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Chikungunya Virus serological cross-reactivity in co-endemic settings: Mechanisms, Diagnostic implications, and epidemiological consequences

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Abstract

Chikungunya virus (CHIKV) causes an acute febrile illness with debilitating arthralgia, and serological assays remain central to its diagnosis and surveillance. However, antibody-based results are complicated by cross-reactivity in settings where multiple arboviruses co-circulate. This review distinguishes three related but distinct phenomena affecting CHIKV serology: biological cross-reactivity, arising from genuine antigenic overlap between related viruses; assay-associated non-target reactivity, in which a diagnostic platform generates a positive signal without an established biological relationship between the viruses; and diagnostic misclassification, the downstream epidemiological consequence of conflating the two. Applying this framework to five virus pairs, we find that CHIKV–O'nyong-nyong virus (ONNV), CHIKV–Mayaro virus (MAYV) and CHIKV–Ross River virus (RRV) relationships are supported by shared alphavirus antigenic architecture and, in several studies, by functional cross-neutralisation. By contrast, CHIKV reactivity reported in dengue virus (DENV)- and Zika virus (ZIKV)-associated sera lacks an established biological basis and is better classified as assay-associated non-target reactivity of unresolved mechanism. These distinctions matter: unadjusted seropositivity can inflate or obscure CHIKV transmission estimates, and no single diagnostic platform resolves the problem for every virus pair. We argue that CHIKV diagnosis in co-endemic settings requires a layered strategy: molecular detection during the acute phase, antigenically informed and multiplex serology thereafter, and neutralisation-based confirmation where attribution is critical, combined with cross-reactivity-aware statistical modelling for surveillance. Strengthening assay validation and confirmatory testing, particularly in African settings such as Nigeria where CHIKV, DENV and related alphaviruses circulate together, is essential for improving diagnostic accuracy and epidemiological inference.

Keywords: Chikungunya Virus; Serological Cross-Reactivity; Arboviruses; Diagnostic Accuracy; Alphaviruses; Epidemiological Surveillance

1. Introduction

Chikungunya virus (CHIKV) is an arthritogenic, positive-sense RNA virus of the genus Alphavirus, family Togaviridae. Infection is classically an acute febrile illness with fever, rash and prominent polyarthralgia, though atypical and severe manifestations occur and joint symptoms can persist for months or years (Bartholomeeusen et al., 2023). Following its re-emergence in the early 2000s, CHIKV underwent substantial geographic expansion and is now an important global arboviral pathogen, with growing concern about continued spread into populations with limited prior immunity (Bettis et al., 2022; Bartholomeeusen et al., 2023).

CHIKV rarely circulates in isolation. In many tropical and subtropical settings, several arboviruses share overlapping human, ecological and vector environments (Sanou et al., 2025; Yman et al., 2026), and the co-circulation of CHIKV with

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dengue virus (DENV), Zika virus (ZIKV) and other alphaviruses creates a diagnostic environment in which similar clinical syndromes and imperfect laboratory tests complicate attribution of infection to a specific virus (Andrew et al., 2022; Bartholomeeusen et al., 2023; Brancini et al., 2026). This is particularly consequential in Africa, where recent seroepidemiological investigations demonstrate exposure to multiple arboviruses while the true extent and geographic distribution of transmission remain incompletely characterised (Parker et al., 2025; Sanou et al., 2025; World Health Organization, 2025a).

Clinical diagnosis of CHIKV is difficult because its acute presentation overlaps substantially with other arboviral febrile illnesses: fever, rash, myalgia and headache occur across CHIKV, DENV and ZIKV infection, and distinction is hardest during outbreaks or when the characteristic arthralgia is absent (Bartholomeeusen et al., 2023; Brancini et al., 2026). Molecular tests offer direct evidence of infection but only within the narrow period of detectable viraemia, so serological testing remains essential once patients present later in illness or where molecular testing is unavailable (Andrew et al., 2022; World Health Organization, 2025b). This reliance on ELISA and related antibody platforms is amplified in resource-constrained settings by cost, infrastructure and specialised-personnel limitations (World Health Organization, 2025b; Yman et al., 2026).

The resulting diagnostic vulnerability is visible in recent African serosurveys. In Dire Dawa, Ethiopia, a population-based survey reported high apparent IgG seropositivity for DENV, CHIKV and ZIKV using a commercial multiplex test, underscoring the importance of assay characteristics when interpreting multi-arbovirus data (Parker et al., 2025). A large multi-regional survey in Burkina Faso similarly found extensive serological evidence of exposure to multiple arboviruses (Sanou et al., 2025). Such misclassification can distort virus-specific seroprevalence estimates, obscure the geography of transmission and influence outbreak interpretation (Hozé et al., 2021; Yman et al., 2026): CHIKV–MAYV investigations show that commercial ELISAs can produce substantial non-target reactivity when antigenically related alphaviruses co-circulate (Fischer et al., 2020), while modelling of CHIKV and O'nyong-nyong virus (ONNV) cross-reactivity shows that derived seroprevalence can differ substantially once cross-reactivity is taken into account (Hozé et al., 2021).

