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Narrative review

Challenges towards serologic diagnostics of emerging arboviruses

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ABSTRACT

Background: Appropriate laboratory diagnostics for emerging arboviruses are key for patient management, surveillance and intervention, including molecular tests and serological tests detecting viral antigen or virus-specific antibodies.
Objectives: We provide an overview of the challenges towards serological testing for the most important emerging arboviruses, including Zika, dengue and chikungunya viruses.
Sources: We retrieved a data set on performance of commercially available antibody- and antigen-detecting tests from 89 peer-reviewed articles conducting a systematic literature research in PubMed.
Content: We identified commonly used antibody- and antigen-detecting tests and analysed their overall performance. We discuss how timing of serological testing and the use of paired samples from acute and convalescent phases of infection are crucial to optimize diagnostic sensitivity and specificity. We then exemplify how serological diagnostics are challenged by the patient's infection history through the 'original antigenic sin' and cross-reactive antibodies in the context of global co-circulation of antigenically related viruses. We highlight how individual infection histories with different arboviruses and with other pathogens such as herpes viruses and Plasmodia can produce inaccurate test results. We show that rapid tests for antibody and antigen detection in point-of-care settings have a significantly lower sensitivity compared with laboratory-based tests such as ELISA. We show that the performance of antibody- and antigen-detecting tests varies greatly between tropical regions of endemic transmission and non-endemic regions. Finally, we highlight that test sensitivity and specificity have to be equilibrated carefully and frequently either of them must be prioritized over the other, depending on disease prevalence and intended use of tests.
Implications: For reliable serological diagnostics, it is essential to be aware of inherent test limitations. Although multiplexed testing and testing of convalescence samples can improve diagnostic performance, global spread of (re-)emerging viruses requires careful implementation and evaluation of serological testing and unambiguous results may not always be achievable. **Carlo Fischer, Clin Microbiol Infect 2021;27:1221**

Introduction

Unprecedented global connectivity, destruction of pristine habitats, population growth and urbanization, global warming, intensification of livestock and anthropogenic landscape changes fuel the spread of emerging infectious diseases (EIDs) globally [1,2]. Among pathogens causing EIDs, arthropod-borne viruses (arboviruses) are particularly important [3], including the recent spread of Zika virus (ZIKV) and chikungunya virus (CHIKV) in the Americas [4], and the re-emergence and spread of dengue virus (DENV) in tropical and subtropical regions globally [4,5]. The emergence of new EIDs in areas harbouring endemic infectious diseases eliciting similar symptoms hampers clinical diagnosis. Diagnostic tests have therefore become increasingly important for

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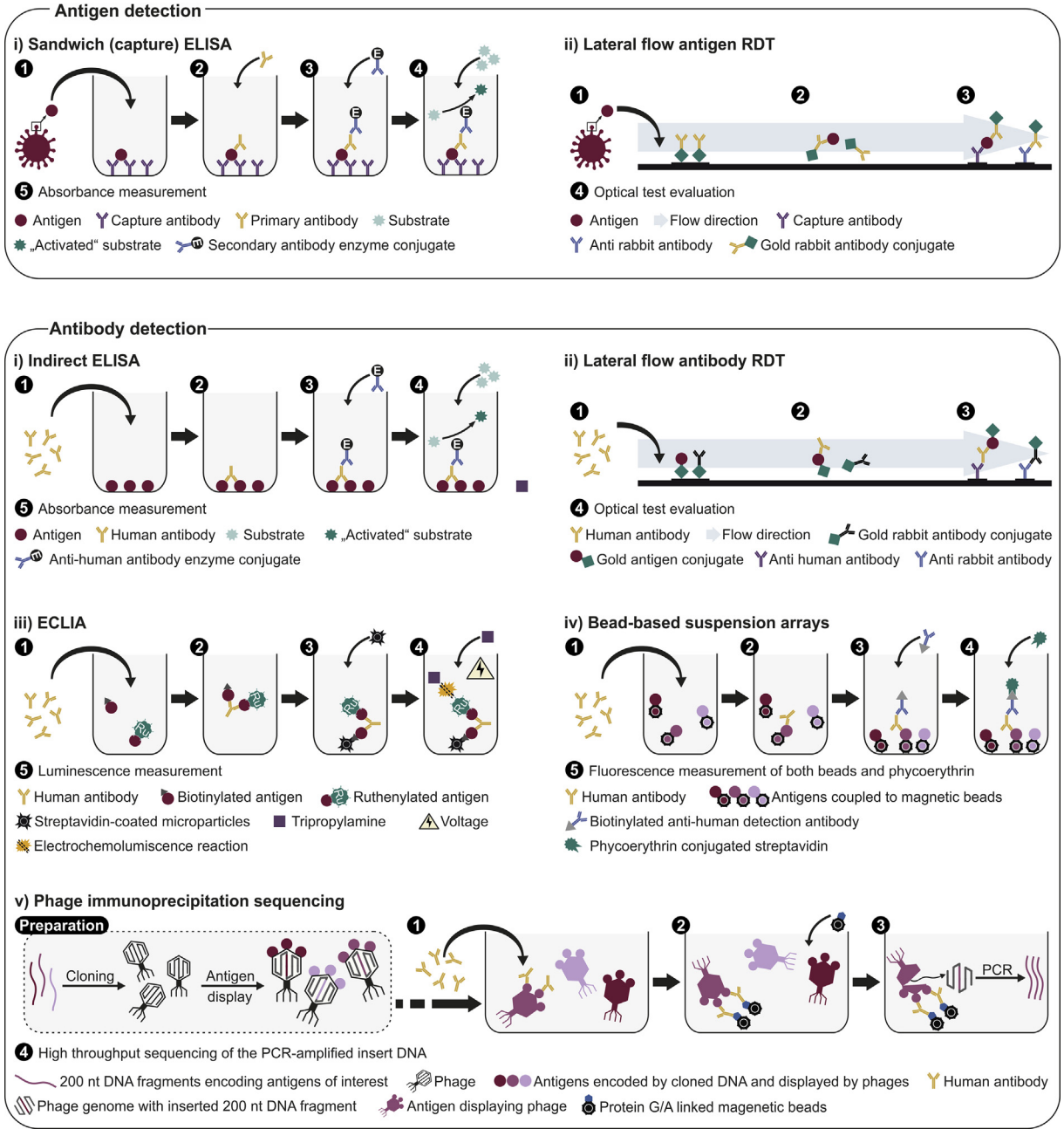

