INTREPID ALLIANCE

INTERNATIONAL READINESS FOR PREVENTING INFECTIOUS VIRAL DISEASE

MAY 2026

Overview of *In Vitro* Assays and Animal Efficacy Models: *Orthoflaviviruses*

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Orthoflavivirus Disease Landscape

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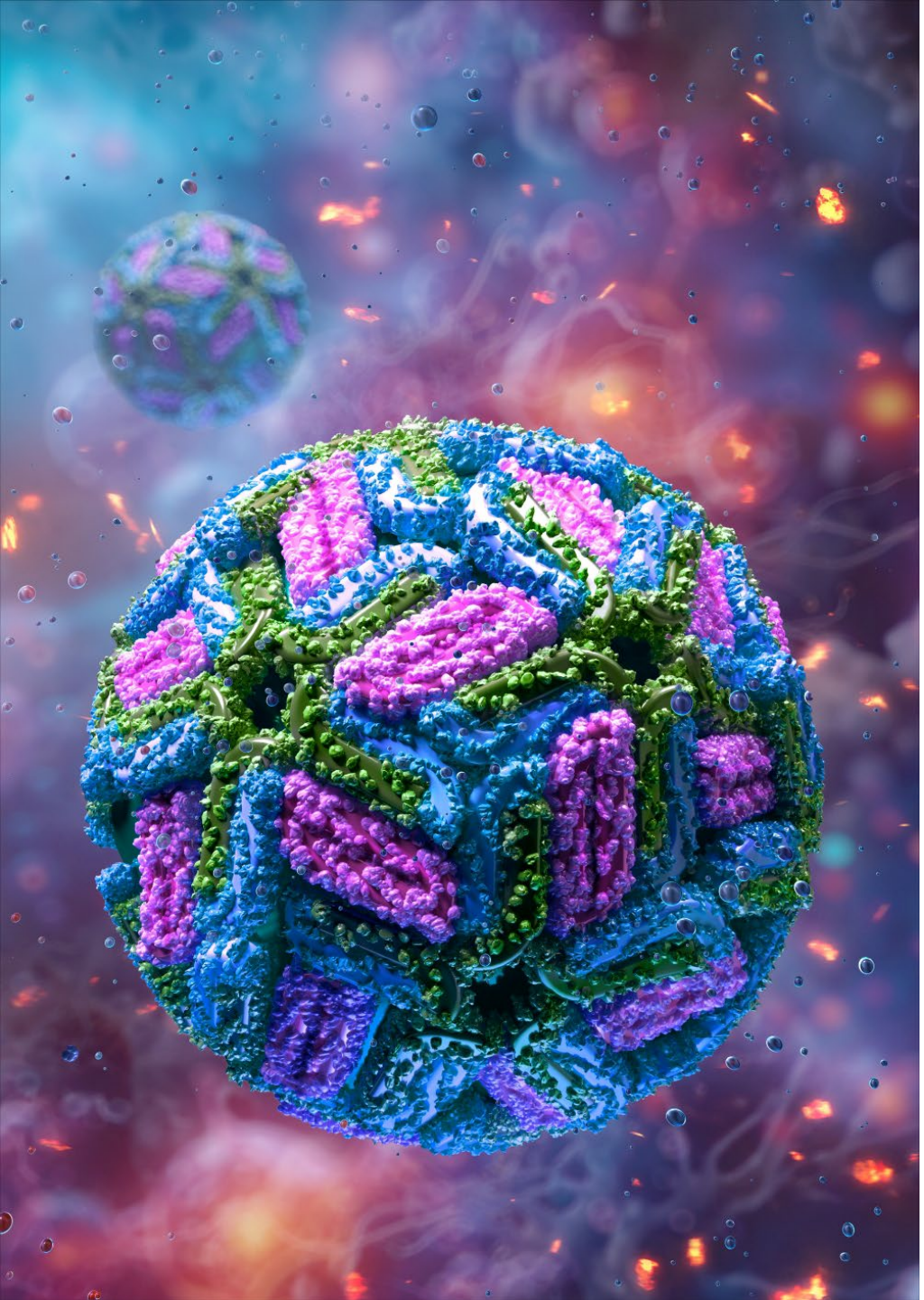

Orthoflaviviruses

- Orthoflaviviruses belong to the *Flaviviridae* family and are a group of enveloped, single-stranded, positive-sense RNA viruses.[1,2]
- Flaviviruses primarily infect mammals and birds, and are mostly transmitted by arthropod vectors, particularly mosquitoes and ticks.[3]
- Notable human pathogens include dengue virus, Zika virus, yellow fever virus, West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis virus.[4]
- Although some vaccines and antiviral treatments have been approved against several orthoflaviviruses, these viruses are becoming an increasing global concern with a high disease burden and unmet need.[5,6,7]
- In 2024, there were 14.6 million reported cases worldwide and more than 12,000 deaths reported for dengue fever alone. The Americas region was especially impacted, reporting over 13 million cases to the World Health Organization (WHO).[8,9]

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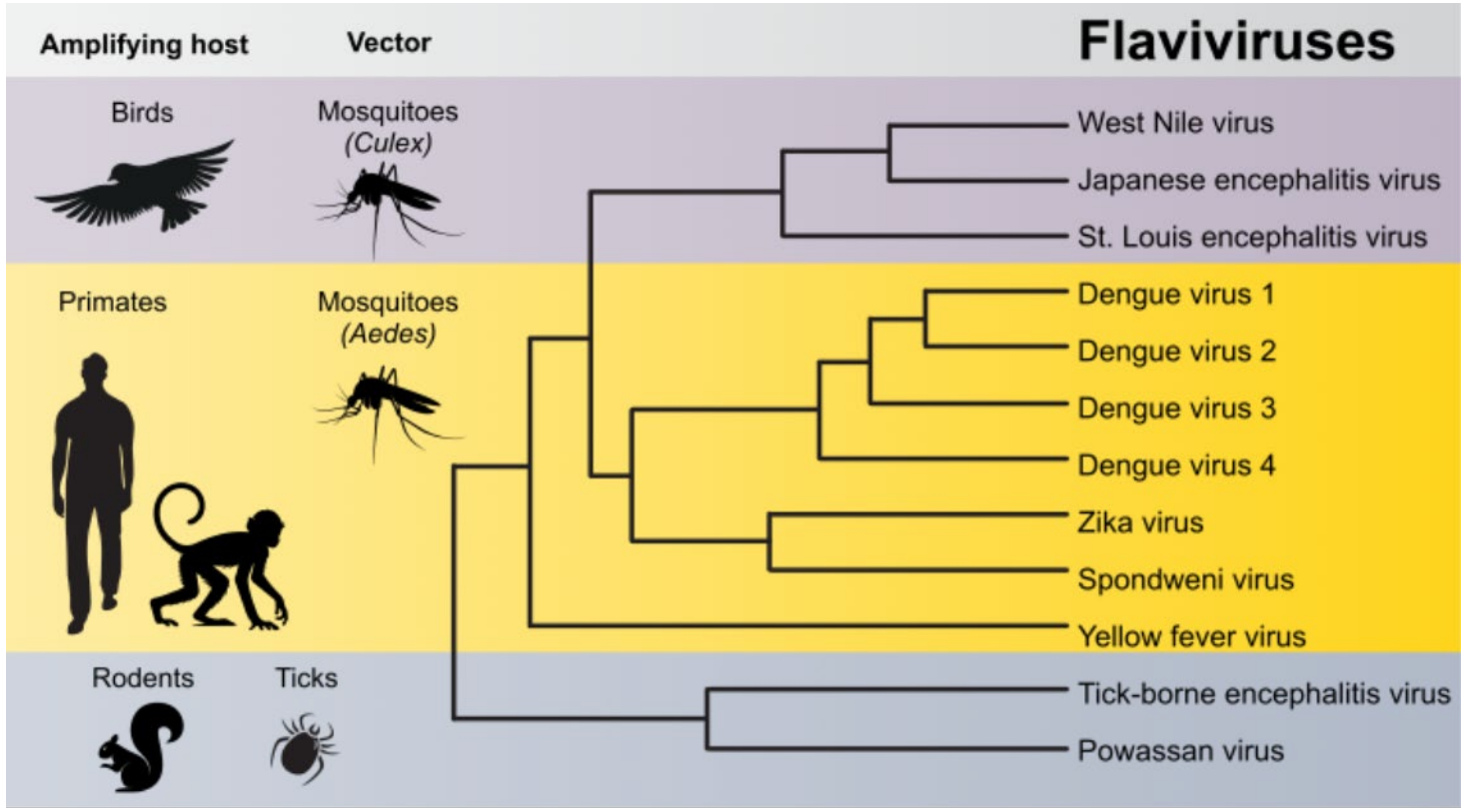
Transmission of Infection with Orthoflaviviruses

- Transmission involves complex life cycles that require both vertebrate hosts (such as humans or birds) and arthropod vectors (mainly mosquitoes or ticks).[10]
- The mosquito-borne group can be further divided into:
  - **Neurotropic viruses**, often associated with meningo-encephalitic disease in humans or livestock. This branch tends to be spread by *Culex* species and to have bird reservoirs.[11]
  - **Non-neurotropic viruses**, associated with human hemorrhagic disease. These tend to have *Aedes* species as vectors and primate hosts.
- Climate change, globalization and urbanization accelerate the spread of the vectors (mosquito, ticks) across the globe.[12]

