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Integrating Molecular Diagnostics and Vector Surveillance for Early Detection of Arboviral Threats in Temperate and Tropical Settings

Moshood Ayinde [1*], Habeeb Damilola Yusuf [2], Kazeem Abdulrazaq [3]
[1] Department of Biological Sciences, Western Illinois University, Macomb, Illinois, USA
[2] Department of Public Health Sciences, New Mexico State University, Las Cruces, New Mexico, USA
[3] University of Ilorin Teaching Hospital, Ilorin, Nigeria

* Corresponding Author: **Moshood Ayinde**

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Abstract
Arboviral early detection depends on two data streams that are usually generated, analysed and governed separately. Molecular diagnostics operate on human specimens, within clinical laboratories, under clinical accreditation regimes, and answer the question of whether a presenting patient is infected. Vector surveillance operates on arthropod collections, within entomology or vector control programmes, under no comparable accreditation regime, and answers the question of whether transmission is occurring in a defined area. Because the arboviral transmission cycle runs through the vector before it reaches the patient, the entomological stream is in principle the leading indicator and the clinical stream the lagging one — yet in practice the clinical stream is the better resourced, the better standardised and the one that triggers response. This review examines what integration of the two would require, and what the evidence says about the performance of each component. It covers the diagnostic architecture of arboviral laboratory confirmation and its constraints, including the viraemic window that limits molecular testing to roughly the first five days of illness and the flavivirus serological cross-reactivity that limits confirmation thereafter; multiplex real-time reverse-transcription PCR and the sensitivity penalty multiplexing imposes; isothermal amplification and CRISPR-based platforms as candidate field-deployable technologies; sequencing and genomic epidemiology as operational rather than research activities; vector trapping methods and the weak evidence base for entomological threshold indices; molecular xenomonitoring and the statistical treatment of pooled infection-rate estimation; and novel modalities including mosquito excreta capture, environmental DNA and wastewater detection. The contrast between temperate and tropical surveillance regimes is treated as structural rather than incidental: a region reporting millions of dengue cases with partial laboratory confirmation faces a different integration problem from one reporting a few hundred West Nile virus cases and relying on animal and vector sentinels for sensitivity. The review concludes that the principal obstacles to integration are not analytical but institutional — differences in accreditation, data standards, turnaround expectations and workforce credentialling between the clinical and entomological arms — and identifies the specific standards, quality systems and workforce investments that would close them.

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1. Introduction

The epidemiology of arboviral disease changed measurably in 2024. The Pan American Health Organization's October 2024 epidemiological alert reported 11,732,921 dengue cases in the Americas through epidemiological week 36, of which 6,253,754 (53%) were laboratory-confirmed, with 17,610 severe cases and 6,650 deaths — a 155% increase over the same period of 2023,

with the Southern Cone accounting for 10,429,118 cases and Brazil alone for 9,569,467 (Pan American Health Organization [PAHO], 2024). The World Health Organization launched a global strategic preparedness, readiness and response plan for dengue and other *Aedes*-borne arboviruses in October 2024, citing more than 12.3 million dengue cases globally by the end of August 2024 — roughly double the total for all of 2023 — approximately four billion people currently at risk rising to five billion by 2050, and endemicity in more than 130 countries (WHO, 2024).

These are figures of a scale that changes what laboratory confirmation means. When incidence is in the low thousands, every case can in principle be confirmed. When it is in the tens of millions, confirmation becomes a sampling exercise, and PAHO's guidance reflects this explicitly: virological assays demonstrating the presence of whole virus, its genetic material or its proteins should be prioritised for case confirmation, serum should be collected within the first five days of symptom onset, rapid immunochromatographic tests should not be relied upon because of low sensitivity, and sampling should target 100% of severe and fatal cases plus 10–30% of non-severe cases (PAHO, 2024). Laboratory confirmation under these conditions is no longer a clinical service; it is a surveillance instrument with a designed sampling frame.

The longer-run baselines against which these figures should be read remain the global dengue burden estimate of 390 million annual infections, of which some 96 million are clinically apparent (Bhatt *et al*., 2013), and WHO's Global Arbovirus Initiative, which frames dengue, chikungunya, Zika and yellow fever as a single integrated preparedness problem rather than four separate programmes (WHO, 2022). Neighbouring epidemics reinforce the point that the 2024 figures are not an isolated anomaly: Bangladesh's worst recorded dengue year in 2023 has been characterised in hospitalised patients (Anika *et al*., 2024), pooled seroprevalence and force-of-infection modelling has quantified the prevalence of chronic post-chikungunya disability across endemic and epidemic settings (Kang *et al*., 2024), and chikungunya mortality appears to have been systematically underestimated (Salje & Cortés Azuero, 2024). Each of these findings depends on laboratory confirmation of a specific kind, and each is therefore a statement about diagnostic capacity as much as about epidemiology.

The temperate picture is structurally different. For the 2023 season across the European Union and European Economic Area, 728 human West Nile virus infections were reported — 709 locally acquired and 19 travel-associated — alongside 153 equid outbreaks in seven countries and 251 bird outbreaks in eight countries (European Centre for Disease Prevention and Control [ECDC], 2024). The human case count is three orders of magnitude below the tropical dengue figure, and the surveillance sensitivity of the European system rests substantially on the animal and vector components. In the United States, ArboNET recorded 1,132 human West Nile virus cases in 2022, a 61% fall from 2,911 in 2021, including 827 neuroinvasive cases, 862 hospitalisations and 93 deaths, alongside 47 Powassan cases, 33 St. Louis encephalitis cases — the highest since 2003 — 22 La Crosse, 12 Jamestown Canyon and one eastern equine encephalitis case (Sutter *et al*., 2024).