The central problem is therefore not simply whether a CHIKV test is positive or negative, but what a positive result actually represents in a given assay and epidemiological context, a question that becomes especially pressing when CHIKV is tested alongside antigenically related alphaviruses or in populations with extensive arbovirus exposure (Fischer et al., 2020; Hozé et al., 2021; Yman et al., 2026). Surveillance and diagnostic limitations remain a recognised obstacle to accurately assessing the global burden and distribution of CHIKV, with under-reporting and incomplete recognition of transmission remaining important challenges even where testing capacity exists (World Health Organization, 2025a).

Although the literature on CHIKV serological cross-reactivity has expanded considerably, the evidence remains fragmented across studies of individual virus pairs, commercial diagnostic assays, outbreak investigations and neutralisation-based research (Andrew et al., 2022; Fischer et al., 2020; Yman et al., 2026). As a result, cross-reactivity is often discussed as a general property of CHIKV serology without sufficient distinction between biologically established alphavirus cross-reactivity and assay-dependent non-target reactivity observed in other diagnostic contexts, and the epidemiological consequences of cross-reactivity are not always integrated with the immunological mechanisms and diagnostic platforms that generate the relevant serological signals (Andrew et al., 2022; Yman et al., 2026).

This review suggests that "cross-reactivity" is not a single, uniform immunological phenomenon and proposes a three-level framework, biological cross-reactivity, assay-associated non-target reactivity, and diagnostic misclassification, for interpreting CHIKV serology. We apply this framework to five clinically important virus pairs (CHIKV–ONNV, CHIKV–MAYV, CHIKV–DENV, CHIKV–RRV and CHIKV–ZIKV), placing particular emphasis on distinguishing demonstrated biological cross-reactivity among related arthritogenic alphaviruses from the more limited and mechanistically uncertain evidence concerning CHIKV reactivity in flavivirus-associated diagnostic settings. It also examines the diagnostic and epidemiological consequences of this heterogeneity and the strategies available for improving virus-specific attribution, with particular relevance to co-endemic African settings such as Nigeria.

2. A Framework for Interpreting Cross-Reactivity: Biological, Assay-Associated, and Diagnostic

A positive reaction in a serological assay may arise through different mechanisms and should not automatically be read as evidence of a shared antigenic relationship between two viruses (Yman et al., 2026); distinguishing these mechanisms is the analytic core of this review.

Biological cross-reactivity occurs when antibodies elicited by one virus recognise structurally or antigenically related epitopes on another (Powers et al., 2023). This is most plausible among alphaviruses, whose envelope glycoproteins E1 and E2 form the principal antigenic surface and can conserve epitopes across related species. Experimental work has identified broadly neutralising monoclonal antibodies that recognise conserved epitopes within the E2 B domain and neutralise multiple arthritogenic alphaviruses (Fox et al., 2015), and human immunological studies confirm that CHIKV infection generates heterotypic cross-neutralising antibodies and memory B-cell responses against related alphaviruses predominantly through this domain (Powers et al., 2023). In such cases, cross-reactivity reflects a genuine, biologically grounded relationship between viral antigens and the antibody response, and, in some instances, direct functional cross-neutralisation. The antigen used in a diagnostic assay also matters independently of virus biology: recombinant proteins, denatured antigens, virus-like particles and whole-virus preparations can expose different antigenic surfaces of the same protein and so interact differently with a given antibody population, meaning the same underlying immune response can appear more or less cross-reactive depending on how the assay presents its target antigen (Andrew et al., 2022; Yman et al., 2026).

Even so, antigen binding and functional neutralisation are not equivalent. A binding assay measures whether an antibody recognises an antigen incorporated into the assay; a neutralisation assay measures whether that antibody functionally blocks infection. Cross-reactive binding can occur even when an antibody has insufficient affinity, targets a non-neutralising epitope, or fails to interfere with a critical stage of the viral life cycle, so ELISA reactivity does not establish cross-protective immunity (Fischer et al., 2020; Yman et al., 2026). The CHIKV–MAYV literature illustrates this clearly: commercial serological assays show substantial CHIKV–MAYV cross-reactivity, but neutralisation-based methods identify a markedly different, generally lower, pattern of attribution (Fischer et al., 2020). Even within the alphavirus group, cross-reactivity is not necessarily symmetric: multiplex serological profiling of CHIKV, MAYV and ONNV found that CHIKV was more prone to induce cross-reactive responses than MAYV, meaning a single antibody signal cannot be read as evidence of equal exposure to both viruses (Yman et al., 2026).