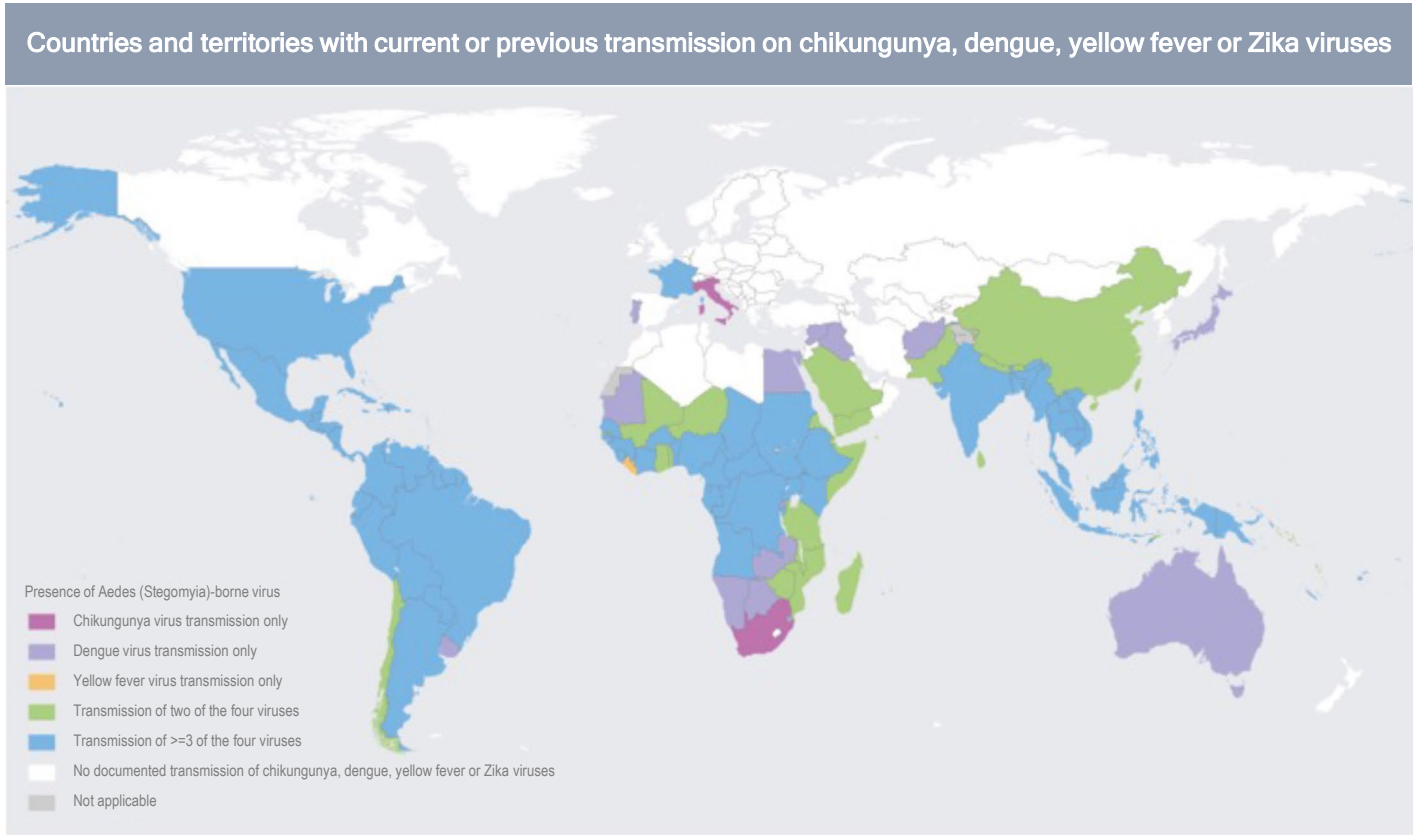


Adapted from Lazear Lab, Examples of Flaviviruses that Cause Human Disease.[13]

Global Distribution of Arboviruses*

- 157-800 million yearly *Orthoflavivirus* infections; mainly driven by dengue infections.* For many of these diseases, including dengue more than 80% of infections remain asymptomatic, but contribute to disease transmission.
- Likely significant underestimation due to widespread underreporting, asymptomatic infections, and limited surveillance capacity in many endemic regions.[14]
- Arboviruses, including many orthoflaviviruses, are important causative agents of global epidemics and pose significant public health challenges due to their potential emergence and re-emergence in new geographic regions.[15]
- **Half of the world's population is at risk of infection.**[16]

*See Supplemental Information for summary annual infection rates per virus.



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Data Source: World Health Organization
Map Production: WHO Health Emergencies Programme
Request ID: RITM00065

World Health Organization

Source: World Health Organization. Global Arbovirus Initiative. 2024.[17]

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Clinical Disease Spectrum of Orthoflaviviruses

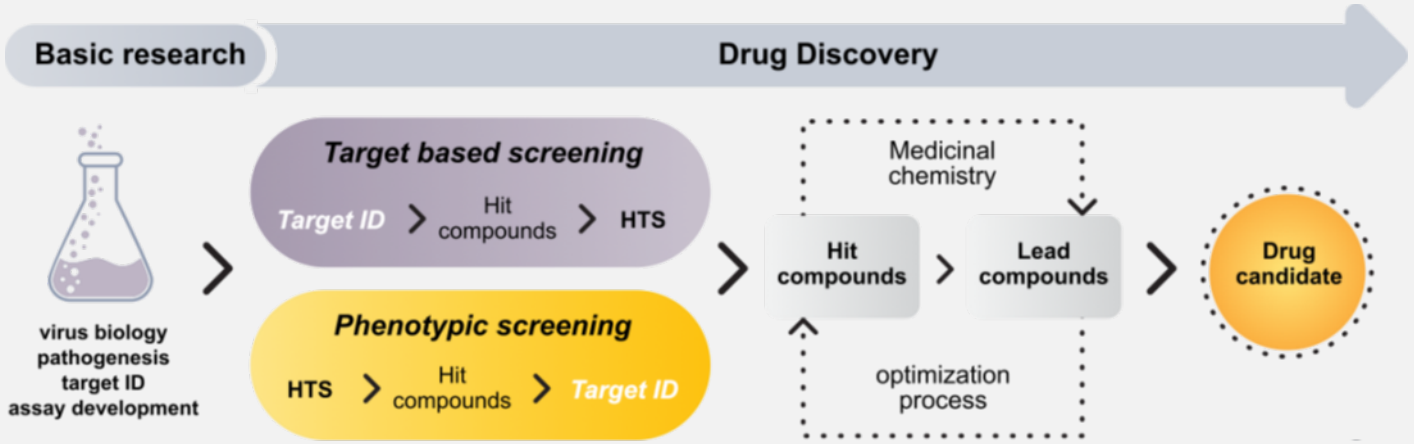
- Infection with orthoflaviviruses can cause a wide spectrum of clinical disease in humans,[18] ranging from asymptomatic to multisystemic disease.
- Symptomatic disease can generally be described as one of three syndromes:
  - Self-limited disease characterized by fever, rash, myalgia and arthralgia
  - Meningoencephalitis
  - Hemorrhagic fever
- There can be considerable overlap of symptoms among these syndromes.
- Approximately 50-80% of all orthoflavivirus infections are asymptomatic[19] (e.g., 80% for West Nile virus and 80% for Zika virus).[20,21]
  - These silent infections play an important role in the persistent circulation of these viruses and contribute to their epidemic potential.[22]

Orthoflavivirus Preclinical & Clinical Antiviral Landscape

Accelerating Unified Antiviral Drug Discovery Efforts to Address *Orthoflavivirus* Unmet Global Health Challenge

Building an efficient antiviral discovery framework applicable to other emerging viruses:

- Platform development: High-throughput screening (HTS) and hit evaluation
- Mechanistic insights: Understand antiviral activity, cytotoxicity, mechanism of action
- Translational relevance: Build *in vivo* models with clinically relevant disease pathogenesis



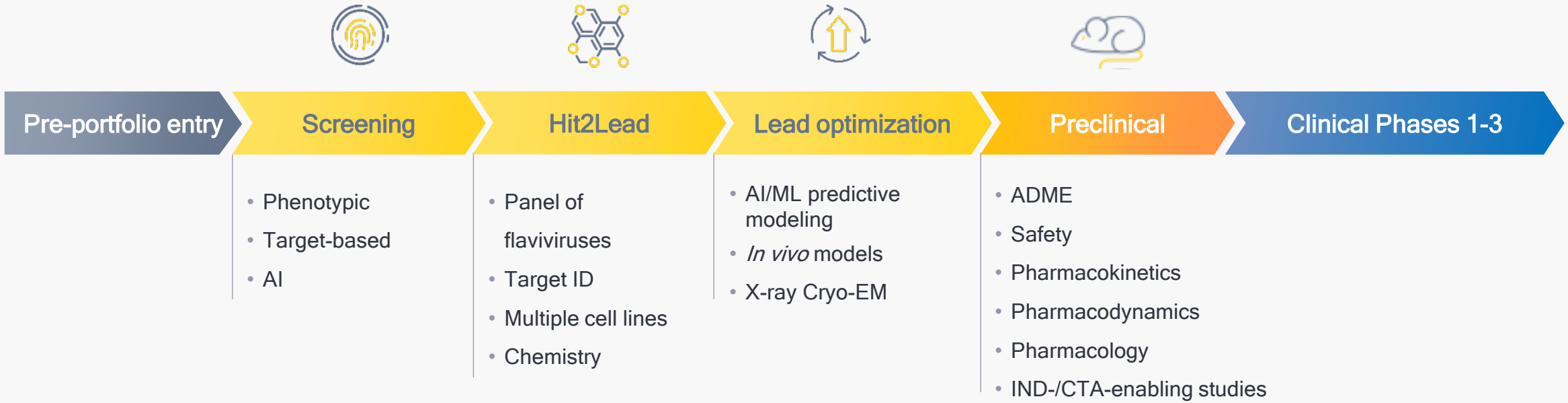
Adapted from Hernandez-Morales I, Van Loock M. *Adv Exp Med Biol*. 2018.[23]

The following slides provide a landscape of *in vitro* antiviral assays and animal efficacy models for discovery and preclinical evaluation of therapeutics focusing on *Orthoflavivirus* representatives:

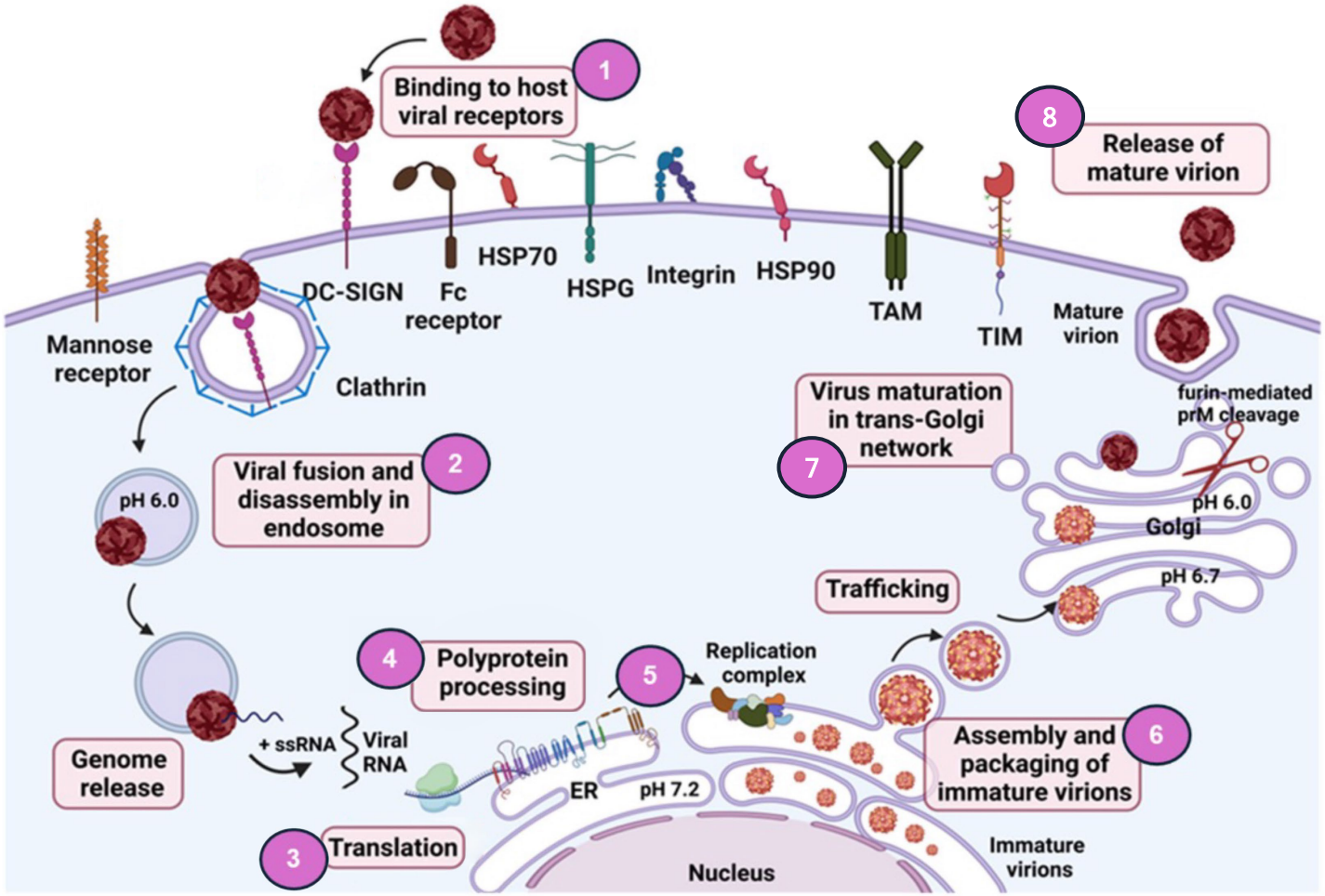
- Dengue virus (**DENV**)
- Japanese encephalitis virus (**JEV**)
- Tick-borne encephalitis virus (**TBEV**)
- West Nile virus (**WNV**)
- Yellow fever virus (**YFV**)
- Zika virus (**ZIKV**)