Between these two regimes sits an emerging intermediate case. France recorded record numbers of both imported and autochthonous dengue cases in 2024 (Arulmukavarathan *et al*., 2024), which means a temperate system must now detect single imported index cases quickly enough to prevent onward transmission by established *Aedes albopictus* populations. That is a detection-speed problem, not a case-counting problem, and it requires the entomological and clinical streams to operate on the same timescale.

This review addresses what such integration requires. Sections 2 through 5 treat the molecular diagnostic component: principles and constraints, multiplex PCR, isothermal and CRISPR platforms, and sequencing. Sections 6 through 8 treat the entomological component: trapping and indices, xenomonitoring and pooled estimation, and novel non-invasive modalities. Sections 9 through 11 treat integration: data systems and early warning, the temperate–tropical contrast, and the quality, biosafety and workforce infrastructure without which neither stream is dependable. Section 12 sets out an integration framework.

2. Principles and Limits of Arboviral Laboratory Diagnosis

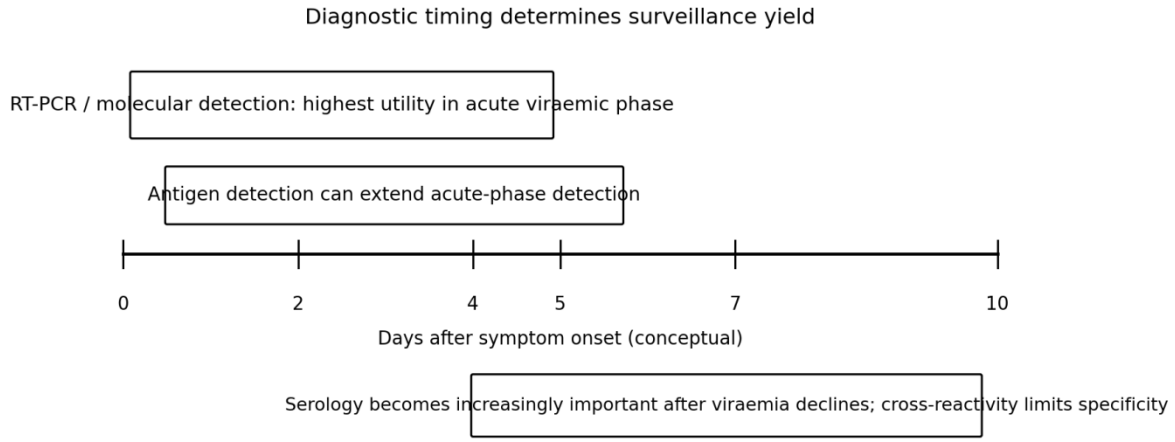


Fig 2: Conceptual diagnostic window for arboviral surveillance, showing the changing utility of molecular, antigen, and serological approaches over time.

2.1. The Viraemic Window

Arboviral molecular diagnosis is constrained by kinetics rather than by assay sensitivity. Tricou *et al.* (2011) characterised dengue plasma viraemia and NS1 antigenaemia in adults and showed that viraemia peaks at or shortly before fever onset and declines steeply from approximately day four to five, while NS1 antigenaemia persists measurably longer; both trajectories are modulated by immune status — primary versus secondary infection — and by infecting serotype. This single observation determines the architecture of arboviral diagnosis. Molecular and antigen testing are informative only in the acute phase; thereafter, confirmation must be serological, with all the interpretive difficulty that entails.

PAHO's five-day rule and its recommended sequence of reverse-transcription PCR, then NS1 antigen, then IgM (PAHO, 2024) is a direct operationalisation of these kinetics. The practical consequence for surveillance is that the diagnostic yield of a surveillance system is a function of health-seeking delay. A population that presents on day two of illness generates confirmable specimens; a population that presents on day six does not, however capable the laboratory. This makes time-to-presentation an entomological-scale variable that laboratory strengthening cannot address.

2.2. Serology and The Cross-Reactivity Ceiling

Flavivirus serology is limited by antigenic relatedness among dengue, Zika, West Nile, Japanese encephalitis and yellow fever viruses. The United States Centers for Disease Control and Prevention's interim algorithm for Zika required IgM MAC-ELISA reactivity to be confirmed by plaque reduction neutralisation testing (Rabe *et al*., 2016), and Lindsey *et al.* (2018) then demonstrated empirically why that algorithm has a ceiling: in United States territories with high prior dengue incidence, neutralisation testing could not distinguish recent Zika infection from prior dengue infection in a substantial proportion of IgM-positive patients. This is the clearest available statement of the limit — serological confirmation of a newly introduced flavivirus in a population with high background flavivirus seroprevalence is frequently impossible with current methods.

The implication for early detection is asymmetric by setting. In a naive temperate population, serology retains discriminatory power and extends the diagnostic window beyond the viraemic phase. In a hyperendemic tropical population, it does not, and the entire burden of specific diagnosis falls on the acute-phase molecular window. That asymmetry is one reason tropical arboviral surveillance depends more heavily on entomological confirmation than temperate surveillance does — a dependency that is rarely made explicit in surveillance guidance.

