deficiencies in analytical performance.

The central challenge for next-generation LFAs is therefore not the discovery of new recognition elements, but their integration into systems designed to evaluate, compare, and deploy them. Where aptamers and nanobodies align with established validation and production frameworks, translation proceeds efficiently. Where such alignment is absent, analytically robust assays may remain confined to laboratory demonstrations despite meeting core performance requirements.

Taken together, the evidence reviewed here indicates that diagnostic development has entered a translational phase in which analytical capability, while necessary, is no longer sufficient. Future impact will depend on access to representative clinical specimens, clarity in regulatory evaluation for non-antibody reagents, manufacturing standards that accommodate synthetic and engineered binders, and data infrastructures that support comparability across platforms and operating environments.

Within this context, the relevance of the WHO REASSURED framework becomes explicit. LFAs are uniquely positioned to address diagnostic inequities because they directly target affordability, rapidity, equipment-free operation, and deliverability in decentralized settings. The extent to which aptamer- and nanobody-based LFAs contribute to equitable diagnostic access will therefore depend less on improvements in binding performance than on whether validation, regulatory, and manufacturing systems evolve to support performance-based evaluation across diverse recognition modalities.

LFAs remain a central technology for expanding diagnostic access beyond centralized laboratories, particularly in resource-limited regions where infrastructure constraints, delayed diagnosis, and specimen access barriers persist. Ensuring that emerging recognition elements can be evaluated and deployed within REASSURED-aligned frameworks is essential for translating analytical innovation into sustained global health.

Author contributions

Evan T. Monk: writing – original draft, writing – review & editing, visualization, investigation, formal analysis, data curation, methodology, conceptualization. Erin McConnell: writing – review & editing. David McMullin: writing – review & editing, supervision, conceptualization. Maria C. DeRosa: writing – review & editing, supervision.

Conflicts of interest

There are no conflicts to declare.

Data availability

No new data were generated or analyzed in this study. All data discussed are derived from previously published literature, which is cited within the article.

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