Simplified Hit-to-Clinical Candidate Roadmap

- Start drug discovery by implementing a HTS campaign using either:
  - Cell-based assays using mammalian cell-lines, with multiplex read-outs for broad-spectrum assessment as recent innovations.[24]
  - Target-based biochemical assays, supplemented with AI/ML to accelerate the turnaround time.
- Assess broad-spectrum activity against clinical isolates early in the process.
- Hits go through multiple med-chem rounds to improve potency and drug-like properties (e.g., ADME, safety).
- Evaluate antiviral activity in relevant animal model to assess PK/PD and human dose predictions.



Orthoflavivirus Life Cycle

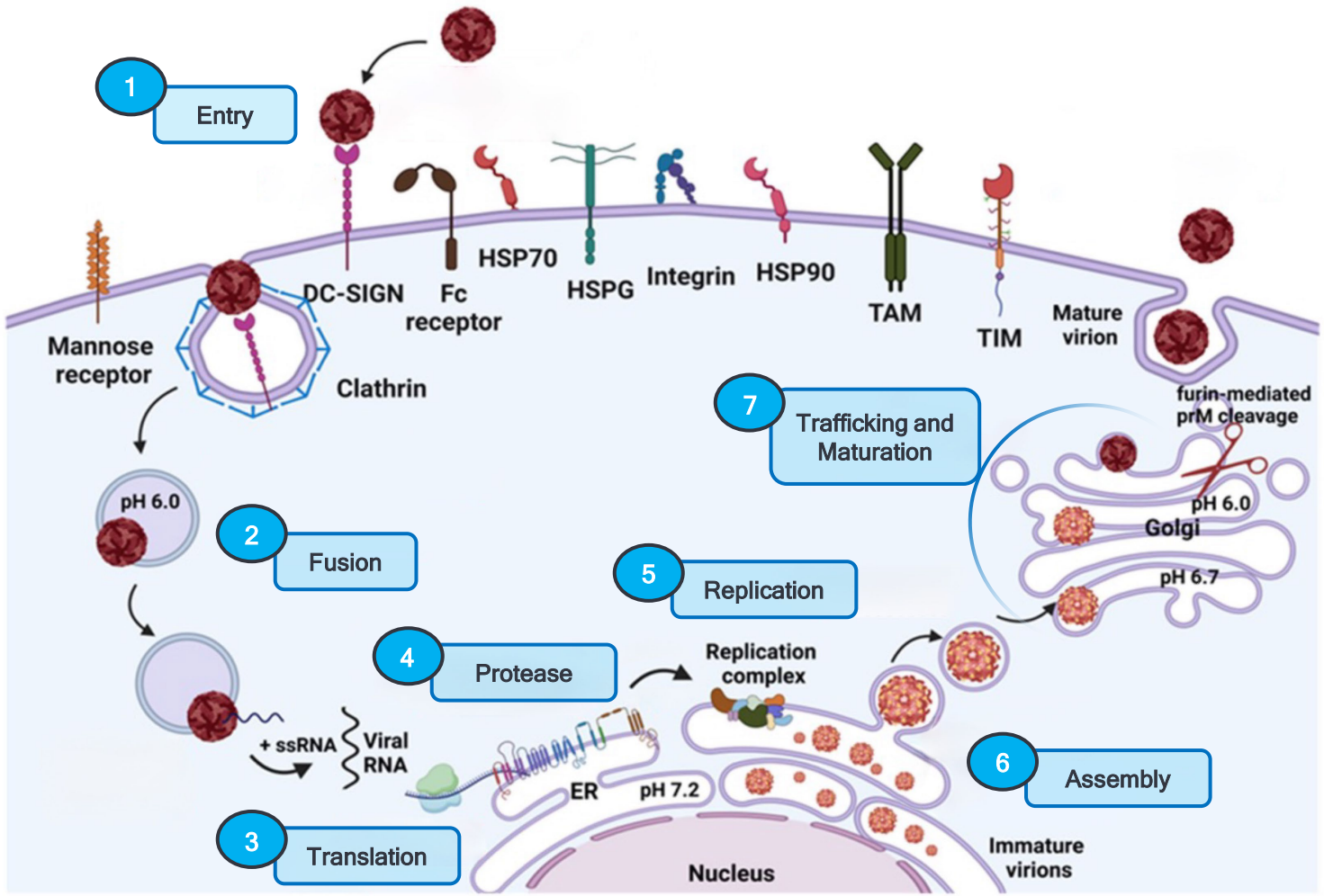


Adapted from Tripathi A, *et al*. *Viruses*. 2025.[25]

Orthoflavivirus life cycle:

1. Viral particles attach to the host cells via interactions between virus surface proteins and their respective host receptors. Virus enters host cells via receptor-mediated or clathrin-dependent endocytosis.
2. pH-dependent membrane fusion occurs in the endosome, and this results in the formation of pores which allows the release of the viral genome into the host cytoplasm.
3. The released viral RNA is translated into a polyprotein.
4. Viral and cellular proteases process the polyprotein into structural and non-structural viral proteins.
5. Viral replication occurs in vesicular packets in the Endoplasmic Reticulum (ER).
6. Viral assembly and packaging takes place at the ER to form immature virions.
7. These immature virus particles travel through the secretory pathway and trans-Golgi network (TGN) to form mature flavivirus virions.
8. Following successful virus maturation, exocytosis of the mature virus particles from the host cells.

Orthoflavivirus Life Cycle: Direct-Acting Antiviral Targets



[Adapted from Tripathi A, *et al*. *Viruses*. 2025.[26]

Targets for direct antivirals:

1. **Entry** inhibitors blocking receptor-mediated binding yet might be bypassed by antibody-dependent enhancement as described for dengue.
2. **Fusion** inhibitors, e.g., beta-OG pocket
3. **Translation** inhibitors
4. **Protease** inhibitors blocking cell-and viral mediated processing.
5. **Viral replication complex**: *KEY TARGET*
    1. Polymerase, methyltransferase
    2. Non-structural proteins: e.g., NS4B
6. **Assembly** inhibitors
7. **Trafficking and maturation** inhibitors

To tackle broad-spectrum activity, targets with high % homology are critical: e.g., RNA-dependent RNA polymerase (RdRp), with high sequence identity, and the 3D structure of the RdRp domain highly conserved, shared among all *Flaviviridae*.

The Majority of Antiviral R&D Activity for Orthoflaviviruses Is in the Preclinical Space

➢ 19/31 evaluations across 4 orthoflavivirus disease indications*

Antiviral Type (# evaluations)	Unapproved Antiviral Evaluations					Indication Expansion Evaluations			
	Preclinical** (n=19)				Clinical (n=1)	Investigational (n=1)		For Approved Compounds (n=10)	
Viral Indication (# evaluations)	Hit (n=1)	Early Lead (n=9)	Late Lead (n=8)	Potential Candidate (n=1)	Clinical Phase (n=1)	Preclinical Exploratory (n=0)	Clinical Study (n=1)	Preclinical Exploratory (n=8)	Clinical Study (n=2)
Dengue (n=12)	x	ZXH-2-107	ASAP-0029002	mCOT466	EYU688 (Phase 2)	x	Mosnodenvir (Phase 2)	Remdesivir	Molnupiravir (Phase 2)
		ZXH-8-004	DV-B-120				x		Zanamivir (Phase 2)
		MLT201	2-Thiouridine						
		DHFLV_003B							
West Nile (n=2)	x	DHFLV_003B	x	x	x	x	x	Etravirine	x
Yellow Fever (n=9)	x	AT-2490	LRP1-Fc Decoy	x	x	x	x	Favipiravir	x
			LRP4-Fc Decoy					Favi/6-MMPr	
			VLDLR-Fc Decoy					Remdesivir	
			BSBI-YF					TRIAC	
Zika (n=6)	x	MLT201	ASAP-0036543	x	x	x	x	Favipiravir	x
		MWAC-4001						Sofosbuvir	
		DHFLV_003B							
Pan-Flavivirus (n=1)	MMV1791425	x	x	x	x	x	x	x	x

Distinct Compounds by Mechanism of Action

- Entry (n=5)
- Protease (n=3)
- Replication (n=15)
- Assembly-Release (n=1)
- Unspecified (n=1)

- 25 distinct antiviral compounds are under evaluation
  - Most compounds are replication inhibitors (15); 7 in preclinical studies and 8 in clinical.
- The number of distinct compounds is insufficient to address the unmet need and account for attrition.

*Based on publicly available information with Airfinity and INTREPID Alliance as of January 2026.
**These preclinical compounds have no clinical or human exposure data.

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“Archived” or “Discontinued” Antivirals for Orthoflaviviruses*

	Preclinical Compounds (n=45)			Clinical Compounds (n=5)	
Viral Indication	Archived (n=40)		Discontinued (n=5)	Archived (n=2)	Discontinued (n=3)
Dengue (n=44)	NITD – Compound 6	Protease inhibitor	NITD - Compound 17	Galidesivir	Balapiravir
	166347	Protinhi DENV	NITD008		AT-752 (Phase 2)
	6A49	Protinhi pan-flavivirus	NITD203		
	ARDP0006	Retrocyclin 1			
	ARDP0009	thiazolidinone-peptide			
	Carnosine	2'-C-Methylcytidine (NM107)			
	cpd 104	7-Fluoro MK608			
	cpd 14	Allosteric NS5 inhibitor			
	cpd 32	NITD - Compound 14a			
	cpd 45a	Compound 24a			
	cpd 7n	Compound 24b			
	Cpd C/D/F	JNJ-A07			
	Cpd 1	Methyl transferase inhibitor			
	cpd1/6/8 – diarylthioethers	MK608			
	Ltc1	NITD-618			
	MB21	RK-0404678			
	Nelfinavir	ST-148			
	Policresulin	ST-610			
	Potegrin 1				
West Nile (n=1)	Protini pan-flavivirus		x	x	x
Yellow Fever (n=2)	x		NITD008	x	Galidesivir
Zika (n=4)	Saliphenylhalamide	Protini pan-flavivirus	NITD008	Galidesivir	x

Distinct Compounds by Mechanism of Action

- Entry (n=1)
- Protease (n=23)
- Replication (n=19)
- Assembly-Release (n=0)
- Vacuolar ATPase (n=1)

*Based on publicly available information with Airfinity and INTREPID Alliance as of January 2026.