2.3. Antigen Detection and Rapid Tests

Dengue NS1 antigen detection extends the acute-phase window and can be performed on platforms requiring less infrastructure than PCR. Meta-analytic estimates of commercial NS1 ELISA accuracy are available (da Costa *et al*., 2014), as are pooled estimates for rapid immunochromatographic NS1/IgM/IgG tests (Macêdo *et al*., 2022). The pooled sensitivity of rapid tests is the empirical basis for PAHO's advice against relying on them for case confirmation (PAHO, 2024). The distinction matters for surveillance design: a rapid test with moderate sensitivity may be entirely adequate for clinical triage and simultaneously inadequate for the confirmed-case denominator that surveillance requires. Conflating the two uses is a recurrent source of surveillance data quality problems.

3. Multiplex Real-Time RT-PCR

Santiago *et al.* (2013) established the analytical and clinical performance of the CDC dengue virus 1–4 real-time RT-PCR, which remains the reference serotyping assay. Santiago *et al.* (2018) then evaluated the CDC Trioplex assay for simultaneous detection of Zika, dengue and chikungunya viruses in a single reaction, and documented the sensitivity penalty that multiplexing imposes relative to the corresponding singleplex assays. This trade-off is the central design decision in arboviral molecular diagnostics: multiplexing reduces specimen volume requirements, reagent cost and turnaround time, and simultaneously reduces analytical sensitivity for each target. In a differential diagnosis where three viruses produce indistinguishable acute febrile illness, the multiplex is operationally preferable; in a setting where one virus is of dominant concern and low-titre detection matters, it is not.

Independent designs confirm both the feasibility and the trade-off. Waggoner *et al.* (2016) developed and clinically applied a single-reaction Zika–chikungunya–dengue assay in Nicaragua, and Alm *et al.* (2014) described a universal single-probe RT-PCR for pan-dengue diagnosis, which trades serotype resolution for pan-serotype sensitivity. The choice between these designs is a surveillance policy decision rather than a laboratory one, because serotype data has epidemiological value — serotype replacement is a driver of epidemic periodicity — that pan-serotype assays discard.

Validation discipline is the underrecognised constraint. Statistical methods and quality engineering principles developed in industrial settings establish what is required for a measurement system to be trustworthy: defined analytical procedures, characterised measurement variability, and control limits derived from the process rather than asserted (Adaramola *et al*., 2023). Arboviral multiplex panels deployed during outbreak surges are frequently validated under emergency conditions and rarely revalidated when reagent lots, extraction platforms or specimen types change. The resulting drift is invisible without the quality architecture that industrial measurement systems take for granted.

4. Isothermal Amplification and CRISPR-Based Detection

4.1. RT-LAMP and RPA

Reverse-transcription loop-mediated isothermal amplification eliminates the thermal cycler, which is the principal infrastructure barrier to molecular testing outside reference laboratories. Lopez-Jimena *et al.* (2018) developed and validated four serotype-specific one-step real-time RT-LAMP assays for dengue virus 1–4, benchmarked against RT-qPCR. Arkell *et al.* (2023) provide the systematic evidence base in a scoping review of dengue RT-LAMP analytical and diagnostic performance framed explicitly around suitability for portable molecular devices, and their central finding is methodological rather than technical: published limits of detection and sensitivity estimates are highly heterogeneous, and reporting quality in this literature is poor enough that cross-study comparison is unreliable. For a technology whose selling point is field deployment, this is a serious gap — a programme cannot choose an assay on the basis of a literature that does not report comparable

performance metrics.

Recombinase polymerase amplification has a parallel evidence base. Abd El Wahed *et al*. (2015) reported a dengue RT-RPA assay for rapid diagnostics; Patel *et al*. (2016) a field-deployable chikungunya RT-RPA; Abd El Wahed *et al*. (2017) RPA detection of Zika virus in acute-phase urine; and Wand *et al*. (2018) a point-of-care Zika RPA assay. The attraction of RPA is run time measured in minutes rather than hours at near-ambient temperature; the constraint is reagent stability and cost.

Gavina *et al*. (2023) situate both platform classes in resource-limited settings and identify the determinants of whether an isothermal assay actually deploys: cold chain requirements, reagent lyophilisation, operator burden and the availability of cartridge-based random-access systems. Their analysis supports a conclusion that the assay-development literature tends to obscure — the binding constraint on field molecular diagnostics is almost never analytical sensitivity, and almost always the supply chain and the operator.

4.2. CRISPR-Based Diagnostics

Gootenberg *et al*. (2017) established Cas13a-based SHERLOCK, demonstrating attomolar nucleic acid detection with discrimination between Zika and dengue viruses. Gootenberg *et al*. (2018) extended this to a multiplexed platform combining Cas13, Cas12a and Csm6 signal amplification with lateral-flow readout, and Chen *et al*. (2018) characterised the Cas12a collateral single-stranded DNase activity underlying the DETECTR platform. The most directly relevant application coupled HUDSON — a heat and chemical treatment that eliminates nucleic acid extraction — to SHERLOCK, enabling detection of Zika and dengue viruses directly in body fluids in under two hours, with discrimination of the four dengue serotypes and of Zika strains differing at single nucleotides (Myhrvold *et al*., 2018). Single-nucleotide discrimination without sequencing is a genuinely new capability with direct surveillance relevance: it would allow lineage assignment at the point of collection rather than after specimen referral. The unresolved questions are regulatory and operational rather than technical — no CRISPR-based arboviral assay has been through the validation, accreditation and proficiency-testing pathway that would permit its use for case confirmation under clinical laboratory regulation. Until that pathway is traversed, these platforms remain surveillance tools rather than diagnostic ones, which is a legitimate use but a narrower one than their developers usually claim.