Fig. 1. Principles of common serological tests. (a) Antigen detection tests. (i) In the sandwich (capture) ELISA viral antigens are specifically captured by antibodies immobilized to the microplate surface. Next, a primary antibody binds to the captured antigen. A secondary enzyme-conjugated antibody binds to the primary antibody. The added substrate reacts to a measurable product in the presence of the enzyme. (ii) In the lateral flow antigen rapid diagnostic test (RDT) a sample is added to a membrane. Flowing along the membrane, viral antigens bind to specific gold-conjugated antibodies. Antibody-bound antigens are captured by immobilized antibodies forming a distinct line on the membrane. Gold-conjugated antibodies not bound to antigens are captured at a second line on the membrane where antibodies targeting the gold-conjugated antibodies are immobilized. (b) Antibody detection tests. (i) In the indirect ELISA, human antibodies are specifically captured by antigens immobilized to the microplate surface. Next, an anti-human primary antibody binds to the captured antibodies. A secondary enzyme-conjugated anti-human antibody binds to the primary antibody. The added substrate reacts to a measurable product in the presence of the enzyme. (ii) In the lateral flow antibody rapid diagnostic test (RDT) a sample is added to a membrane. Flowing along the membrane, human antibodies bind to specific gold-conjugated antigens. Antigen-bound antibodies are captured by immobilized antibodies forming a distinct line on the membrane. Gold-conjugated rabbit antibodies are captured at a second line on the membrane where antibodies targeting the rabbit antibodies are immobilized as control. (iii) In electrochemiluminescence immunoassays (ECLIA), human antibodies bind to both biotinylated and ruthenylated antigens. The antibody complexes are captured with streptavidin-coated microparticles, which bind to the biotin. Added tripropylamine reacts with the ruthenium while voltage is applied, producing measurable electrochemiluminescence. The figure represents an ECLIA, other test formats such as CLIA or other chemistry are not shown for clarity of presentation. (iv) In bead-based suspension arrays, human antibodies bind to specific antigens coupled to magnetic beads. Biotinylated detection antibodies bind to the human antibodies. Next, added phycoerythrin-conjugated streptavidin binds to the biotin. Measurement of fluorescence allows the quantitative detection of the bound phycoerythrin. As the magnetic beads can be dyed with different fluorescence dyes, different antibodies can be tested in parallel. (v) In phage immunoprecipitation sequencing, a library of phages expressing and presenting epitopes encoded by short DNA fragments is prepared. Antibodies of a clinical sample bind to specific antigens presented by phages. The bound antibodies are captured by protein G/A linked to magnetic beads, allowing the isolation of the phages expressing the antigens that were targeted by the antibodies in the clinical sample. The genome of the phages is isolated and the inserted DNA fragment encoding the antigen is amplified by PCR. The amplified insert DNA is analysed by high-throughput sequencing.

clinical medicine to allow adjusted treatment, to provide medical prognoses and, in the case of ZIKV, to guide decisions about abortion and family planning [6]. Adequate tests are also crucial for disease surveillance [7], to support DENV vaccination, which is currently recommended only in individuals with pre-existing dengue immunity [8], to clarify potential associations between previous infections and prolonged disease, such as CHIKV-associated chronic arthralgia or ZIKV-associated congenital Zika syndrome [9] and for disease control, e.g. to interrupt transmission chains [10]. Antibody-detecting tests may also offer the chance to predict disease severity and to identify patients at risk for severe disease in secondary infections. For example, low levels of pre-existing DENV antibodies have been identified as a major risk factor for severe dengue [11]. Similarly, ZIKV exposure may increase the risk of severe dengue infections [9].

Direct pathogen detection is commonly achieved by molecular testing or by detection of viral antigens. Beyond antigen-detecting tests, serological testing chiefly includes indirect testing by detection of immunoglobulins produced by the patient's adaptive immune system in response to an infection [12] in serum, plasma, saliva, urine or cerebrospinal fluid [13,14]. Detection of virus-specific antibodies is crucial in arbovirus infections because viraemia is usually short-lived [15], making serological testing the only suitable solution in returning travellers or in remote areas, where clinical samples are collected several days or even weeks after symptom onset [16,17].

This narrative review focuses on serological diagnostics of the most important emerging arboviruses, namely CHIKV, DENV and ZIKV.