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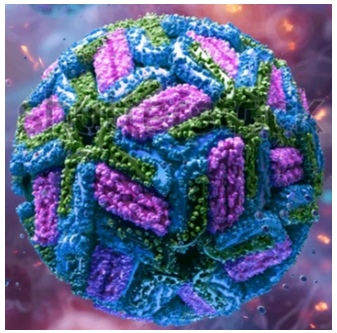
Orthoflavivirus Drug Discovery:
Selected *In Vitro* Viruses, Assays & Cells

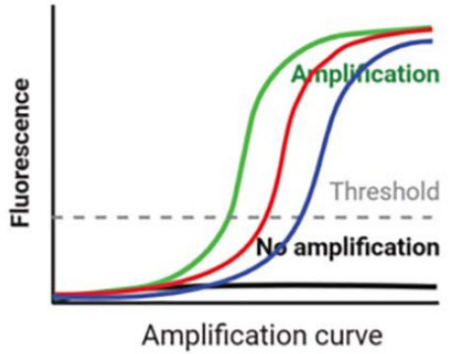
Guiding Principles for Antiviral Assay Development:

Viruses, Assays & Cells

Viruses

- Multiple flavivirus species testing for broad-spectrum activity[27,28]
- Clinical isolate validation beyond laboratory strains[29]
- Geographic variant testing for global applicability[30]
- Drug-resistant strain profiling





Antiviral Assays

- Dose-Response Characterization:[31]
  - Full dose-response curves with at least 8-10 concentrations
  - Hill slope analysis to understand cooperativity
- Parallel Cytotoxicity Assessment:
  - Always run cytotoxicity assays alongside antiviral screens[32]
  - Calculate selectivity index (SI) = $CC_{50}/EC_{50} \geq 4$ as minimum threshold
  - Use multiple cell lines to assess cytotoxicity spectrum

Translation Considerations
(i.e., in vitro, physiological relevance):

- Primary cell cultures when possible
- Blood brain barrier assessment for neurotropic flaviviruses[33]
- Co-culture systems mimicking tissue environment
- 3D cell culture models for enhanced physiological relevance (See Organoids slides 21-22)

Guiding Principles for Antiviral Assay Development:

Assay Robustness and Scalability

Robust assay validation, based on the following parameters:

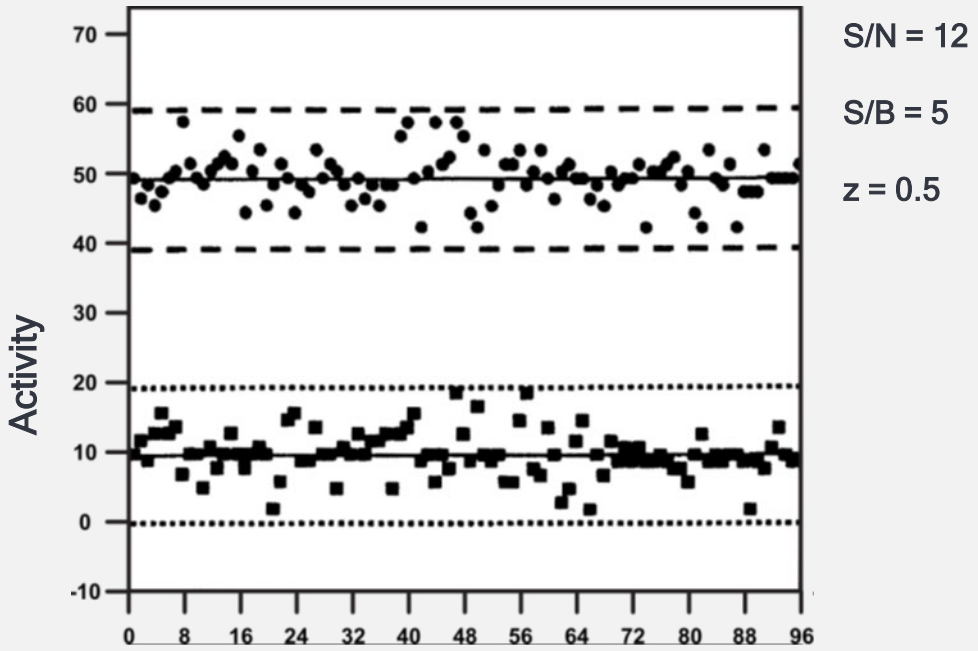
- **Z-factor** ≥ 0.5 for high-throughput screening suitability[34]
- Signal-to-background ratio (**S/B**) ≥ 3:1
- Coefficient of variation (CV) ≤ 20% for reproducibility
- Dynamic range spanning at least 2 log units

Standardization Requirements[35]

- Standardized virus stocks with consistent titer and passage history
- Controlled infection conditions (MOI, temperature, timing)
- Consistent cell passage numbers to minimize variability
- Environmental controls (CO_2, humidity, temperature)

Quality Control Measures

- Reference compounds in every assay plate
- Positive and negative controls with acceptance criteria
- Inter- and intra-assay precision monitoring
- Regular assay performance review



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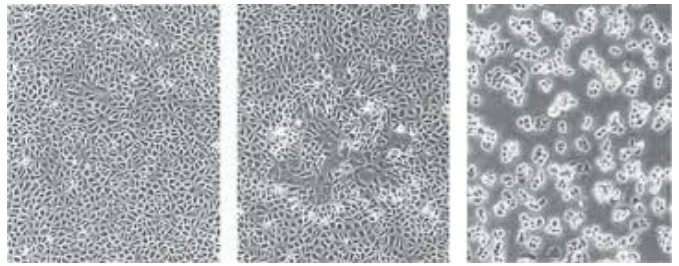
Primary Antiviral Screening Assays for Orthoflaviviruses

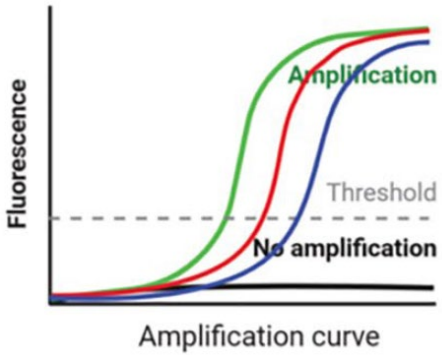
Cytopathic Effect (CPE) Inhibition Assays

- The most fundamental approach involves measuring the ability of compounds to protect cells from virus-induced cytopathic effects. These assays typically use:
  - Vero cells (African green monkey kidney cells) – commonly used for dengue, Zika, and Yellow fever viruses[36]
  - BHK-21 cells (baby hamster kidney cells) – useful for multiple flaviviruses[37]



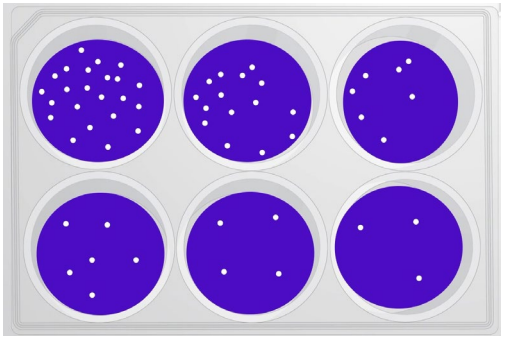
Viral Replication Inhibition Assays

- More specific assays that directly measure viral RNA or protein production:
  - qRT-PCR-based assays measuring viral RNA levels[38]
  - Immunofluorescence assays detecting viral proteins (NS1, E protein)[39]
  - Reporter virus systems using luciferase or GFP-expressing viruses[40,41]



Plaque Reduction Assays

- Gold standard for determining antiviral efficacy:
  - Plaque Reduction Neutralization Test (PRNT) for measuring infectious virus reduction[42]
  - Focus Forming Unit (FFU) assays as alternatives to traditional plaque assays[43]



Target-Based Assays for Orthoflaviviruses

Enzymatic Activity Assays

- The NS3 protease is essential for viral polyprotein processing and represents a critical target.
  - NS3 Protease Assay: Fluorogenic or FRET-based peptide substrates mimicking viral polyprotein cleavage sites provide continuous readout of cleavage kinetics.[44,45]
- The NS5 polymerase contains both methyltransferase and RdRp activities that are critical for viral replication.
  - NS5 RNA-Dependent RNA Polymerase (RdRp) Assay: Incorporation of labelled nucleotides (fluorescent, radiometric, or luminescent) into RNA templates can be configured as primer-extension or de novo synthesis formats.[46,47]
- The NS3 helicase domain is essential for viral replication and unwinds double-stranded RNA intermediates.
  - NS3 Helicase Assay: Unwinding of duplex RNA or DNA substrates with fluorescent/quencher pairs, or ATPase-coupled luminescence, infers helicase activity.[48,49]

Protein–Ligand Binding Assays

- Surface Plasmon Resonance (SPR): Real-time measurement of compound binding kinetics to recombinant target proteins.[50]
- Microscale Thermophoresis (MST): Detection of binding-induced changes in thermophoretic mobility of fluorescently labelled proteins.[51]
- Differential Scanning Fluorimetry (DSF; Thermal Shift): Monitoring protein melting temperature shifts upon ligand binding to detect stabilization.

Guiding Principles for Antiviral Assay Development:

Target-Based Assays

Biochemical Relevance and Specificity

- Use substrates and cofactors closely mimicking the viral context.[52]
- Validate hits in orthogonal formats (e.g., confirm protease inhibition in different assays).[53]

Robustness and Statistical Quality

- Optimize signal window (fold-change between positive and negative controls) to achieve a Z′-factor ≥ 0.5.[54]
- Ensure low coefficient of variation (CV ≤ 10%) across wells and plates.

Kinetic and Mechanistic Characterization

- Determine enzyme kinetics under assay conditions.[55]
- Choose enzyme/substrate concentrations to operate in the initial-velocity regime (<10% substrate turnover).

DMSO and Compound Interference Tolerance

- Validate assay performance across typical DMSO concentrations (0.1-2%).[56]
- Include counter-screens for common interference mechanisms

Miniaturization and Throughput

- Adapt to 384- or 1536-well formats with automated liquid handling.
- Balance throughput with data quality–maintain Z′-factor and CV while scaling.[57]

Control Design

- Positive controls: known inhibitors or active-site mutants.
- Negative controls: no enzyme, no substrate, or inactive analogs.
- Vehicle controls: buffer-only and DMSO-matched wells.

Orthogonal and Secondary Assays

- Rapid counter-screen to eliminate false positives via a different technology.
- Cell-based reporter assays or viral replication assays to confirm antiviral activity and assess permeability/toxicity.