5. Sequencing and Genomic Epidemiology

Quick *et al*. (2017) published the tiling multiplex PCR protocol for MinION and Illumina sequencing of Zika and other viral genomes directly from clinical samples at low viral titre — the methodological ancestor of the ARTIC amplicon schemes now standard in outbreak genomics. Faria *et al*. (2016) described the mobile laboratory model in which sequencing capacity travels to the outbreak, and Faria *et al*. (2017) used it to demonstrate that Zika virus had circulated cryptically in north-east Brazil for roughly a year before detection. Grubaugh *et al*. (2017) resolved multiple independent Zika introductions into Florida, attributing local transmission chains that case surveillance alone could not distinguish. For West Nile virus, Hadfield *et al*. (2019) assembled twenty years of American spread and evolution into a continuously updated phylodynamic build — the clearest example of genomic epidemiology functioning as an operational surveillance product rather than a retrospective research output.

Patiño *et al*. (2024) provide the current synthesis of what routine genomic arbovirus surveillance requires in a public health laboratory: the choice between amplicon and metagenomic strategies, minimum viral load and coverage thresholds, bioinformatic capacity, and the institutional preconditions — sustained funding, data-sharing agreements, trained personnel — that determine whether sequencing capacity built during a surge survives it. Naveca *et al*. (2024) demonstrate the application that only sequencing delivers, identifying a novel reassortant Oropouche virus lineage driving persistent Amazonian outbreaks; and Benitez *et al*. (2024) demonstrate the minimum viable version during acute response, using 626-base-pair nucleocapsid fragments for lineage assignment in Cuba's first detected Oropouche outbreak, in which 74 of 89 acute serum samples (83.1%) were RT-PCR positive and all were negative for dengue, Zika, chikungunya, yellow fever, Mayaro and enteroviruses.

Two constraints deserve emphasis. First, bioinformatic interpretation is the rate-limiting step in most settings, not sequencing itself. Work on human-centred automated annotation using generative models is relevant here, establishing that keeping a trained human in the loop improves annotation quality relative to both fully manual and fully automated approaches (Ladapo *et al*., 2024) — a finding that applies directly to variant calling and lineage assignment workflows where automated pipelines produce confident but occasionally wrong outputs. Second, the African experience indicates that surge sequencing capacity does not automatically become routine capacity: Onywera *et al*. (2024) document the pathogen genomics and bioinformatics workforce built across Africa during and after the COVID-19 pandemic, and Mercy *et al*. (2024) document why that capacity has not yet translated into dengue detection across the continent. The gap between the two is a workforce deployment and priority-setting problem rather than an equipment problem.

6. Vector Surveillance: Methods and the Index Problem

6.1. Trapping methods and their comparability

Ramírez *et al*. (2018) frame the central difficulty accurately: arbovirus vector surveillance is a search for rare infection in large vector populations, and the statistical properties of that search determine what the resulting data can support. The method-comparison literature is substantial but fragmented. Farajollahi *et al*. (2009) field-compared the BG-Sentinel trap against industry-standard traps for *Aedes albopictus* and West Nile virus surveillance in New Jersey; Li *et al*. (2016) compared BG-Sentinel, CDC light trap and oviposition trap efficiency for vector mosquito surveillance in China. Both report species-specific differentials large enough that catch data from different trap types are not interchangeable — a fact that undermines most multi-site and multi-year comparisons in the published literature. For ticks, Salomon *et al*. (2020) provide the standardised drag and flag protocol that permits questing density to be reported per unit area, which is the minimum requirement for comparability and is not universally followed.

6.2. The Weakness of Entomological Thresholds

The most consequential negative finding in this literature concerns indices. Bowman *et al*. (2014) systematically

reviewed the relationship between vector indices — Breteau, house, container and pupal indices — and dengue transmission, and found the evidence for predictive thresholds weak and inconsistent. Codeço *et al*. (2015) compared the classical House Index against four alternative adult and oviposition traps for *Aedes aegypti* surveillance and quantified how poorly larval indices track adult abundance. Against this, Sanchez *et al*. (2010) derived locally calibrated Breteau Index threshold levels indicating risk in a low-infestation setting in Havana, demonstrating that thresholds can be established locally even where they do not generalise. The synthesis is uncomfortable but clear: the entomological indices embedded in most national dengue programmes were not validated as predictors of transmission and mostly do not function as such, but locally derived thresholds can work. Programmes therefore need local calibration studies rather than imported cut-offs, and an index reported without local validation should be treated as a measure of programme activity rather than of risk.

7. Xenomonitoring and Pooled Infection-Rate Estimation

Molecular xenomonitoring — testing vector pools for pathogen nucleic acid — is the entomological analogue of clinical molecular diagnosis, and it is where the leading-indicator promise of vector surveillance is realised or not. Cameron and Ramesh (2021) review it as a surveillance modality across pathogen groups. Bigeard *et al*. (2024) provide the flagship recent application, demonstrating real-time surveillance of West Nile and Usutu viruses in French mosquito populations through molecular xenomonitoring, with detection frequency and turnaround data bearing directly on whether xenomonitoring can lead human case detection.