Assay-associated non-target reactivity describes a different situation: an assay produces a positive signal in the presence of antibodies raised against another virus, without the available evidence demonstrating a biologically meaningful shared antigenic relationship. This is the most defensible description of the CHIKV–DENV problem. CHIKV and DENV belong to different genera and do not share the antigenic architecture underlying alphavirus cross-reactivity, yet a commercial anti-CHIKV IgM ELISA evaluated in dengue-positive Brazilian samples showed a specificity of only 25.3%, with cross-reactivity in 31.6% of acute dengue cases (Lima et al., 2021). This demonstrates that a CHIKV-reactive signal can occur in dengue-infected populations without establishing a conserved CHIKV–DENV epitope. Candidate explanations include non-specific IgM reactivity, acute-phase immune activation, and assay matrix or antigen-preparation effects (Lima et al., 2021). Polyclonal B-cell activation is biologically plausible, DENV infection is known to generate broad, cross-reactive memory B-cell responses that intensify after secondary infection (Andrade et al., 2020), but this has not been shown to specifically cause CHIKV-reactive ELISA signals, so it should be treated as a hypothesis rather than an established mechanism. These explanations are not mutually exclusive and may operate jointly; direct experimental work is needed to weigh their relative contributions. The timing of specimen collection likely matters too, since the balance between early, broadly reactive antibody populations and later affinity-matured responses may affect the probability of non-target reactivity (Andrew et al., 2022).

Diagnostic misclassification is the downstream, epidemiological consequence of treating either of the above as definitive evidence of CHIKV infection when it is not. It is at this level that the practical stakes become clear: misclassification can inflate or obscure CHIKV seroprevalence, distort the apparent geography of transmission, and complicate individual clinical and public-health attribution (Hozé et al., 2021; Yman et al., 2026).

The immunological basis of assay-associated non-target reactivity remains an important unresolved question more broadly. One proposed explanation is that acute viral infection can generate broad, poorly restricted antibody responses that increase the probability of non-specific binding in highly sensitive immunoassays, a possibility particularly relevant to IgM-based testing, since IgM antibodies can show lower antigenic specificity and greater poly-reactivity than affinity-matured antibody populations. Polyclonal B-cell activation has likewise been proposed as a possible context for broad non-specific antibody production, but the available CHIKV–DENV evidence does not directly demonstrate that this mechanism is responsible for the CHIKV-reactive ELISA results observed in dengue-positive patients; it should be regarded as biologically plausible rather than established. This distinction matters for evidence calibration: a mechanism can be plausible on general immunological grounds while remaining unproven for a specific virus pair or platform, and the interpretation of CHIKV–DENV reactivity should avoid presenting polyclonal activation, non-specific IgM binding and assay matrix effects as mutually exclusive rather than potentially joint explanations (Correa et al., 2015).

These three levels, antibody–antigen recognition, test-system behaviour, and epidemiological interpretation, are not interchangeable, and conflating them risks converting an assay artefact into an unsupported immunological claim. The framework also clarifies why the same word, "cross-reactivity," can describe phenomena resting on markedly different levels of mechanistic evidence: strong structural and functional support in the alphavirus literature, versus unexplained assay behaviour in the CHIKV–DENV literature. The remainder of this review applies this framework to five virus pairs, beginning with those for which biological cross-reactivity is best established.

3. Comparative Analysis by Virus Pair

3.1. CHIKV–ONNV: Biologically Grounded and Asymmetric Alphavirus Cross-reactivity

CHIKV and ONNV are closely related arthritogenic alphaviruses whose shared antigenic architecture, particularly within the E2 glycoprotein, provides a plausible mechanistic basis for heterotypic antibody recognition (Pezzi et al., 2019; Powers et al., 2023). In a model-based analysis of CHIKV and ONNV circulation in Mali, Hozé et al. (2021) estimated that CHIKV infection generated an ONNV-cross-reactive response in approximately 80% of cases, while ONNV infection generated a CHIKV-cross-reactive response in only about 22% of cases a striking asymmetry demonstrating that cross-reactivity between related viruses need not be reciprocal or quantitatively equivalent. Kenyan febrile-patient data corroborate the practical difficulty of distinguishing CHIKV and ONNV exposure using conventional serology in settings where their circulation overlaps, with serological evidence of exposure to both viruses detected within the same patient population (Masika et al., 2022).