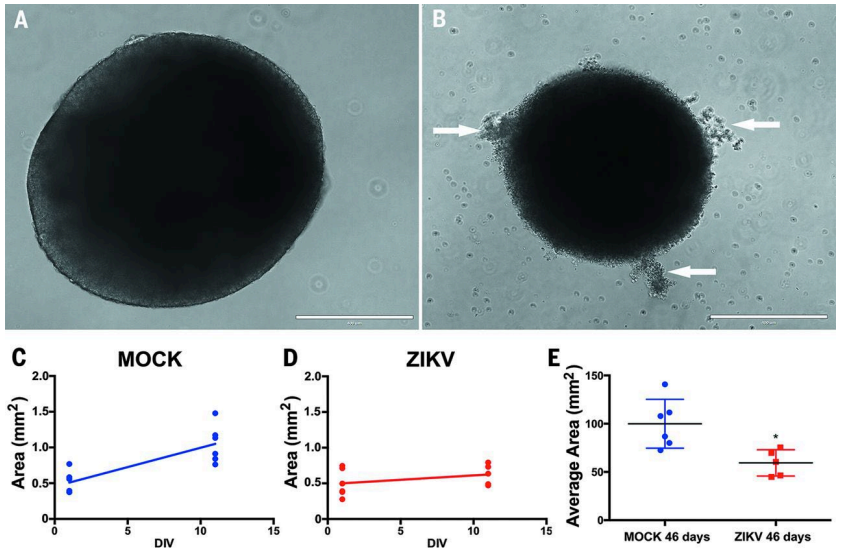
Data Management and Hit Validation Cascade

- Implement real-time data capture and automated analysis.
- Prioritize hits based on potency, selectivity index, and biophysical confirmation.
- Advance validated hits through dose-response, mechanism-of-action studies, and ADME profiling.

Available Organoids Models for Orthoflaviviruses

Brain Organoids

- Human brain organoids are used for studying neurotropic flaviviruses, particularly Zika virus (ZIKV).
  - These three-dimensional cultures derived from induced pluripotent stem cells (iPSCs) recapitulate key features of human brain development and are highly susceptible to ZIKV infection.[58,59,60]
  - Brain organoids have been successfully used to:
    - Model ZIKV-induced microcephaly and neuronal damage[61,62,63]
    - Screen direct-acting antiviral compounds, and alternative strategies including TLR3 inhibitors and RNAi enhancers like enoxacin[64]



Source: Garcez PP, *et al*. *Science*. 2016.[67]

Other Organ-specific Organoid models

- In absence of a clear link with their pathology, kidney and intestinal organoid models are not employed, yet liver organoids have been used to recapitulate dengue virus infection and antiviral screening[65]

Air-liquid interface models

- Primary human nasal epithelial cells (NECs) cultured at the air-liquid interface have demonstrated susceptibility to multiple flaviviruses including ZIKV, JEV, WNV, and Usutu virus[66]

Guiding Principles for Antiviral Assay Development:

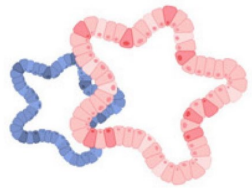
Organoids

Establish standardized protocols for organoid generation, maintenance, and characterization[68,69]

- Implement quantitative and qualitative assessment methods for organoid maturity
- Ensure consistent cell composition and morphology across batches
- Validate organoid phenotype against *in vivo* tissue characteristics

Culture Standardization:

- Use defined, reproducible media formulations with validated growth factors
- Pre-test extracellular matrix lots for consistent performance
- Maintain controlled culture conditions including temperature, CO_2, and humidity
- Document all protocols with minimal information standards (MIAOU - Minimal Information About an Organoid and its Use)[70]



Organoid Quality Control

Organoid Evaluation Methods	Organoid Quality Requirements
Morphological evaluation Viability evaluation Biomarker-based evaluation Gene Expression-based evaluation Reproducibility evaluation In vivo similarity evaluation	Morphology **Qualitative Method:** Microscopy, Transmission electron microscopy, Confocal microscopy, Immunofluorescence staining **Quantitative Method:** Size and diameter, Organoid count, Proliferation/Differentiation evaluation, Volume, Nucleus/Cytoplasm ratio, Proportionality and Symmetry, Differentiation pathway Biomarker **Qualitative Method:** Immunofluorescence staining, Immunohistochemistry, Western blot, In situ hybridization **Quantitative Method:** ELISA, qPCR, Flow cytometry, Mass spectrometry, RNA sequencing, Proteomics analysis

Source: Ahn SJ, *et al*. *Int J Stem Cells*. 2024.[71]

Orthoflavivirus Drug Discovery: *In Vivo* Preclinical Efficacy Models

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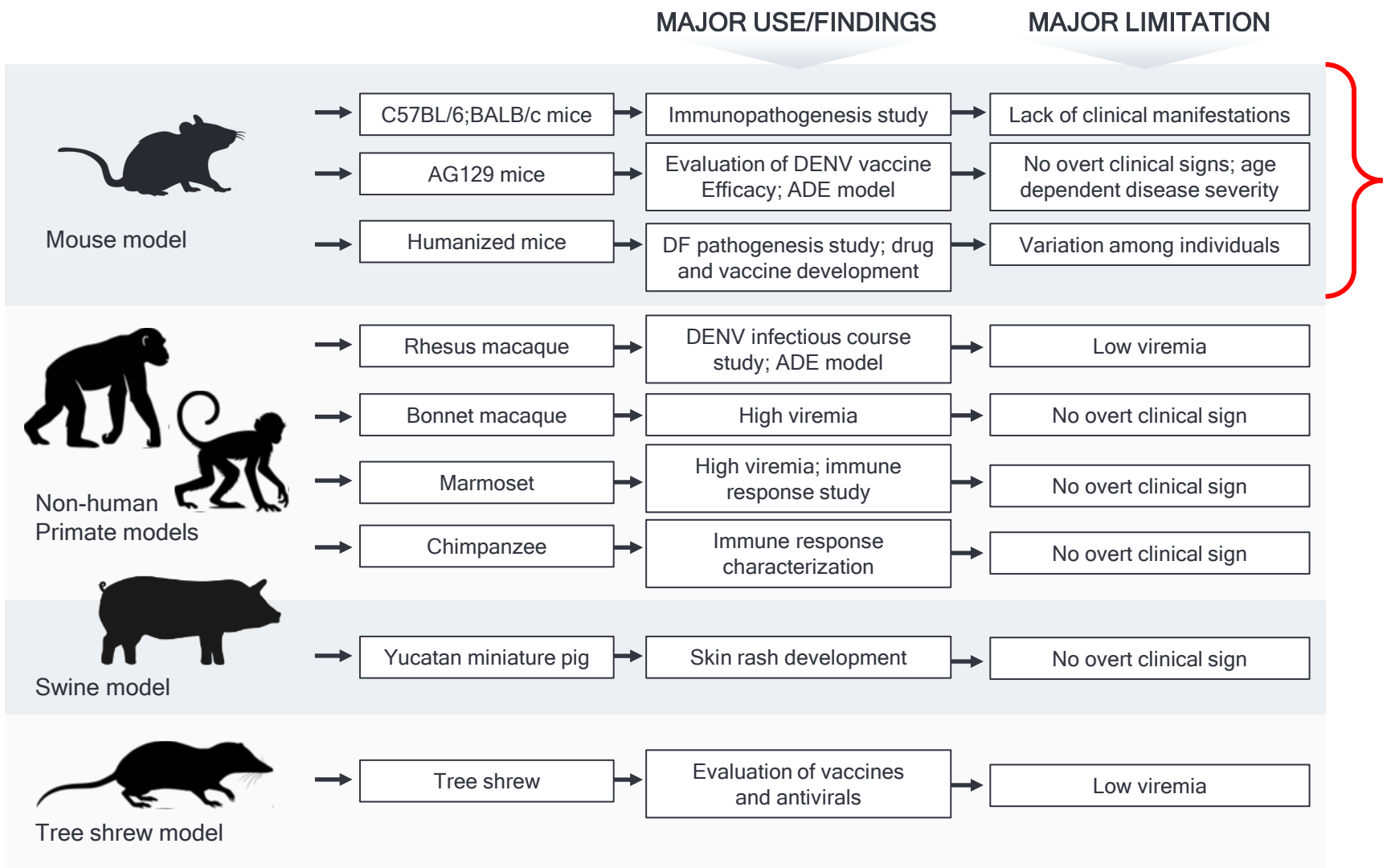
In Vivo Preclinical Efficacy Models

- *In vivo* preclinical models commonly used for *orthoflavivirus* antiviral drug development, along with exemplary references (non-exhaustive), are listed in several tables in the Supplemental Information section of this presentation.
- Most are lethal challenge models that allow testing of antiviral agents.
  - Disease models that fully replicate human disease have not been described for flaviviruses in any species.
  - Aspects of disease may be modeled in specific settings (e.g., infection of pregnant mice with ZIKV).
- Two rodent strains, **BALB/c** and **AG129**, allow *in vivo* efficacy studies on most currently circulating human-pathogenic flaviviruses.
- These strains can be infected with a variety of viral strains ranging from commonly used laboratory strains to clinical isolates.
  - Sometimes adaptation of the viral inoculum to the selected host may be required.
- While non-human primate models do not recapitulate the course of human disease, they are still valuable for translating preclinical data to humans.

Example of the DENV in Animal Efficacy Models



The animal model overview section that follows focuses on murine models for evaluating the efficacy of direct-acting antivirals with consideration to:

- Ease of evaluation
- Cost
- Historical data

Adapted from Kayesh, MEH, *et al*. *Arc Virol*. 2022.[72]

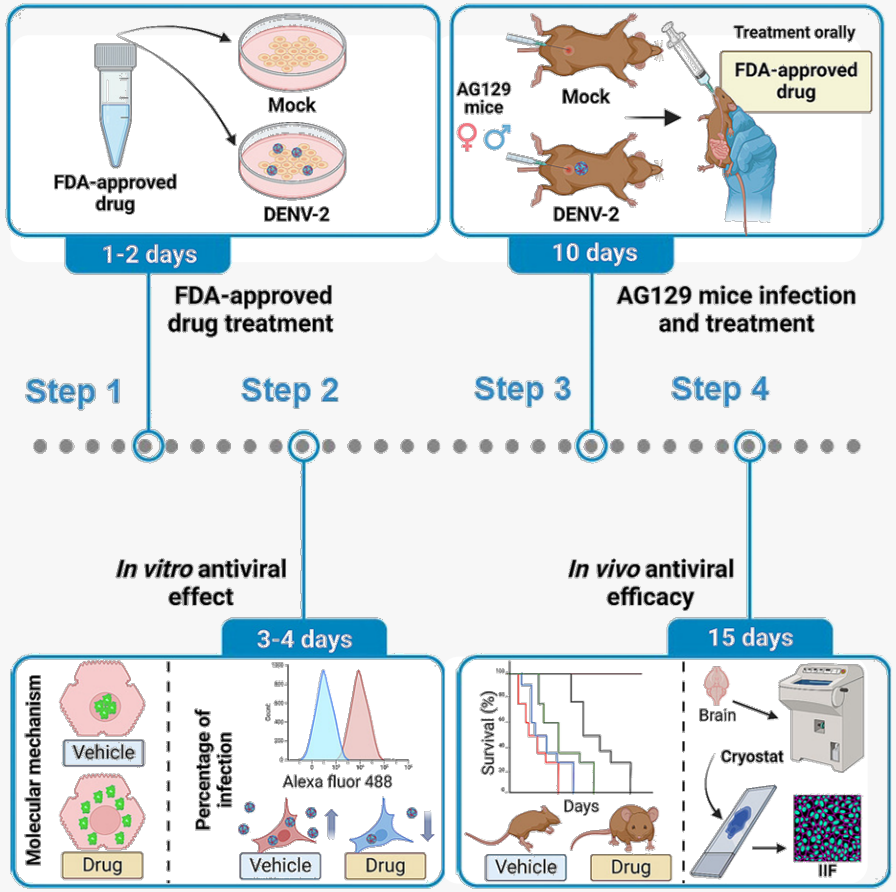
Guiding Principles for *In Vivo* Model Development