The statistical treatment of pooled data is a persistent source of error. Gu *et al*. (2003) showed that the Minimum Infection Rate is a biased estimator whose bias grows with true infection prevalence and with pool size, and Gu *et al*. (2004) extended the analysis to variable-size pooling, establishing maximum likelihood estimation as the correct default and quantifying the conditions under which MIR and MLE diverge materially. The practical rule is straightforward — at low prevalence MIR is an acceptable approximation, and as prevalence rises it progressively underestimates infection — yet MIR remains widely reported without qualification. Because infection rates are the quantity on which entomological risk thresholds and intervention triggers depend, a biased estimator propagates directly into operational decisions.

On the human side, ArboNET operates as a passive system collecting demographics, onset date, clinical syndrome classification and outcome under standardised case definitions from state health departments (Sutter *et al*., 2024), and its European analogue for vector data is VectorNet, which has assembled approximately 475,000 records for more than 330 arthropod species since 2010 and publishes validated distribution maps for 42 species — five to ten times the record density of general biodiversity aggregators for the same taxa (Wint *et al*., 2023). The existence of both systems, and the absence of a formal linkage between them, is a reasonable summary of the state of integration.

Specimen and data traceability across these multi-tier systems is an unglamorous but decisive requirement. End-to-end visibility frameworks developed for complex multi-tier supply operations establish the principles — unique identification, custody logging at every transfer, and reconciliation between upstream and downstream records (Nnabueze *et al*., 2021) — that a specimen referral chain running from a field trap through a district laboratory to a national reference centre must satisfy if pool-level results are to be attributable to collection sites at all. Where that chain is undocumented, xenomonitoring generates prevalence estimates without a valid denominator.

8. Novel and Non-Invasive Surveillance Modalities

8.1. Mosquito Excreta and Sugar-Bait Capture

Hall-Mendelin *et al*. (2010) established the principle that mosquitoes expectorate virus while sugar-feeding, so that a honey-baited nucleic acid preservation card converts a mosquito trap into an arbovirus detector without any processing of mosquito bodies. van den Hurk *et al*. (2014) scaled this into an operational sugar-based surveillance system tracking arboviruses in wild Australian mosquito populations across multiple jurisdictions, and Flies *et al*. (2015) evaluated the conversion of routine mosquito surveillance into arbovirus surveillance using honey-baited cards. The appeal is substantial: no sorting, no pooling, no cold chain for mosquito bodies, and a single card representing the aggregate output of many mosquitoes.

The limiting case is equally important. Wipf *et al*. (2019) tested honey-baited cards against several trap types in an area of low arbovirus prevalence in Switzerland and demonstrated the sensitivity constraint that matters for temperate programmes: where prevalence is very low, the aggregation that makes the method efficient also dilutes the signal. Sugar-bait surveillance is therefore well suited to confirming and tracking established transmission and poorly suited to detecting first incursions — which is the opposite of what a temperate early-detection system most needs.

8.2. Environmental DNA

Schneider *et al*. (2016) demonstrated detection of invasive mosquito vectors from environmental DNA in water samples, establishing eDNA as a larval-habitat surveillance tool for *Aedes* incursions. The method addresses a real gap: the earliest stage of an invasion, when adult densities are below trap detection thresholds but breeding sites exist. Its constraints are eDNA persistence and degradation kinetics, which determine whether a positive result indicates current or recent-past presence, and the absence of standardised sampling protocols, which limits comparability between programmes.

8.3. Wastewater-Based Detection

Wolfe *et al*. (2024) detected dengue virus RNA in United States wastewater solids and related the signal to local case activity, and Monteiro *et al*. (2024) reported detection of both dengue and chikungunya virus RNA in Portuguese wastewater, including in a setting without recognised local transmission. Both are proof-of-concept studies. Neither establishes an operational decision threshold, and this is the honest limit of the current evidence: wastewater arboviral surveillance has demonstrated detectability but not yet demonstrated lead time or actionability. Given that arboviral shedding into wastewater is incidental rather than a primary excretion route — unlike enteric viruses — the signal-to-noise properties may prove less favourable than for the pathogens where wastewater surveillance has succeeded.

8.4. Vector Identification and Age-Grading

Two entomological measurement technologies deserve mention because they address variables that current surveillance mostly ignores. Joy *et al*. (2022) assessed near-infrared spectroscopy for evaluating *Aedes aegypti* population age structure, including the degradation of predictive accuracy beyond binary young/old classification. Age structure matters because the extrinsic incubation period means only mosquitoes that survive long enough after an infectious blood meal can transmit; a population's age distribution is therefore closer to a determinant of transmission than its abundance. Rakotonirina *et al*. (2022) built and validated a MALDI-TOF mass spectrometry reference-spectrum approach for dengue vector species identification across multiple countries, offering a cheaper and higher-throughput alternative to morphological identification or DNA barcoding. Species misidentification is a known and under-quantified source of error in vector surveillance data, particularly for cryptic species complexes, and a scalable identification method addresses it directly.