Intrinsic properties affecting the performance of different test formats

Available test formats for antigen and antibody detection include enzyme-linked immunosorbent assays (ELISA), (electro-) chemiluminescence immunoassays ((E)CLIA), chemiluminescent

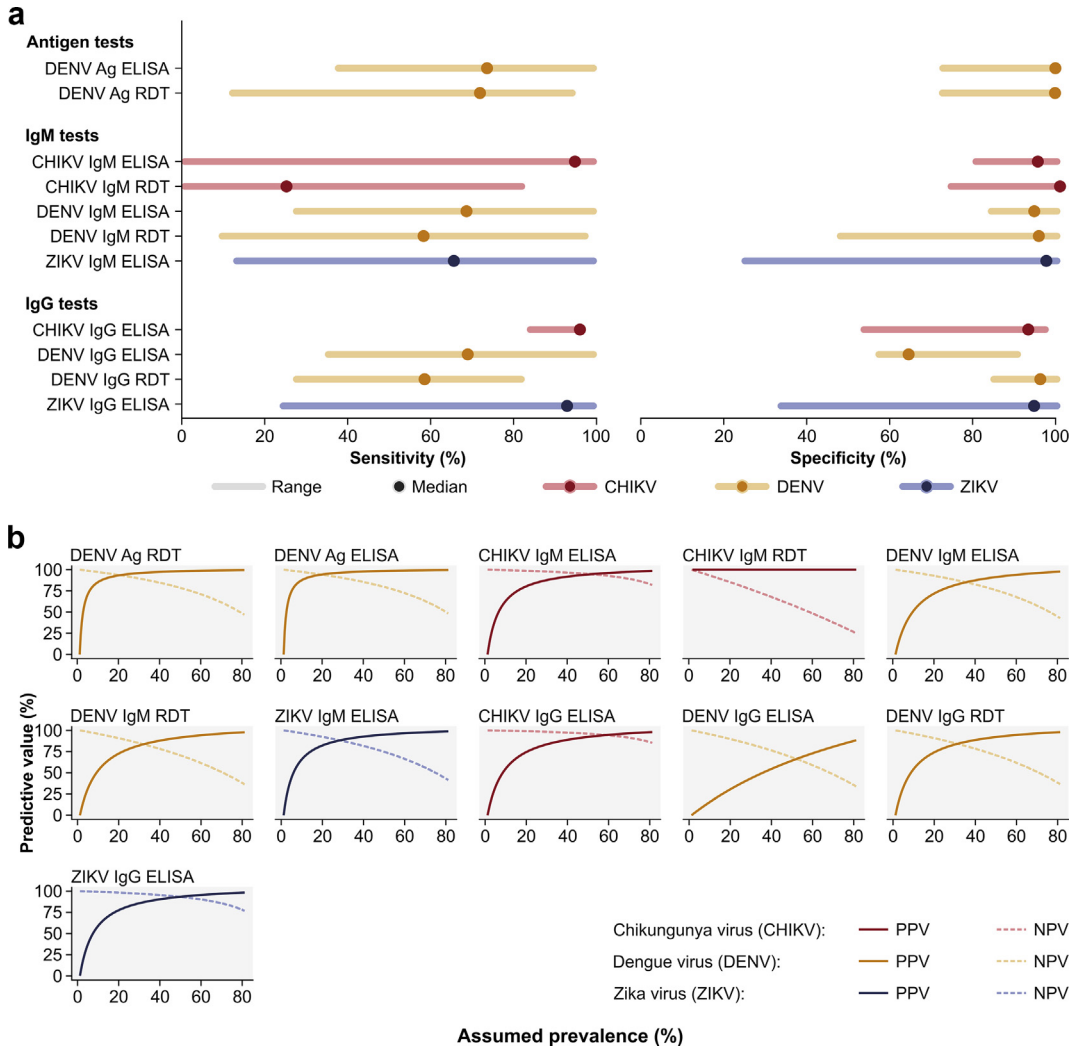

Fig. 2. Performance of antigen- and antibody-detecting tests for arboviruses. (a) Median sensitivity and specificity on antigen, IgM and IgG tests. (b) Positive predictive value (PPV) and negative predictive value (NPV) of antigen, IgM and IgG tests based on an assumed prevalence range. In total we analysed 247 entries from 89 publications on the performance of serological test identified by PubMed and Google Scholar research by 21 April 2021. For PubMed searches, combinations of virus names or abbreviations, ELISA, RDT, POC, rapid test, serologic test, antigen test and antibody test were used. Studies were selected for those evaluating commercial assays and for providing conclusive data on sensitivity and specificity. For statistical analyses, publications were included only if infection was confirmed by PCR, seroconversion, plaque reduction neutralization testing (PRNT), haemagglutination inhibition (HI) testing, or CDC IgM antibody capture ELISA (MAC-ELISA). If studies included several cohorts that were confirmed by different tests, only those cohorts matching the inclusion criteria were considered. If the test performances were provided for acute and convalescence samples, the performance with acute phase samples was considered for antigen tests and the performance with convalescence samples for antibody tests. The PPV and the NPV were calculated as follows: $PPV = \frac{Sensitivity*Prevalence}{Sensitivity*Prevalence+(1-Specificity)*(1-Prevalence)}$, $NPV = \frac{Specificity*(1-Prevalence)}{(1-Sensitivity)*Prevalence+Specificity*(1-Prevalence)}$.