Model Selection Criteria:

- Disease Relevance: No model can effectively reproduce disease pathology; therefore, choose models that recapitulate human pathophysiology as close as possible.[73]
- Immune Competence: Balance between susceptibility and immune response similarity to humans
- Route of Infection: Mimic natural transmission (mosquito bite vs. injection)
- Outcome Measures: Define clear, quantifiable endpoints (viremia, survival, neuro-invasion)[74]

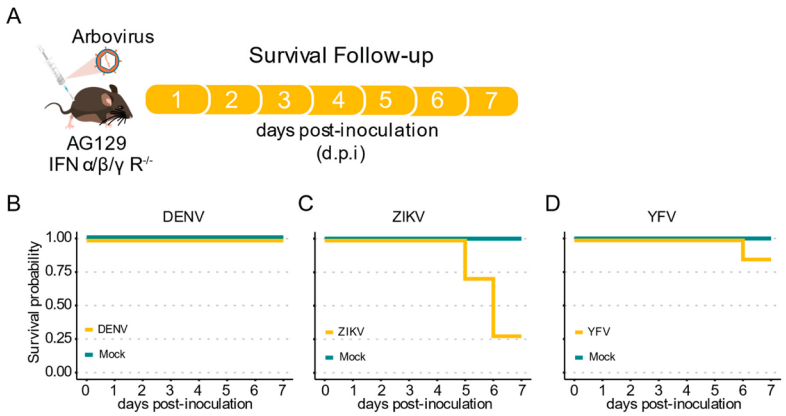
Experimental Design Considerations:

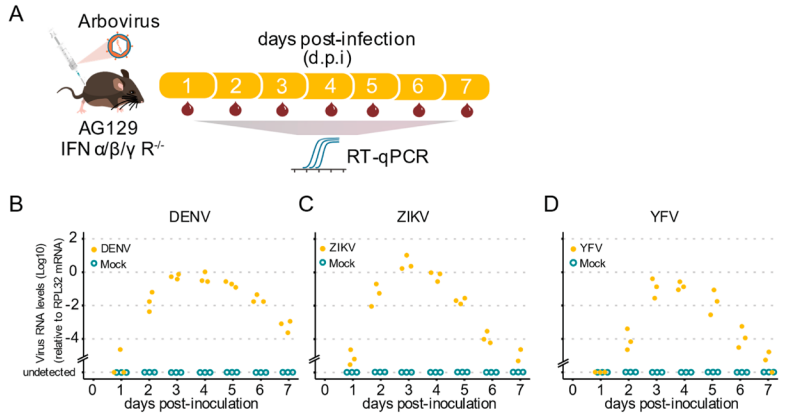
- Dose-Response Studies:
  - Establish minimum effective dose and therapeutic window
  - Test multiple dosing regimens (prophylactic, therapeutic, post-exposure)
  - Include vehicle and positive control groups[75]
- Pharmacokinetic/Pharmacodynamic (PK/PD) Analysis:[76]
  - Correlate drug exposure with antiviral efficacy[77]
  - Assess tissue distribution, especially for neurotropic viruses
  - Monitor for drug-drug interactions with standard of care



Source: Palacios-Rápalo SN, *et al.* *STAR Protoc*. 2024.[78]

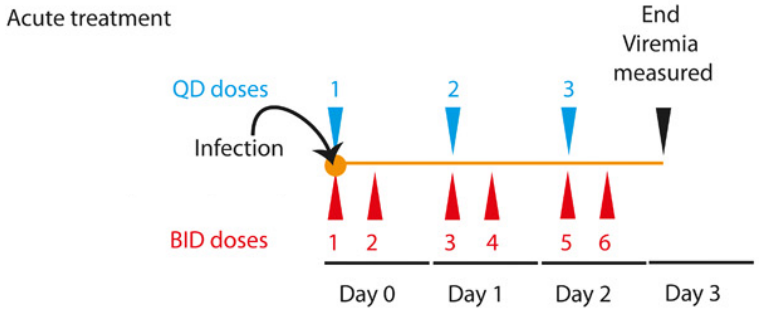
Additional Details for Animal Efficacy Models

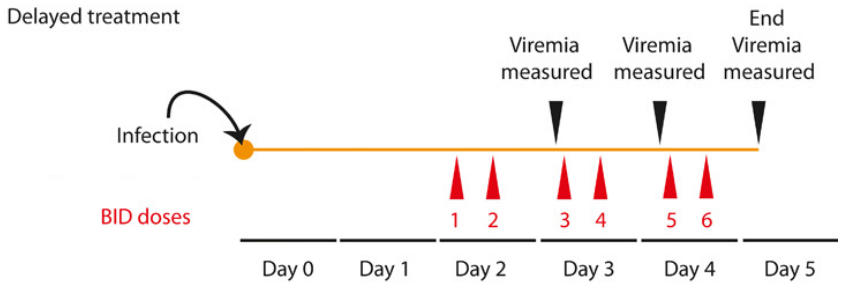




- Exemplary mortality and viremia time course plots after infection with DENV, ZKV and WNV.

Source: Baldon LVR, *et al.*, *Pathogens.*, 2022.[79]





Source: Moquin SA, *et al.* *Sci Transl Med*. 2021.[80]

- Evaluation of antiviral activity *in vivo* can be done by post-prophylaxis treatment (i.e., first treatment together or shortly after infection), or therapeutic treatment (i.e., treatment after viremia onset), which is desirable for translatability toward the treatment indication in humans.

Diagnostics for *Orthoflavivirus*

Guiding Principles for *Orthoflavivirus* Diagnostics

Temporal Considerations

- Acute phase testing (0-7 days) should prioritize direct detection methods including RT-PCR, NS1 antigen detection, or combination tests.[81] The viremic phase typically lasts up to 10 days from symptom onset, during which molecular methods achieve optimal sensitivity.[82]
- Convalescent phase testing (>7 days) relies primarily on serological methods, with IgM detection becoming increasingly reliable as the immune response develops. Paired sera testing at 10-14 days intervals enables confirmation of seroconversion and differentiation between primary and secondary infections.[83]

Sample Selection and Handling

- Serum or plasma represents the preferred specimen type for most diagnostic approaches.[84,85] Whole blood can be used for certain point-of-care tests, while urine samples may extend the detection window for specific viruses like Zika. Cerebrospinal fluid (CSF) should be collected for suspected neurological complications.[86]

Biosafety Requirements

- Most orthoflavivirus diagnostic procedures require Biosafety Level 2 (BSL-2) containment.[87] However, virus isolation and PRNT procedures may require BSL-3 facilities, particularly for handling infectious viruses.[88] Personal protective equipment, biosafety cabinets, and appropriate decontamination procedures are essential components of safe testing protocols.

Quality Assurance and Validation

- Diagnostic tests should undergo independent, comprehensive assessment of quality, safety, and performance before implementation. External Quality Assessment programs and standardized protocols with appropriate controls help ensure reliable results.

Guiding Principles for *Orthoflavivirus* Diagnostics (cont'd)

Tiered Diagnostic Approach

- Primary level: Point-of-care rapid tests for initial screening[89,90]
- Secondary level: RT-PCR and ELISA-based confirmation[91]
- Tertiary level: PRNT and specialized testing for complex cases

Quality Management

- Standardized protocols with appropriate controls
- Staff training programs for test performance and interpretation
- External quality assessment participation
- Equipment maintenance and calibration procedures

Contextual Adaptation

- Local epidemiology consideration for test selection
- Resource availability assessment for sustainable implementation
- Cross-reactivity patterns evaluation based on co-circulating viruses[92]
- Vaccination program impact on serological interpretation

Data Integration

- Laboratory information systems for result tracking
- Surveillance network connectivity for outbreak detection
- Clinical correlation protocols for result interpretation
- Public health reporting mechanisms for disease control

Diagnostic Armamentarium for Orthoflaviviruses:

Antigen- and Serological-Based Methods

Point-of-Care and Rapid Testing

- Lateral flow assays enable rapid diagnosis without sophisticated laboratory infrastructure, meeting ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Robust and rapid, Equipment-free, Deliverable[93.] Combination tests that simultaneously detect NS1 antigen and IgM antibodies enhance diagnostic accuracy across different phases of infection.[94,95]

Antigen Detection Methods

- Non-structural protein 1 (NS1) antigen detection serves as a critical diagnostic tool, particularly for dengue virus. NS1 is secreted from infected cells within hours of infection and remains detectable for 1-18 days post-symptom onset.[96,97]
- Enzyme-linked immunosorbent assays (ELISA) provide quantitative NS1 detection with high sensitivity, while lateral flow rapid diagnostic tests (RDTs) offer point-of-care capabilities with results available in 15-30 minutes.[98,99]
- Recent innovations include gold nanorod-based detection systems that achieve detection limits as low as 100 PFU/mL for orthoflaviviruses in both human serum and mosquito samples.[100]

Serological Testing Methods

- IgM and IgG antibody detection through ELISA represents the most widely used serological approach. IgM antibodies typically appear 3-8 days after symptom onset and persist for 30-90 days, making them suitable for acute infection diagnosis.[101] However, cross-reactivity among flaviviruses presents significant challenges, particularly in endemic regions.[102]

Plaque Reduction Neutralization Tests (PRNT)

- Constitute the gold standard for serological diagnosis due to their high specificity and minimal cross-reactivity between different flaviviruses. The PRNT50 value, representing the serum dilution that reduces plaque formation by 50%, provides quantitative measurement of neutralizing antibodies.

Diagnostic Armamentarium for Orthoflaviviruses:

Molecular Detection Methods

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

- Represents the gold standard for acute-phase detection of orthoflaviviruses. This technique offers high specificity and sensitivity, with commercial RT-PCR tests achieving 83.9-90.3% sensitivity and 100% specificity.[103] Real-time quantitative RT-PCR (qRT-PCR) provides additional advantages through rapid results and quantitative viral load determination.

Pan-flavivirus Assays

- Enable broad-spectrum detection of multiple orthoflavivirus species simultaneously. These multiplex approaches use conserved genome regions to detect various flaviviruses, with some systems capable of identifying mixed infections within a single assay. Advanced molecular platforms include multiplex molecular diagnostic assays that provide sample-in-answer-out results in under one hour, making them suitable for near point-of-care testing.