A parallel to instrument-based condition monitoring in engineered systems is instructive here. Predictive maintenance and condition monitoring frameworks developed for critical infrastructure rest on the principle that continuously measured state variables permit failure to be anticipated rather than merely recorded (Adaramola *et al*., 2018), and sensor-based fault detection demonstrates the same logic in distributed building systems (Sunday & Omoegun, 2022). Applied to vector surveillance, the analogue is that measuring the state variables that govern transmission — age structure, infection rate, host-feeding pattern — yields anticipation, whereas measuring abundance alone yields description. Machine-learning approaches to predictive maintenance in operational systems, including cloud-analytic implementations that keep model outputs continuously available to operators (Mayo *et al*., 2021), illustrate the architecture such a surveillance system would need. The relevant lesson from that literature is also cautionary: model-based anticipation is only as good as the instrumentation behind it, and data-driven methods applied to poorly characterised measurement systems produce confident and wrong predictions (Adaramola *et al*., 2020).

9. Integration: Data Systems and Early Warning

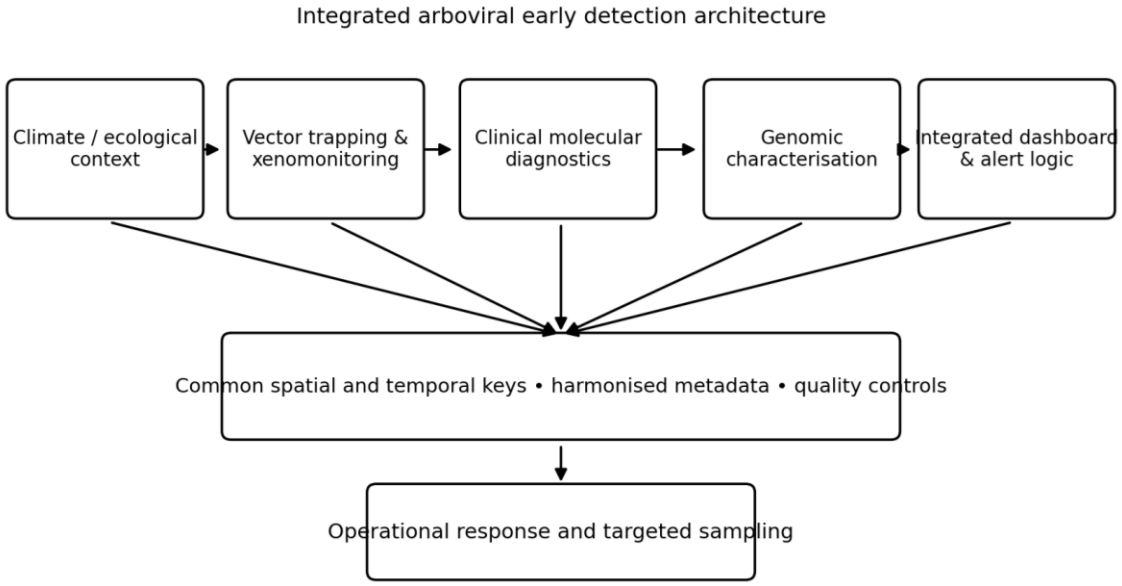


Fig 1: Integrated arboviral early-warning architecture linking ecological, entomological, clinical, genomic, and operational data streams.

9.1. Climate-Driven Forecasting

Lowe *et al*. (2016) built and prospectively evaluated a probabilistic dengue early-warning system for Brazil, reporting forecast skill against observed epidemic thresholds, and established the benchmark for threshold-based alerts driven by seasonal climate forecasts. Lowe *et al*. (2021) then quantified the combined effects of hydrometeorological hazards — drought and extreme rainfall, with distinct lag structures — and urbanisation on dengue risk in Brazil, supplying the mechanistic covariates that operational models require. The significance of the second paper for integration is that it shows the relevant covariates are not solely climatic: water supply interruption and urban form mediate the climate–dengue relationship, which means an early-warning system needs infrastructure data as well as meteorological data.

9.2. Combining Indicator-Based and Event-Based Streams

The clearest demonstration of surveillance-stream integration is Serres *et al*. (2024), who trained ecological niche models jointly on ECDC indicator-based human case data for 2006–2021 and automatically harvested event-based news signals, together with environmental covariates, and identified areas of previously unrecognised West Nile virus risk in Spain, Italy, France and Greece. The authors conclude that integrating both streams proved useful despite the epidemiological unverifiability of event-based data. That qualification is the important part: event-based signals cannot be validated case by case, but they carry information at the aggregate level that formal notification data does not, because they are generated by a different and faster process.

9.3. What an Integrated System Would Look Like

Drawing the components together, an integrated arboviral early-detection system requires five linked elements. First, a clinical molecular stream operating within the viraemic window, with defined sampling targets rather than opportunistic confirmation. Second, an entomological stream reporting locally calibrated infection rates estimated by maximum likelihood rather than minimum infection rate. Third, a genomic layer applied selectively to characterise lineage and reconstruct introduction events. Fourth, environmental and climate covariates at a resolution matched to the vector's response. Fifth, a data architecture in which these streams share spatial and temporal keys, so that an entomological signal and a clinical signal from the same locality can be compared without manual reconciliation.