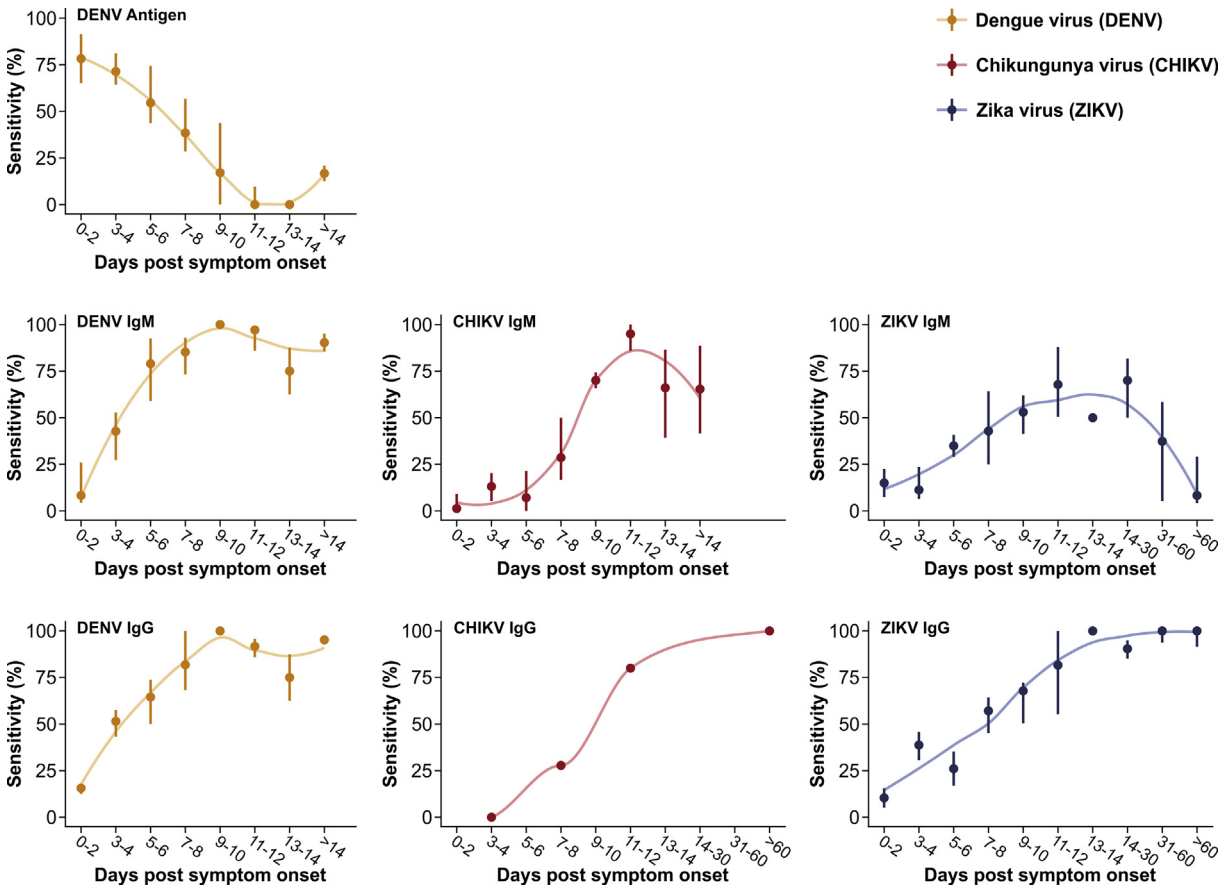

Fig. 3. Sensitivity of antibody- and antigen-detecting tests over time. Median sensitivity and quantiles (25%–75%) are shown by dots and bars. The analysis was performed based on the identified studies from Fig. 1. If the collection time-point of samples was provided as interval only, it was set to the mean days post onset of symptoms (dpo).

microparticle immunoassays, indirect immunofluorescence tests, Western blots and rapid diagnostic tests (RDTs), which commonly apply lateral flow immunochromatographic techniques (Fig. 1). However, some of those test formats are not available as commercial tests or have not been evaluated sufficiently in scientific studies. The test formats that have been evaluated most extensively for the selected viruses are ELISAs and RDTs. We identified 89 peer-reviewed studies published between 2006-2021 and mostly conducted in endemic regions evaluating IgM and IgG ELISAs for all three arboviruses, IgM RDTs for DENV and CHIKV, as well as IgG RDTs, antigen-detecting ELISAs and antigen-detecting RDTs for DENV (Table S1). Among those studies, serological and antigen-detecting tests show a broad range of sensitivity and specificity. In general, the median sensitivity is higher among ELISAs compared with RDTs for the same diagnostic target (Fig. 2a). The difference was strongest for CHIKV IgM tests—showing a median sensitivity of 94.8% for ELISAs and 25.3% only for RDTs (p 0.001, Wilcoxon rank sum test). For DENV antigen-detecting tests, the difference was smallest—being 71% for RDTs and 72.2% for ELISAs. Among antibody tests, the median sensitivity was significantly higher for IgG tests compared with IgM tests (p 0.002, Wilcoxon rank sum test), albeit this trend was not consistent for all three viruses. While the sensitivity of IgM and IgG tests was very similar for DENV and CHIKV, it was significantly higher for ZIKV IgG ELISAs compared with IgM ELISAs (p 0.003, Wilcoxon rank sum test). In contrast to sensitivity, specificity was generally higher among RDTs compared with ELISAs. The median specificity was very high for DENV antigen-detecting tests and CHIKV IgM RDTs (>98.5%) and it was low for DENV IgG ELISAs (63.5%). The positive predictive value (PPV), which indicates the proportion of true positives, is commonly higher among RDTs whereas the negative predictive value (NPV), which indicates the proportion of true negatives, is higher among ELISAs (Fig. 2b).