The epidemiological consequence is that populations with substantial CHIKV transmission may appear to carry a greater ONNV serological burden than is biologically accurate; incorporating cross-reactivity into model-based analyses materially altered estimates of CHIKV and ONNV circulation in the Malian dataset (Hozé et al., 2021). This problem has historically been compounded by the absence of high-throughput assays able to reliably distinguish CHIKV, ONNV and MAYV in co-circulation settings, a gap identified in the GloPID-R biological diagnostics assessment as a priority for future assay development (Pezzi et al., 2019). A duplex RT-qPCR assay developed by Wesselmann et al. (2024), capable of detecting and differentiating ONNV and CHIKV, now offers a molecular route around this limitation where serological discrimination fails. Overall, CHIKV–ONNV is best classified as genuine biological cross-reactivity whose central scientific challenge is not whether the two viruses cross-react, but how the magnitude and asymmetry of the response should be accounted for in individual diagnosis and population-level seroepidemiology.

3.2. CHIKV–MAYV: Biological Cross-reactivity With a Critical Distinction Between Binding and Neutralisation.

CHIKV and MAYV both belong to the Semliki Forest virus antigenic complex and possess sufficient antigenic relatedness to generate overlapping antibody responses (Pezzi et al., 2019; Powers et al., 2023) a relationship of growing importance as MAYV emerges in regions where CHIKV already circulates. Using patient-derived sera, Fischer et al. (2020) observed approximately 50% false-positive results with commercial ELISAs based on structural antigens, concentrated in IgM testing, while parallel testing for both viruses markedly improved positive predictive value; plaque-reduction neutralisation testing identified cross-neutralisation at substantially lower frequencies than ELISA cross-reactivity, direct evidence that antigen binding and functional neutralisation are not equivalent endpoints. Human immunological studies again implicate the E2 B domain in genuine heterotypic cross-neutralisation between CHIKV and MAYV (Powers et al., 2023), yet a 2018 study using recombinant CHIKV E2 antigen reported no detectable cross-reactivity in tested MAYV and other alphavirus hyperimmune sera (Fumagalli et al., 2018) — illustrating that antigen selection, and not virus biology alone, shapes the observed cross-reactivity profile. Separately, experimental work using E2 B-domain nanoparticle immunogens showed that antibody responses could bind CHIKV and MAYV E2 B-domain antigens without producing equivalent cross-neutralising activity (Tong et al., 2024), reinforcing that even a region associated with cross-neutralisation can generate antibodies that bind without neutralising. Multiplex serological work further shows the relationship is directionally asymmetric, with CHIKV more prone than MAYV to induce cross-reactive responses, and that cross-reactivity-adjusted estimates better reflect local transmission dynamics (Yman et al., 2026). CHIKV–MAYV is therefore biologically genuine cross-reactivity, but treating serological binding, cross-neutralisation and cross-protection as interchangeable overstates the extent of functional heterotypic immunity. In practice, this means a laboratory reporting a positive CHIKV IgM in a region where MAYV also circulates should qualify that result rather than treat it as conclusive, since roughly half of such positives in the Fischer et al. (2020) cohort did not hold up under neutralisation testing.

3.3. CHIKV–DENV: Assay-Associated Reactivity With An Unresolved Mechanism

Unlike the alphavirus pairs above, CHIKV and DENV belong to different genera and families and lack an established shared antigenic basis. Nonetheless, Lima et al. (2021) documented that a routinely used commercial anti-CHIKV IgM

ELISA showed markedly reduced specificity (25.3%) in dengue-positive Brazilian samples, with cross-reactivity in 31.6% of acute dengue cases. This demonstrates an important empirical phenomenon, DENV infection can be associated with a CHIKV-reactive assay result, without establishing a conserved CHIKV–DENV epitope or direct CHIKV–DENV cross-neutralisation. Candidate mechanisms include non-specific IgM reactivity, assay matrix effects, and immune activation during acute infection; DENV infection generates robust cross-reactive memory B-cell responses, particularly after secondary infection (Andrade et al., 2020), but this has not been shown to directly cause the observed CHIKV-reactive ELISA signal. The distinction from flavivirus-flavivirus cross-reactivity is also important: DENV–ZIKV serological cross-reactivity is well recognised within the flavivirus diagnostic context, but this should not be extrapolated to CHIKV, an alphavirus, without direct evidence (Yman et al., 2026). CHIKV–DENV reactivity therefore belongs in the assay-associated, mechanistically unresolved category, and the term "cross-reactivity" should be applied cautiously here, accompanied by a description of the specific assay phenomenon observed. Because antibody composition shifts over the course of infection, from early, broadly reactive populations toward later affinity-matured responses, a single cross-sectional serological result may not adequately capture the dynamic nature of the antibody response, reinforcing the importance of interpreting assay performance alongside the timing of specimen collection and clinical context (Andrew et al., 2022).