Isothermal Amplification Techniques

- Such as reverse transcription loop-mediated isothermal amplification (RT-LAMP) and reverse transcription insulated isothermal PCR offer field-deployable alternatives that require only basic heating equipment rather than expensive thermal cyclers.[104]

▶ *A listing of currently available diagnostics aligned with orthoflavivirus antiviral drug development needs are provided in the Supplemental Information section of this presentation.*

Supplemental Information

INTREPID ALLIANCE

Addressing the Unmet Need for *Orthoflavivirus* Direct-Acting Antivirals

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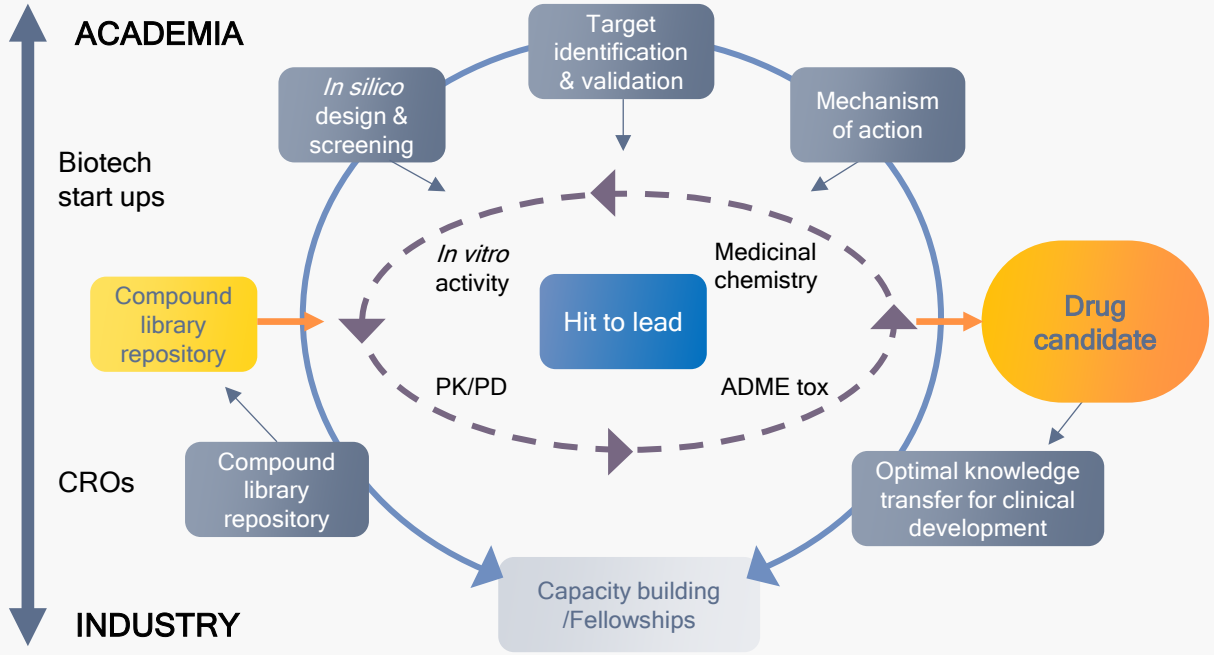
Addressing the Unmet Need

Reasons to Believe

- Decades of research against orthoflaviviruses has identified highly conserved proteins critical for viral replication:
  - Enzymatic targets: RNA-dependent RNA polymerase, protease, methyltransferase
  - Non-structural proteins: e.g., NS4B
  - Structure proteins: Envelope, pre-M
- Broad-spectrum orthoflavivirus compounds have been identified, with pan-serotype dengue compounds successfully entered clinical trials.
- Private-public partnerships can accelerate direct-acting antiviral R&D, including cross-validating assays, animal models, and targets.



Adapted from Hernandez-Morales I, Van Loock M. *Adv Exp Med Biol*. 2018.[105]

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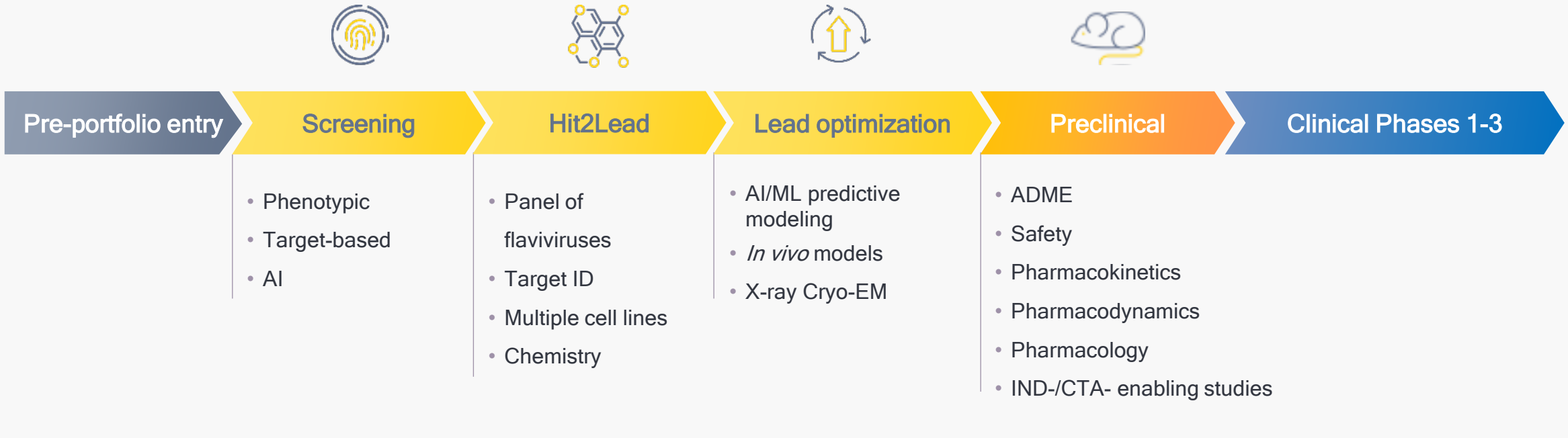
Simplified Hit-to-Clinical Candidate Roadmap

- Start drug discovery by implementing a HTS campaign using either:
  - Cell-based assays using mammalian cell-lines, with multiplex read-outs for broad-spectrum assessment as recent innovations.[106]
  - Target-based biochemical assays, supplemented with AI/ML to accelerate the turnaround time.
- Assess broad-spectrum activity against clinical isolates early in the process.
- Hits go through multiple med-chem rounds to improve potency and drug-like properties (e.g., ADME, safety).
- Evaluate antiviral activity in relevant animal model to assess PK/PD and human dose predictions.



Orthoflavivirus Epidemiology

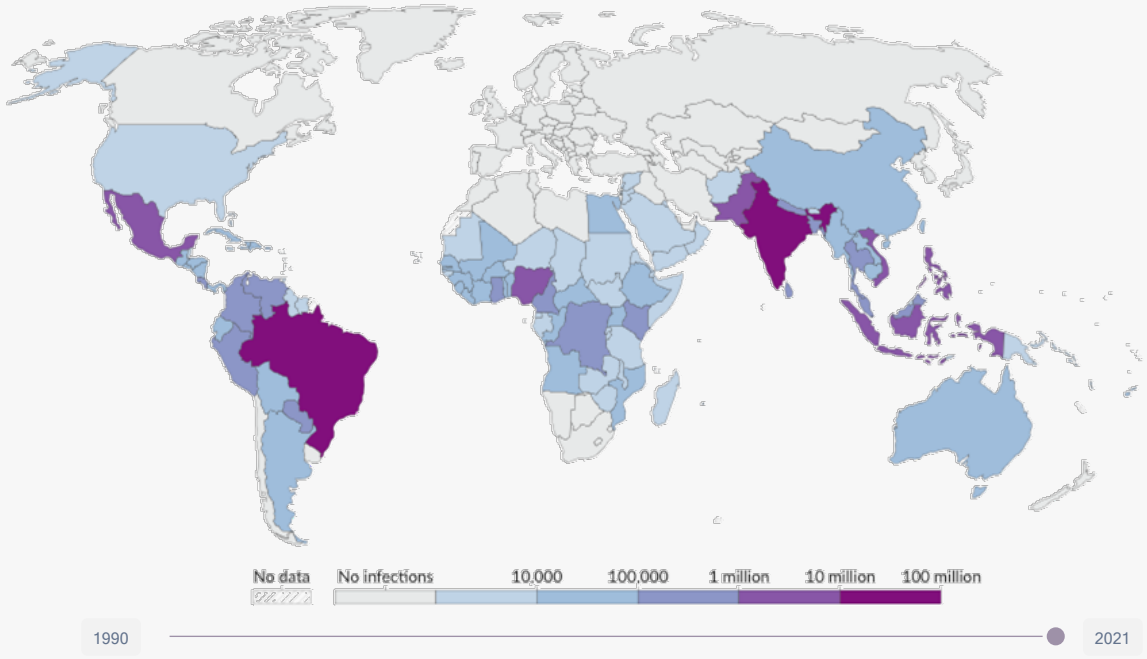
Annual Infections:

Dengue Virus (DENV)

- WHO estimates approximately 390 million dengue virus infections occur per year globally, with 96 million manifesting clinically.[107,108] Many infections remain undiagnosed, and/or are underreported.
- In the pathogenesis of the disease, cases could be asymptomatic, or manifest as dengue fever, with the most severe of cases evolving to dengue hemorrhagic fever / dengue shock syndrome.
- About 2.5 billion people live in dengue-endemic areas.[109]
- From January to July 2025, over 4 million cases and over 3,000 deaths were reported to WHO from 97 countries.[110]

Dengue fever infections, 2021

Estimated annual number of new symptomatic dengue infections.