Timing of serological testing is crucial

Direct viral detection

During acute infection, viral nucleic acid and proteins (antigens) can be detected in clinical samples [18]. Concentrations of viral antigens and of viral RNA are commonly correlated [18–21] but the period of detectability of viral antigens is often shorter compared with viral nucleic acids [22,23]. The applicability of antigen-detecting tests is therefore limited to a short time frame. The main target for DENV antigen-detecting tests is the non-structural protein 1 (NS1). Among the analysed studies, the median sensitivity of NS1 antigen-detecting tests for DENV was above 70% with samples collected 0–4 days post onset of symptoms (dpo). Afterwards it declined rapidly to 54.6% at 5–6 dpo and to 0% at 11–12 dpo (Fig. 3). Notably, diagnostic usability of NS1 is not granted for all flavivirus infections. Compared with DENV, ZIKV NS1 antigen concentrations in clinical samples are ten-fold

Table 1
Median sensitivity and specificity of different serological tests based on published studies

	Test	Sensitivity in % (interquartile range, *n*)	Specificity in % (interquartile range, *n*)
Antigen tests	DENV Ag RDT	71.9 (61.8–84.7, 63)	98.8 (92.0–100, 54)
	DENV Ag capture ELISA	73.9 (60.0–84.4, 41)	99.0 (97.1–100, 32)
Antibody tests	CHIKV IgM RDT	25.3 (14.8–47.5, 10)	100 (91.1–100, 11)
	CHIKV IgM ELISA	94.8 (83.6–100, 16)	94.7 (87.7–97.0, 14)
	DENV IgM RDT	58.3 (36.0–79.0, 31)	94.9 (85.0–98.9, 27)
	DENV IgM ELISA	68.7 (42.8–83.9, 14)	93.8 (87.3–97.6, 7)
	ZIKV IgM ELISA	65.6 (45.6–85.7, 29)	96.7 (94.5–99.1, 21)
	CHIKV IgG ELISA	96.0 (95.0–96.3, 5)	92.4 (79.0–96.8, 4)
	DENV IgM RDT	58.6 (41.7–74.1, 8)	95.3 (92.2–96.5, 4)
	DENV IgG ELISA	69.0 (55.4–92.1, 6)	63.5 (59.6–77, 3)
	ZIKV IgG ELISA	92.9 (73.2–100, 23)	93.8 (65.5–99.9, 12)

Abbreviations: CHIKV, chikungunya virus; DENV, dengue virus; ELISA, enzyme linked immunosorbent assays; n, number of studies; RDT, rapid diagnostic test; ZIKV, Zika virus. Median sensitivity and specificity were calculated using the database in the Supplementary material (Table S1).

lower, parallel to lower RNA concentrations in ZIKV infections compared with DENV infections [24]. Accordingly, antigen tests for ZIKV suffer from very low sensitivity, which limits their clinical usability, although both ZIKV and DENV are genetically related and part of the same viral family *Flaviviridae* [25]. Although at least one commercial CHIKV antigen-detecting test is available [26], robust data for diagnostic CHIKV antigen detection over time are missing (see Table 1).

Detection of virus-specific immune responses

In response to acute infections, the host's adaptive immune response includes production of different classes of immunoglobulins by B lymphocytes, the first of which is usually IgM [27]. Among the analysed studies, the median DENV IgM sensitivity increased at 3–4 dpo to 42.8%, reaching 100% at 9–10 dpo (Fig. 3). Compared with DENV, median ZIKV IgM sensitivity increased more slowly, reaching 34.9% at 5–6 dpo and reaching its maximum of 70% at 14–30 dpo. The increase of CHIKV IgM sensitivity was delayed compared with DENV and ZIKV, reaching 28.6% at 7–8 dpo but then increased rapidly towards 70.1% at 9–10 dpo and 95% 11–12 dpo.

A few days or weeks after infection, IgM- and IgA-producing B cells are subject to a process called immunoglobulin class switch, resulting in IgG-generating B cells [28,29]. In consequence, IgM and IgA titres stagnate or decrease (Fig. 3) and IgG titres increase. Surprisingly, the increase of sensitivity of IgG tests was not delayed compared with IgM tests. The median DENV IgG test sensitivity was even higher at 3–4 dpo compared with IgM, reaching 51.5%. Similar to IgM, the median IgG sensitivity reached 100% at 9–10 dpo. For ZIKV IgG testing, the median sensitivity increased parallel to the IgM sensitivity. However, ZIKV IgG testing showed higher sensitivities being >80% for all samples collected at 11 dpo or later. In contrast to IgM, the sensitivity of ZIKV IgG testing further increased for samples collected >60 dpo. Compared with IgM, the sensitivity of CHIKV IgG tests increased more slowly and reached its maximum of 100% with samples collected >60 dpo. The rapid increase of IgG class antibodies is probably a consequence of secondary or heterologous infections among study participants because almost 80% of the studies were conducted in regions of endemic transmission [30,31].

As antibody kinetics can vary dramatically among viruses and among individual patients, antibody tests are often negative in clinical samples collected early during the acute infection phase. Paired testing of acute phase and convalescence samples to show seroconversion is therefore a common procedure [28,32] that significantly increases the sensitivity of antibody testing [15,32,33].