3.4. CHIKV–RRV: Biologically Plausible But Less Well Quantified

Ross River virus (RRV) is an arthritogenic alphavirus for which broader antigenic overlap with CHIKV among arthritogenic alphaviruses is biologically plausible. Contemporary serological studies have shown that commercial alphavirus ELISAs can produce substantially higher seropositivity than neutralisation assays in populations exposed to multiple alphaviruses, suggesting cross-reactive antibody detection (Kizu et al., 2023). Direct experimental evidence supports biological CHIKV–RRV antibody overlap: Fox et al. (2020) demonstrated that a cross-reactive anti-CHIKV monoclonal antibody could limit RRV-associated musculoskeletal disease in mice despite only moderate in vitro neutralisation, and that human CHIKV-immune IgG weakly neutralised RRV and improved clinical outcomes in the experimental model — mechanistic, if not routine clinical, evidence that CHIKV-elicited antibodies can recognise biologically relevant RRV epitopes. Kizu et al. (2024) subsequently compared ELISA with neutralisation results and reported potential serological misclassification of RRV and Barmah Forest virus infections as CHIKV, indicating that positive alphavirus ELISA results may not reliably identify the infecting virus where multiple related alphaviruses circulate. CHIKV–RRV cross-reactivity is thus best classified as biologically plausible and experimentally supported, although its epidemiological magnitude in natural human infection remains less well quantified than for CHIKV–ONNV or CHIKV–MAYV. The practical implication is similar to that for MAYV: a positive alphavirus-panel ELISA in a region where RRV or Barmah Forest virus circulate should prompt confirmatory testing before CHIKV is reported as the infecting agent, particularly for travel-associated or returning-traveller cases where the true exposure history may be uncertain.

3.5. CHIKV–ZIKV: No Established Direct Biological Cross-Reactivity

The CHIKV–ZIKV relationship demands particular caution. CHIKV and ZIKV belong to different virus families and genera, and the well-established serological cross-reactivity associated with ZIKV involves chiefly other flaviviruses, particularly DENV, with extensive documented DENV–ZIKV cross-reactive memory B-cell responses and persistent difficulty harmonising ZIKV assays for this reason (Andrade et al., 2020; Sapkal et al., 2024). By contrast, direct biological CHIKV–ZIKV antibody cross-reactivity has not been demonstrated in the evidence reviewed here. Nigerian seroepidemiological data show antibody evidence of both CHIKV and ZIKV circulation and meaningful co-seropositivity (Mac et al., 2023), but co-seropositivity in a population does not itself establish an antigenic cross-reaction between the two viruses. The more defensible interpretation is that CHIKV and ZIKV create diagnostic complexity in multi-arbovirus testing environments through shared exposure risk in the same populations, rather than through a demonstrated antibody cross-reaction a distinction obscured when "arboviral cross-reactivity" is used as a catch-all term regardless of the viruses' underlying antigenic biology.

3.6. Synthesis

Across the five pairs, the strength of evidence for genuine biological cross-reactivity tracks phylogenetic relatedness. It is strongest for CHIKV–ONNV and CHIKV–MAYV, supported by shared alphavirus antigenicity and, in relevant studies, functional cross-neutralisation (Hozé et al., 2021; Fischer et al., 2020; Powers et al., 2023; Yman et al., 2026); plausible and experimentally supported but less quantified for CHIKV–RRV (Fox et al., 2020; Kizu et al., 2024); and unestablished for CHIKV–DENV and CHIKV–ZIKV, where apparent reactivity is better explained as assay-associated non-target signal or as a consequence of shared exposure risk rather than shared antigenicity (Lima et al., 2021). This distinction has direct consequences for how a positive CHIKV serological result should be interpreted, which the following section addresses.