The fifth element is where most systems fail, and it is a data engineering problem with well-characterised solutions elsewhere. Integrated visualisation frameworks for continuous monitoring demonstrate how heterogeneous streams can be rendered into a single operational view with defined refresh and alerting logic (Ogbole *et al*., 2021), and real-time machine-learning risk dashboards in health system operations show the same pattern with clinical and logistics data combined (Filani *et al*., 2022). Neither is technically difficult. Both require a single institutional owner with authority over all the contributing streams, which arboviral surveillance rarely has.

Data availability and assurance are the governance counterpart. Risk-based assurance frameworks that treat data availability as a first-class security property rather than a byproduct (Fadayomi *et al*., 2019) and privacy-centric security engineering approaches for distributed and cloud-hosted systems (Okoruwa *et al*., 2020) address the objections most commonly raised against cross-sectoral surveillance data sharing. Where those objections are not addressed architecturally, they are resolved by default in favour of non-sharing.

10. Temperate and Tropical Surveillance Architectures

The contrast established in Section 1 can now be stated structurally. The tropical regime, exemplified by PAHO (2024), is characterised by case counts too large for universal confirmation, laboratory confirmation operating as a designed sample, serological confirmation compromised by background seroprevalence, and entomological data used primarily for programme management rather than early warning. The temperate regime, exemplified by ECDC (2024), is characterised by case counts small enough for universal confirmation, surveillance sensitivity resting substantially on animal and vector sentinels, serology retaining discriminatory power in a mostly naive population, and the operative question being incursion detection rather than transmission quantification.

These are different problems requiring different system designs, and guidance that treats them as the same problem at different scales is unhelpful. The tropical problem is a sampling and representativeness problem. The temperate problem is a sensitivity and speed problem. The emerging intermediate case — record imported and autochthonous dengue in France in 2024 (Arulmukavarathan *et al*., 2024) — requires elements of both simultaneously, and it is the case for which existing guidance is least adequate.

Resource constraints shape platform choice differently in each regime. Gavina *et al*. (2023) review the determinants in resource-limited settings: cartridge-based random-access platforms, cold chain dependency, reagent supply and clinical impact. The supply-side vulnerability is real and under-managed. Work on healthcare supply chain configuration, including the application of advanced materials to delivery systems and the broader question of how health commodity chains are structured (Ike *et al*., 2024), and on supplier relationship management as the basis of supply chain resilience (Ike *et al*., 2021), establishes that resilience derives from supplier structure and redundancy rather than from stockholding. Reagent supply interruption is among the most common causes of arboviral testing gaps in low- and middle-income settings, and it is treated in the literature as an operational accident rather than as a designable property of the system.

The COVID-19 legacy is the most consequential recent change in the tropical picture. Onywera *et al*. (2024) document the pathogen genomics and bioinformatics workforce built across Africa through the Africa Centres for Disease Control and Prevention's Pathogen Genomics Initiative, and Mercy *et al*. (2024) document the dengue burden across Africa that remains under-recognised and under-reported. The juxtaposition identifies the opportunity precisely: substantial molecular and sequencing capacity now exists in settings where arboviral surveillance remains minimal, and the binding constraint is priority-setting and specimen flow rather than capability.

11. Quality Systems, Biosafety and Workforce

11.1. External Quality Assessment as A Capacity Measure

Declared laboratory capacity and demonstrated laboratory capacity are different quantities, and external quality assessment is what distinguishes them. Squires *et al*. (2020) reported the performance of Eastern Mediterranean Region laboratories in the WHO external quality assessment programme for arbovirus diagnostics, providing a direct measure of whether national reference laboratories can detect what they claim to detect. Mikaty *et al*. (2022) evaluated diagnostic capacity for emerging arboviral diseases across the MediLabSecure network from 2014 to 2018 and argue explicitly that EQA is the instrument that exposes capacity gaps which self-assessment conceals. This is a general point about surveillance evaluation: capacity assessed by questionnaire and capacity assessed by proficiency panel diverge, and the divergence is not random.

11.2. Accreditation Pathways

Normative requirements for medical laboratory quality and competence are set by ISO 15189 (International Organization for Standardization, 2022), and in the United States by the Clinical Laboratory Improvement Amendments regulations at 42 CFR Part 493, which govern personnel qualifications, proficiency testing and quality control for high-complexity testing (Centers for Medicare & Medicaid Services, 2024), with the College of American Pathologists Laboratory Accreditation Program as the principal deemed alternative (College of American Pathologists, 2024). Arboviral molecular testing is high-complexity testing, which means that the personnel credentialling and quality control obligations attaching to it are not discretionary.

In low- and middle-income settings the pathway to accreditation runs through stepwise improvement schemes. Datema *et al*. (2020) critically review the WHO Regional

Office for Africa Stepwise Laboratory Quality Improvement Process Towards Accreditation against ISO 15189 requirements, and Odhiambo *et al*. (2023) examine seven years of quality management system implementation across sub-Saharan African medical laboratories and quantify progression toward accreditation. The relevance to arboviral surveillance is direct: a laboratory that cannot sustain a quality management system cannot produce confirmation data that a surveillance system should rely on, and stepwise schemes are the realistic route to that capability.

11.3. Biosafety

Containment requirements are agent-specific and jurisdiction-dependent. Green and Ronca (2023) set out practical procedures for handling West Nile virus in biosafety level 3 facilities, and the CDC and National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories* provides the agent summary statements and risk assessment framework that assign arboviruses to containment levels (Centers for Disease Control and Prevention & National Institutes of Health, 2020). The common arrangement — serology and RT-PCR on inactivated material at BSL-2, virus propagation and animal work at BSL-3 — has an important surveillance consequence: molecular surveillance can be decentralised far more readily than virus isolation, which is one reason molecular xenomonitoring has expanded while virus isolation from vector pools has not.