Individual infection histories affect diagnostic test performance

Secondary infections and the original antigenic sin

A patient's immune response can be strongly affected by previous infections or vaccinations. This is particularly the case in subsequent infections with the same or antigenically closely related viruses. Diagnostics of CHIKV and DENV, which are the most common arboviruses in tropical and subtropical regions and which are becoming increasingly important in southern Europe, can therefore be hampered by co-circulation with other arboviruses and re-infections (Fig. 4) [34]. Serological testing in flavivirus infections is particularly challenged by previous infections. In DENV and ZIKV patients who have been infected with DENV before, IgM titres are significantly reduced, increasing the risk of false-negative test results [35,36]. Among the analysed studies, the median sensitivity of both CHIKV IgM ELISAs and ZIKV IgM tests was higher with patients living in non-endemic regions (e.g. infected travellers) compared with patients in endemic regions (Fig. 5a). In secondary DENV infections, the sensitivity of antigen-detecting tests is reduced by roughly 30%, probably because of antigen depletion by pre-existing antibodies [35,37]. In regions with high DENV seroprevalence, negative antigen tests may thus have to be confirmed by antibody tests or replaced by PCR tests. Although IgM titres are reduced in secondary arbovirus infections, IgG titres are often higher and can increase more quickly [28,29]. Accordingly, the sensitivity of ZIKV IgG tests was significantly higher in patients living in endemic regions compared to patients living in non-endemic regions when we analyzed the selected studies (p 0.024, Wilcoxon rank sum test). One reason for the altered immune responses in secondary or subsequent infections with antigenically related flaviviruses is the 'original antigenic sin'. During a primary infection, long-lasting memory B and T cells are formed that mature over time to become highly specific. During a subsequent infection with an antigenically related virus, those existing memory B and T cells can be re-activated. The response of those re-activated immune cells is often faster than the response of naive cells, causing a less specific immune response of lower avidity for antigens of the sequentially infecting virus [38,39].

Global mixing of antigenically related viruses

In consequence of the global spread of EIDs, many regions of the world are affected by co-circulation of multiple closely related viruses. Because of the high antigenic conformity of some viruses, pre-existing antibodies can cause false-positive results in serological tests against closely related viruses [7,40,41]. This is particularly