4. Diagnostic and Epidemiological Implications

4.1. Cross-reactivity and its effects on diagnostic accuracy and epidemiological surveillance

Serological testing remains widely used in CHIKV diagnosis and surveillance, particularly when patients present after the viraemic phase or where molecular capacity is limited, so the diagnostic consequences of cross-reactivity extend well beyond an isolated false-positive result (Andrew et al., 2022). At the individual level, cross-reactivity and non-target reactivity reduce the specificity of a CHIKV antibody result, particularly where ONNV, MAYV or RRV may have circulated, and particularly when antigen-binding assays are interpreted without an appropriate confirmatory method (Andrew et al., 2022). Diagnostic accuracy also varies with the timing of specimen collection: early serological testing may have limited accuracy because the antibody response is still developing, so a positive or negative result must be interpreted against the clinical and temporal context of infection (Andrew et al., 2022). A false-positive CHIKV result does not automatically produce a specific adverse outcome, since management of uncomplicated CHIKV infection is generally supportive (Powers et al., 2023), but misclassification can still shift the differential diagnosis a clinician pursues and the public-health reporting pathway that is activated. The consequences are more acute in pregnancy: CHIKV infection around the time of delivery carries a documented risk of neonatal infection and symptomatic neonatal disease, particularly with intrapartum maternal infection (Contopoulos-Ioannidis et al., 2018), so an isolated IgM result should not be treated as definitive evidence of recent maternal CHIKV infection where co-circulating arboviruses are plausible; confirmatory and temporally aware testing matters most precisely in this population.

At the population level, misattributed antibodies distort seroprevalence in either direction: if antibodies generated by ONNV, MAYV or another related alphavirus are attributed exclusively to CHIKV, apparent CHIKV seroprevalence may be inflated; if CHIKV-specific antibodies are missed because of inadequate assay sensitivity, transmission may be underestimated. Hozé et al. (2021) demonstrated that incorporating cross-reactivity into epidemiological analyses can materially alter estimates of virus-specific exposure, and multiplex serological work reinforces the value of modelling cross-reactive responses explicitly rather than treating every positive antigen-binding signal as independent evidence of target-virus infection (Yman et al., 2026). These errors extend beyond prevalence to estimates of transmission intensity and geographic distribution, with consequences for outbreak preparedness and resource allocation, the problem is ultimately one of public-health interpretation, not just measurement (Hozé et al., 2021; Yman et al., 2026). This is particularly relevant in Africa, where the geographic distribution of multiple arthritogenic alphaviruses is incompletely characterised and surveillance systems risk aggregating a mixture of CHIKV, ONNV and other alphavirus exposures under a single diagnostic label (Simo et al., 2019). Where an area is classified as having active or historical CHIKV transmission on the basis of an unadjusted antibody signal that partly reflects exposure to a related virus, the resulting map of arboviral risk can misdirect outbreak preparedness and diagnostic investment even when every individual assay result was performed correctly.

The CHIKV–DENV relationship requires separate treatment. Because reactivity here reflects assay-associated non-target signal of unresolved mechanism rather than conserved antigenicity, DENV-associated CHIKV reactivity should not automatically be read as evidence that DENV infections are being systematically converted into CHIKV infections (Lima et al., 2021). In Nigeria specifically, where CHIKV, DENV and other arboviruses circulate against a backdrop of uneven clinical recognition, competing febrile illnesses such as malaria, and heterogeneous diagnostic approaches, cross-reactivity should be treated as a measurement-error source when comparing CHIKV seroprevalence estimates across studies (Simo et al., 2019). The overlap of febrile clinical presentations with malaria and other common infections may already reduce the number of patients investigated for arboviral disease in the first place; where serological testing is then performed using assays of imperfect specificity, the resulting data can combine clinical under-recognition with serological misattribution in ways that are difficult to disentangle retrospectively.

4.2. Diagnostic approaches for improving specificity in CHIKV diagnosis

No single platform resolves cross-reactivity across the CHIKV–ONNV, CHIKV–MAYV, CHIKV–DENV and CHIKV–RRV relationships; the appropriate method depends on whether the objective is acute-infection detection, recent-infection diagnosis, serological attribution, or population surveillance.

Molecular detection (RT-qPCR) remains the most direct route to virus-specific diagnosis during the acute, viraemic phase, since it detects viral RNA rather than an antibody response and so avoids antibody-mediated cross-reactivity altogether; its principal limitation is temporal, as diagnostic yield declines once viraemia resolves (Andrew et al., 2022). Multiplex molecular assays that detect CHIKV alongside DENV serotypes or ZIKV extend this advantage to differential diagnosis in acute febrile illness and outbreak settings (Belem et al., 2024), and a duplex RT-qPCR assay specifically differentiating CHIKV and ONNV illustrates how molecular approaches can resolve a problem that is difficult using

conventional serology alone (Wesselmann et al., 2024). Molecular assays are not immune to error; their performance depends on primer and probe design, validation, and the possibility of contamination or non-specific amplification, but their specific advantage in the context of this review is more precise than general infallibility: they avoid antibody-mediated cross-reactivity during the diagnostic phase, which is the particular problem this review addresses.