11.4. Workforce

The workforce constraint is the least well documented and probably the most binding. Peer-reviewed vacancy and attrition data for the clinical laboratory workforce is thinner than the operational significance of the problem warrants; the available material is substantially grey literature, including professional association analyses of laboratory staffing shortages (Association for Diagnostics & Laboratory Medicine, 2023; American Society for Clinical Laboratory Science, 2024). This evidentiary weakness should be stated plainly rather than papered over: assertions about laboratory workforce shortage in the arboviral surveillance literature generally rest on advocacy documents rather than on surveys with published methods.

What can be said with more confidence concerns the training and development side. Professional development delivered through structured continuous learning has a documented effect on teaching and practice effectiveness in technical fields (Joseph & Nwankwo, 2024), and the digital divide in technical education is a measurable constraint on who can acquire computational and instrument-based competencies at all (Joseph & Uzondu, 2024). Both findings bear on arboviral surveillance workforce development, where the required competency set now spans molecular technique, bioinformatics, entomological taxonomy and data management — a combination that no single existing training pathway produces.

Clinical-side determinants of surveillance sensitivity deserve a final mention. The speed with which an acutely febrile patient moves from presentation to specimen collection determines whether a confirmable specimen is obtained at all, and that interval is governed by front-line clinical process rather than by laboratory capability. Evidence from emergency nursing interventions in sepsis management demonstrates that time-critical clinical pathways are systematically improvable through protocol and workflow redesign, with measurable reduction in time to definitive intervention (Ohunyon *et al*., 2024), and nurse-led quality improvement in clinical decision units shows the same mechanism operating on triage and escalation of undifferentiated presentations (Ayinde *et al*., 2023). Infection control leadership during healthcare crises is the related capability that determines whether specimen handling and biosafety practice hold up under surge conditions (Ohunyon *et al*., 2023). Arboviral early detection depends on all three, and none is a laboratory function.

12. An Integration Framework and Recommendations

Six recommendations follow from the evidence reviewed.

Report pooled vector infection rates by maximum likelihood estimation: The bias properties of the minimum infection rate are established (Gu *et al*., 2003, 2004), and continued reporting of MIR without qualification propagates a known error into intervention thresholds. This is a costless change.

Require local calibration before using entomological thresholds: Given that vector indices do not generalise as transmission predictors (Bowman *et al*., 2014; Codeço *et al*., 2015) but can be calibrated locally (Sanchez *et al*., 2010), programmes should either conduct calibration studies or report indices explicitly as activity measures rather than risk measures.

Standardise trap type and reporting units within surveillance networks: The magnitude of between-trap differentials (Farajollahi *et al*., 2009; Li *et al*., 2016) means that multi-site networks using heterogeneous trapping cannot support spatial comparison. Standardisation at network level is more valuable than optimisation at site level.

Extend external quality assessment from reference laboratories to the full testing tier: EQA reveals what self-assessment conceals (Mikaty *et al*., 2022; Squires *et al*., 2020). The same logic should be applied to entomological laboratories, which currently operate under no comparable proficiency regime — an asymmetry with no scientific justification.

Treat specimen traceability and reagent supply as designed system properties: End-to-end visibility architecture (Nnabueze *et al*., 2021) and supplier resilience structure (Ike *et al*., 2021) are the applicable frameworks, and both are absent from most arboviral surveillance planning.

Define the temperate and tropical problems separately in normative guidance: The two regimes differ in what limits them — sampling representativeness in the tropics, incursion sensitivity in temperate settings — and a single set of recommendations serves neither well.

13. Conclusion

Molecular diagnostics and vector surveillance are, in principle, complementary instruments measuring the same transmission process at different points. In practice they are separately owned, separately funded, separately standardised and separately interpreted, and the entomological stream — the one that in principle leads — is the less regulated, less quality-assured and less consequential of the two.

The technical components of an integrated system largely exist. Multiplex molecular panels, isothermal platforms and CRISPR-based assays span the full range from reference laboratory to field deployment. Amplicon sequencing protocols and phylodynamic pipelines make lineage-level

surveillance operationally feasible. Molecular xenomonitoring has demonstrated real-time detection of arboviral circulation in vector populations, and novel modalities from sugar-bait capture to environmental DNA extend the reach of entomological detection into regimes where conventional trapping fails. Early-warning models integrating indicator-based and event-based streams with environmental covariates have identified risk areas that neither stream identified alone.

What is missing is at the level of institutions and standards rather than methods. There is no proficiency testing regime for entomological laboratories comparable to that governing clinical ones. There is no shared spatial and temporal data key linking human case reporting to vector infection data in most jurisdictions. The estimator most widely used to report vector infection rates is known to be biased. The entomological indices embedded in national programmes were never validated as transmission predictors. And the workforce competency set that integration requires — molecular technique, bioinformatics, entomological taxonomy, data engineering — is not produced by any single existing training pathway. Addressing these is neither technically difficult nor expensive relative to the burden at stake, but it requires treating arboviral surveillance as one system with one set of quality obligations rather than as two adjacent programmes that occasionally exchange reports.

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