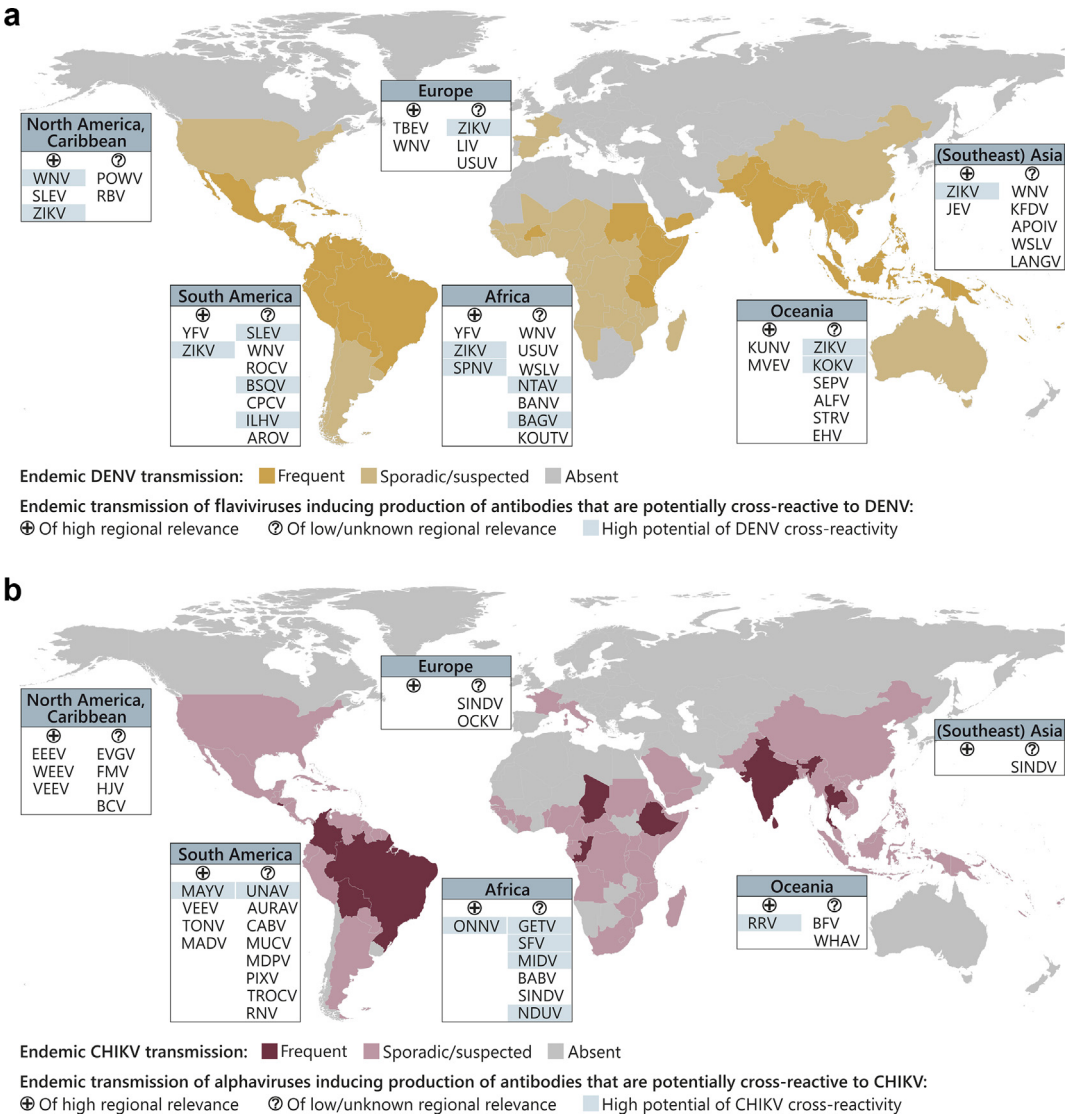

Fig. 4. Global distribution of DENV and CHIKV and the co-occurrence of genetically related potentially cross-reactive viruses. Transmission frequency is shown based on information provided by the World Health Organization/Pan American Health Organization [61,62], the US Centers for Disease Control and Prevention [63,64] and the European Centre for Disease Prevention and Control [65,66]. AURAV, Aura virus; BABV, Babanki virus; BCV, Buggy Creek virus; BFV, Barmah forest virus; CABV, Cabassou virus; EEEV, Eastern equine encephalitis virus; EVGV, Everglades virus; FMV, Fort Morgan virus; GETV, Getah virus; HJV, Highland J virus; MADV, Madariaga virus; MAYV, Mayaro virus; MIDV, Middelburg virus; MDPV, Mosso das Pedras virus; MUCV, Mucambo virus; NDUV, Ndumu virus; OCKV, Ockelbo virus; ONNV, Onyong-nyong virus; PIXV, Pixuna virus; RNV, Rio Negro virus; RRV, Ross River virus; SFV, Semliki Forest virus; SINDV, Sindbis virus; TONV, Tonate virus; TROCV, Trocara virus; UNAV, Una virus; VEEV, Venezuelan equine encephalitis virus; WEEV, Western equine encephalitis virus; WHAV, Whataroa virus.

problematic in serological diagnostics of DENV or CHIKV (Fig. 4). In Africa, DENV is co-endemic with at least ten other human-pathogenic flaviviruses including ZIKV. In Latin America, CHIKV is co-endemic with at least 12 other human-pathogenic alphaviruses including the closely related Mayaro virus. In Africa, O'nyong-nyong virus is the major alphavirus beside CHIKV [42]. CHIKV antibody tests are often susceptible to cross-reactivity with antibodies against Mayaro virus or O'nyong-nyong virus [7,40] and DENV antibody tests are susceptible to cross-reaction with antibodies against ZIKV and vice versa because of structural and antigenic similarities [41,43]. Accordingly, the specificity of DENV and ZIKV ELISAs was lower in patients living in regions of endemic arbovirus transmission compared to patients living in regions without endemic transmission, although the difference was significant only for ZIKV IgG (p 0.007, Wilcoxon rank sum test) (Fig. 5B). Notably, the specificity of ZIKV ELISAs was generally higher than for DENV ELISAs. This might be explained by the usage of different viral antigens. While ZIKV ELISAs commonly use NS1 as viral antigen, DENV ELISAs commonly use full viral particles or the envelope protein which is known for potentially broad cross-reactivity.

Polyclonal B-cell stimulation and environmental factors

Beyond the cross-reactivity with antigenically related viruses, serological tests can also be affected by infections with entirely different pathogens potentially due to polyclonal B-cell stimulation [44,45]. Although this is well known for Epstein–Barr virus infections [45], infections with parasites such as *Plasmodium* sp. (malaria) have also been found to cause false-positive results in ZIKV [44] and DENV [46] ELISAs. In contrast, previous infections with measles virus can diminish the pre-existing humoral immune