Where viraemia has resolved, serological testing remains essential, and antigen selection becomes central to specificity. The divergent CHIKV–MAYV results obtained with a recombinant E2-based assay showing no detectable cross-reactivity (Fumagalli et al., 2018) versus commercial structural-antigen ELISAs showing substantial cross-reactivity (Fischer et al., 2020) demonstrate that assay design, not virus biology alone, shapes observed cross-reactivity. The E2 glycoprotein remains of particular interest because it contains both conserved and virus-specific determinants, though the same E2 B domain implicated in genuine cross-neutralisation may also contribute to cross-reactive signal (Powers et al., 2023), so each candidate antigen still requires direct empirical validation.

Neutralisation assays (PRNT/FRNT) provide the strongest functional evidence of cross-reactivity, since they measure whether antibodies actually inhibit infection rather than merely bind antigen a distinction particularly important given that ELISA reactivity can exceed the proportion of samples demonstrating cross-neutralisation (Fischer et al., 2020). Reciprocal neutralisation testing against suspected virus pairs, such as CHIKV and ONNV, can provide information unavailable from a single CHIKV antigen-binding assay (Hozé et al., 2021), though cross-neutralisation can itself occur among related alphaviruses and titre thresholds vary between laboratories, so neutralisation should be viewed as a high-specificity confirmatory tool rather than a universal gold standard. Its principal operational limitation is scalability: these assays are technically demanding and difficult to implement routinely in resource-limited settings, so their greatest value is as reference methods for assay validation and difficult individual samples.

Multiplex serology addresses the problem analytically rather than eliminating it. Yman et al. (2026) profiled antibody responses to 28 antigens from nine arboviruses across more than 4,000 samples in multiple epidemiological settings, combining competitive immunoassays, mixture modelling and receiver operating characteristic analysis to define seropositivity thresholds and characterise cross-reactive responses directly, rather than treating them as unexplained error. This approach may be particularly valuable for surveillance where CHIKV, ONNV and MAYV exposure overlap, though interpretation remains complex and depends on the antigen panel, population-specific exposure history and analytical model used, so multiplex platforms should still be validated against well-characterised samples and, where possible, neutralisation-based reference methods. Pseudovirus-based neutralisation assays, which substitute a non-replicating surrogate system for live virus, offer a further option where biosafety constraints limit conventional PRNT (Chung et al., 2020); like other neutralisation methods, however, they require specialised validation and remain more suited to research and reference-level investigation than to routine diagnostic use.

Table 1 summarises these strategies against the diagnostic problem each best addresses. No single approach resolves all CHIKV cross-reactivity problems; the appropriate choice depends on the virus pair, specimen timing and diagnostic objective.

Table 1 Diagnostic strategies for improving CHIKV specificity and serological attribution in co-endemic arbovirus settings.

Diagnostic strategy	Primary problem addressed	Best use	Main advantage	Principal limitation
RT-qPCR/RT-PCR	Avoids antibody-mediated cross-reactivity during acute infection	Acute CHIKV infection	Direct RNA detection with high analytical specificity	Narrow detection window; sensitivity declines as viraemia resolves
Multiplex molecular testing	Clinical overlap among CHIKV, DENV and ZIKV	Acute febrile illness and outbreak investigation	Simultaneous multi-arbovirus detection in one workflow	Requires validated multiplex design and molecular capacity
PRNT/FRNT	Serological attribution among related alphaviruses	Confirmatory and reference testing	Functional neutralisation endpoint stronger than antigen binding alone	Labour-intensive and difficult to scale

Recombinant-antigen ELISA	Non-specific or broad cross-reactive binding	Serological diagnosis and surveillance	Defined antigen composition; scalable	May reduce but not eliminate cross-reactivity
Multiplex serology	Multi-virus exposure and complex cross-reactive profiles	Comparative seroepidemiology and surveillance	Simultaneous multi-antigen antibody profiling	Requires complex interpretation and population-specific validation
Competitive/inhibition assays	Distinguishing virus-specific from cross-reactive binding	Specialist confirmatory testing and assay development	Identifies the contribution of competing antigenic responses	Requires validated antigen pairs and standardised interpretation
Cross-reactivity-adjusted statistical modelling	Distortion of virus-specific seroprevalence estimates	Population surveillance and epidemiological inference	Explicitly incorporates cross-reactivity into prevalence estimation	Requires reliable cross-reactivity parameters and appropriate model assumptions

4.3. Towards an integrated diagnostic algorithm

No single platform is likely to replace serology universally; the most practical approach is a layered diagnostic algorithm keyed to specimen timing and local arboviral ecology. Acute-phase samples collected during expected viraemia are appropriate for RT-qPCR, while samples collected later in illness require serological testing interpreted against the patient's clinical history and local circulation of related viruses. Where a CHIKV serological result is obtained in a setting with substantial ONNV, MAYV or RRV circulation, a confirmatory strategy reciprocal neutralisation testing, a validated recombinant-antigen assay, or a multiplex serological platform should be considered depending on the purpose of testing and available laboratory capacity. For population surveillance, cross-reactivity-adjusted statistical models should be incorporated to prevent simple seropositivity counts from being read as direct estimates of virus-specific exposure (Hozé et al., 2021; Yman et al., 2026).