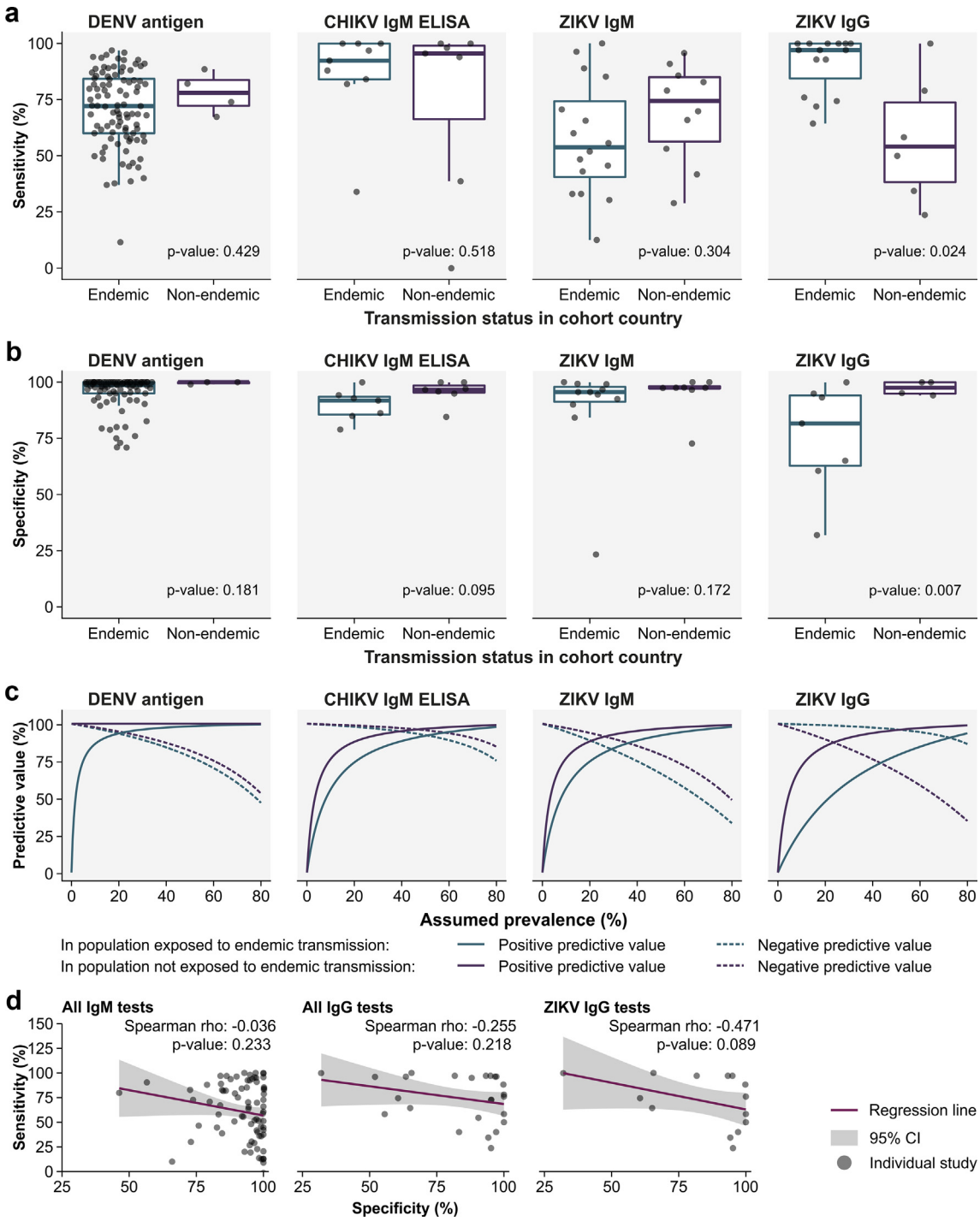

Fig. 5. Performance of antibody- and antigen-detecting tests in regions of endemic and non-endemic transmission. (a) Sensitivity of tests with at least four values for each endemic and non-endemic transmission as Tukey's boxplots. Single study results are indicated as grey dots. Significance was calculated by Wilcoxon rank sum test. (b) Specificity of tests with at least four values for each endemic and non-endemic transmission as Tukey's boxplots. Single study results are indicated as grey dots. Significance was calculated by Wilcoxon rank sum test. (c) Predictive values for tests shown in (a) and (b) for a range of assumed prevalence. (d) Negative correlation of sensitivity and specificity. Significances were calculated using the Spearman's rank correlation test.

memory, hampering seroprevalence studies [47]. Diagnostics can also be affected by climatic factors, e.g. loss of sensitivity in DENV RDTs when stored at 35°C [48].

In summary, serological testing can be challenged by the patient-specific infection histories and it is essential to be aware of the potential regional limitations of serological tests [49]. Specific detection of antibodies is particularly challenging when antigenically closely related viruses co-circulate in the same region. In consequence, the PPV is higher with patients living in regions lacking endemic arbovirus transmission because the risk of false-positive results due to recent heterologous arbovirus infections is lower. Although the NPV of antigen-detecting and IgM tests is also higher in regions without endemic arbovirus transmission, the NPV for IgG tests is higher in regions of endemic transmission because

IgG responses are commonly stronger in secondary infections (Fig. 5C). Antibody test results for ZIKV, DENV and CHIKV must therefore be interpreted cautiously, including interpretation of regionally co-circulating anitgenically related arboviruses.

Balancing sensitivity and specificity

A major challenge in serological testing is to equilibrate sensitivity and specificity. In arbovirus tests, these intrinsic properties are negatively correlated (Fig. 5d), which also applies to non-vector-borne viruses such as the severe acute respiratory syndrome coronavirus 2 [50]. Prioritization of either sensitivity or specificity can be done according to the desired usage and can include selection of individual tests with defined performance, or adaptation of test-specific cut-offs, the defined values at which a test is considered positive or negative [50]. Additionally, the selection of antigens or antibodies used in serological tests can have a major impact on the test accuracy. In flavivirus infections, both NS1 and the envelope protein (E) are immunogenic and widely used [38]. However, the detection rate of anti-ZIKV E IgM in infected patients may be higher compared with anti-ZIKV NS1 IgM, hinting at differential immune responses and so limited usage of some antigens for long-term follow up and serology-based surveillance [15]. Similarly, the durability of IgG antibody responses generally varies among the targeted antigens [51]. Using specifically mutated recombinant envelope proteins increased the specificity of DENV ELISAs, allowing the differentiation of serotypes for IgM [52].

If possible, combining different tests can increase the diagnostic accuracy. A common approach to increase sensitivity of serological testing is combining antigen and antibody tests for acute phase samples [53,54]. For co-endemic and antigenically related viruses, antibody tests against both viruses can be combined and compared, considering the higher test value positive. This approach has been used to differentiate DENV from ZIKV [55] and CHIKV from Mayaro virus [7] infections.

In summary, the balance of sensitivity and specificity is challenged by co-circulation of antigenically closely related viruses. Beyond the equilibration of a test's sensitivity and specificity, a stepwise testing algorithm combining a very sensitive screening test for first-line testing and a more specific test for confirmatory testing is a commonly used solution, but it is not always affordable and generally delays test results [7,56].

Conclusion and outlook

Serological tests are key for diagnostics and epidemiological studies of EIDs. Test selection and timing are crucial for high test accuracy and optimized diagnostic protocols may vary among regions of endemic and non-endemic arbovirus transmission. Serological diagnostics will be increasingly challenged by the changing endemicity and spread of emerging viruses, which may interfere with regionally established tests. Unambiguous results may not always be achievable in hyperendemic regions, particularly if only single samples collected during the acute infection phase are tested. To overcome those limitations, simultaneous detection of different antigens or antibodies is currently a central aim in the development and adaptation of new techniques for infectious disease diagnostics [57–59], including bead-based assays or suspension array technologies [57,58] and bacteriophage library display [59], albeit the diagnostic applicability of these new techniques remains unknown [60].

Transparency declaration

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Authors' contributions

JFD and CF were responsible for conceptualization, writing and editing; CF performed the formal analysis; CF, WKJL, VH, EFO and AMS contributed to data curation; CF was responsible for visualization and JFD provided supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.05.047.

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