5. Conclusion

This review examined the diagnostic and epidemiological implications of serological cross-reactivity involving Chikungunya virus (CHIKV). CHIKV–ONNV and CHIKV–MAYV, and to a lesser and less-quantified extent CHIKV–RRV, reflect genuine biological cross-reactivity rooted in shared alphavirus antigenicity and, in places, direct evidence of cross-neutralisation (Powers et al., 2023). CHIKV–DENV and CHIKV–ZIKV instead involve assay-associated non-target reactivity or diagnostic confounding whose immunological basis remains unresolved (Lima et al., 2021). Collapsing this distinction risks converting assay artefacts into unsupported immunological claims and obscures where diagnostic development is genuinely needed. Antigen-binding reactivity and functional neutralisation are not interchangeable measures of antibody specificity, and treating them as such overstates the extent of functional heterotypic immunity between related alphaviruses. Diagnostic specificity, in short, is not solely a property of the assay in isolation but the product of assay design, antigen selection, specimen timing, local arboviral ecology, and the analytical framework used to interpret the result.

The practical consequences are substantial for both individual diagnosis and population surveillance, particularly in co-endemic settings such as Nigeria and other African countries, where limited confirmatory testing capacity magnifies the cost of misclassification and where surveillance systems risk aggregating CHIKV, ONNV and other alphavirus exposures under a single diagnostic label. These consequences are not evenly distributed across the clinical population: they matter comparatively little for an uncomplicated febrile adult, whose management is supportive regardless of the precise infecting alphavirus, but matter considerably more for pregnant women near delivery, for whom accurate attribution can influence anticipated neonatal risk and clinical monitoring. Improving CHIKV diagnosis will not come from a single new assay but from an integrated strategy: molecular testing during the acute phase, antigenically informed and multiplex serology thereafter, neutralisation-based confirmation where attribution is critical, and cross-reactivity-aware modelling wherever seroprevalence is used to infer transmission.

Recommendations

These recommendations are addressed jointly to diagnostic laboratories, assay developers, surveillance programmes and researchers, since no single actor can resolve the cross-reactivity problem alone. Based on the evidence reviewed, we recommend that:

- Diagnostic laboratories should avoid interpreting a single positive CHIKV antibody result as definitive evidence of infection in co-endemic settings, interpreting it instead alongside clinical presentation, specimen timing and local arbovirus circulation.
- Molecular testing should be prioritised for suspected acute CHIKV infection where specimens are collected during the viraemic phase, with RT-qPCR and validated multiplex molecular assays incorporated into arbovirus diagnostic algorithms where laboratory capacity allows.
- CHIKV serological assays should undergo systematic evaluation against panels of sera from patients with confirmed ONNV, MAYV, RRV, DENV and ZIKV infection, to distinguish biological cross-reactivity from assay-associated non-target reactivity.
- Assay developers should prioritise antigenically informed design, evaluating recombinant antigens and defined viral domains for their ability to improve CHIKV-specific recognition while reducing detection of heterotypic antibodies.
- Neutralisation-based assays should be used as confirmatory or reference methods where virus-specific serological attribution is critical, with reciprocal testing against relevant alphaviruses considered when CHIKV, ONNV, MAYV or RRV exposure is epidemiologically plausible.
- Multiplex serological platforms should be further investigated for arbovirus surveillance in co-endemic regions, accompanied by population-specific validation and appropriate statistical modelling of cross-reactive responses.
- Cross-reactivity should be explicitly incorporated into the interpretation of CHIKV seroprevalence studies, with clear reporting of diagnostic platform, antigen used, confirmatory strategy and known cross-reactivity limitations.
- Nigeria and other African countries should strengthen integrated arbovirus surveillance systems that move beyond isolated virus-specific testing toward frameworks capable of distinguishing multiple clinically and serologically overlapping arboviruses.
- Further research should investigate the mechanistic basis of apparent CHIKV–DENV and CHIKV–ZIKV assay-associated reactivity, combining well-characterised clinical sera, antigen mapping, inhibition assays and neutralisation testing to determine whether observed non-target reactivity reflects polyclonal B-cell activation, non-specific immunoglobulin binding, assay matrix effects, or other mechanisms.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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