Adapted from Dattani S, *et al.* Neglected Tropical Diseases. 2024. OurWorldinData.org. [111]

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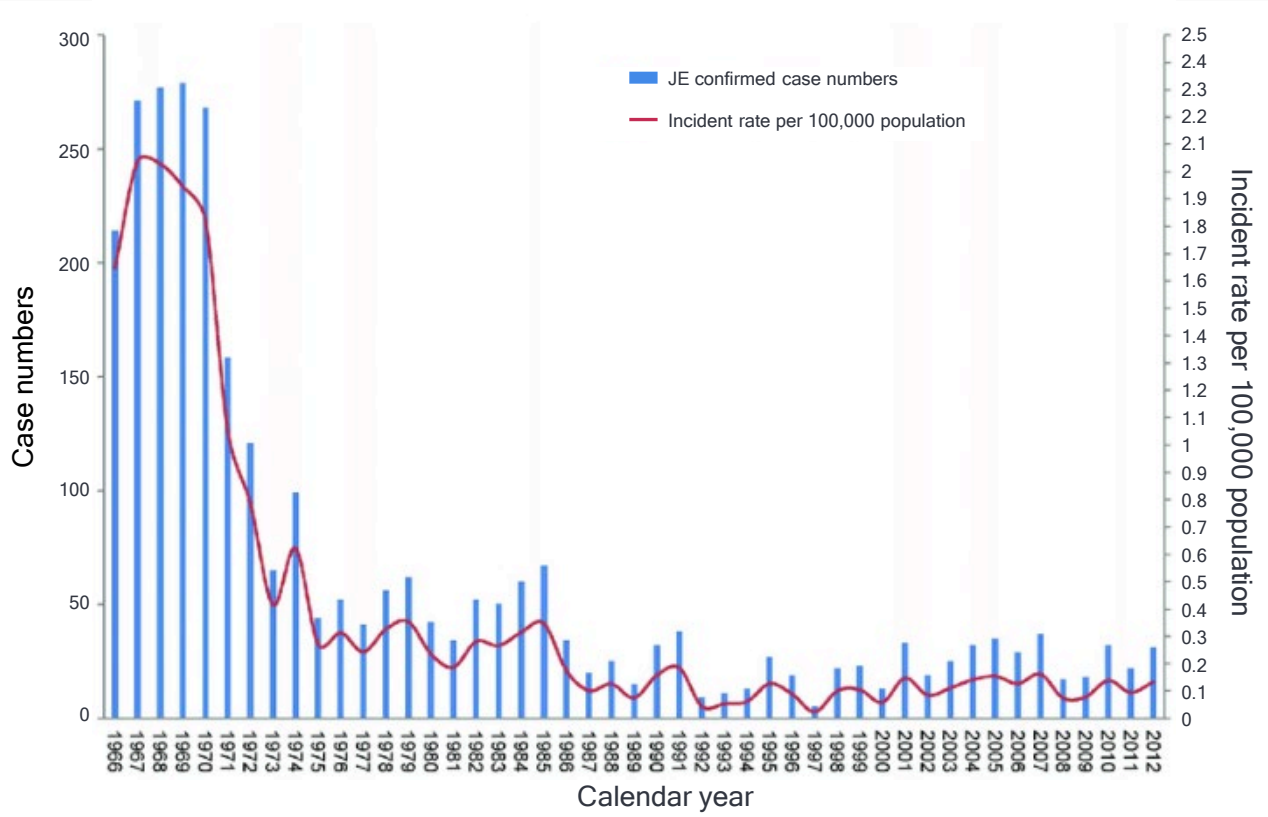
Annual Infections:

Japanese encephalitis virus (JEV)

- Current estimates suggest approximately 56,847 clinical cases of JEV in 2019, down from 81,258 cases in 2010.[112]
- WHO literature review estimates about 100,000 clinical cases of JEV globally each year.[113]
- Over 3 billion people live in JEV-endemic countries.[114]



The cases numbers of Japanese encephalitis and incidence rate in Taiwan during 1966-2012.

Source: Hsu LC, *et al.* *PLoS Negl Trop Dis*. 2014.[115]

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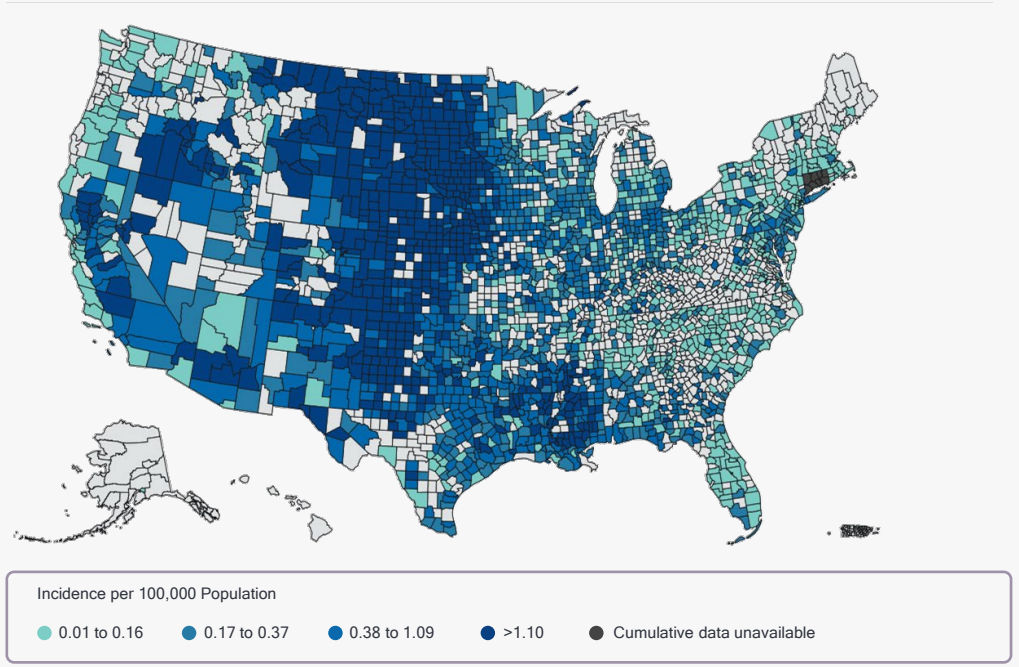
Annual Infections:

West Nile virus (WNV)

- In the United States alone, 51,607 WNV cases were recorded through October 2019.[116]
- In 2022, the US reported 1,132 human cases.[117]
- Europe reports approximately 460 cases per year on average, with 5,514 total cases from 2012-2023.[118]

West Nile virus human neuroinvasive disease average annual incidence per 100,000 population by county of residence, 1999-2024



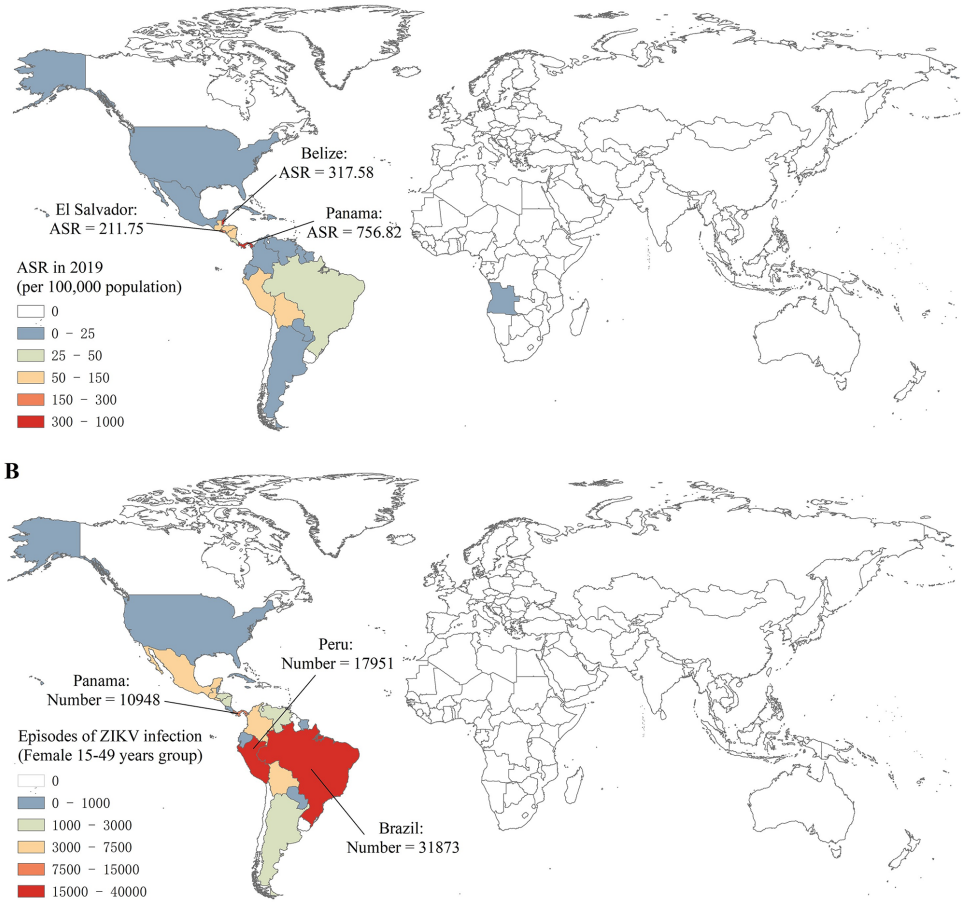
Source: U.S. Centers for Disease Control and Prevention. West Nile Virus: Historic Data (1999-2024). 16 January 2026.[119]

Annual Infections:

Yellow Fever (YFV) & Zika (ZIKV) Viruses

- **YFV**
  - A global burden analysis estimates 67,000-173,000 severe yellow-fever infections and 31,000-82,000 deaths per year in Africa and the Americas combined, with about 90% of this burden in Africa.[120,121]
  - An estimated total of 900 million people, living in 47 countries, are high risk of YF infections.[122]

- **ZIKV**
  - In 2021, there were an estimated 169,734 ZIKV cases globally.[123]
  - Peak year for ZIKV cases was 2015 with 1,519,501 cases, declining to 268,643 in 2019.[124]
  - Cases continue to be reported in 14-15 countries annually, with the Americas remaining the highest burden region.[125]



Source: Guo Z, *et al*. *PLoS Negl Trop Dis*. 2022.[126]

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In Vitro Antiviral Assays

In Vitro: Cells, Viruses, & Assays*

Key Cell Lines

Phylum	Species	Cell Line & Tissue Source	Common Use Case	Notes
Arthropod	Aedes albopictus (mosquito)	**C6/63** Larval tissue	Highly permissive cell line that is the gold standard for flaviviruses cultivation in insects	Lack of a functional RNAi response, insect specific glycosylation of viral proteins
	Culex quinquefasciatus (mosquito)	**Hsu** Larval tissue	Permissive cell line of *Culex* origin for viruses	Functional RNAi response allows insect immune responses, insect specific glycosylation of viral proteins
	Ixodidae ricinus (tick)	**IRE/CTVM19** Larval tissue	Permissive cell line of tick origin for tick-borne viruses	One of several tick derived cell lines, arthropod specific glycosylation of viral proteins
	Chlorocebus sabaeus (African green monkey)	**Vero** Kidney epithelium	Highly permissive cell line for flavivirus replication. Used in virus production and plaque assay	No interferon production, remain responsive to exogenous interferon
Chordata	Macaca mulatta (Rhesus monkey)	**LLC-MK2** Kidney epithelium	Permissive cell line for flavivirus replication. Used in virus production and plaque assay	Functional interferon response in response to infection
	Human	**HeLa** Cervical epithelium	Permissive cell line for flavivirus replication. Used in virus production and plaque assay	Common laboratory cell line
	Human	**Huh7** Liver hepatoma	Permissive cell line for flavivirus replication. Used in virus production and plaque assay, resistance mutant selection	Liver origin cell line
	Human	**HepG2** Liver hepatoma	Permissive cell line for flavivirus replication. Used in virus production and plaque assay	Liver origin cell line

*Based on references as of November 2024.

In Vitro: Cells, Viruses, & Assays*

Key Virus Isolates

Virus	Isolate	Source	Sequence Reference	Notes	References
YFV	17D	ATCC VR-1506	X03700.1	Vaccine strain	Stokes A, *et al.* 1997.
	176-Asibi	Academic	AY640589.1	WT virus	Whitman L, 1937.
DENV1	Hawaii	ATCC VR-1856	KM204119.1	Isolated from pooled patient sera in Hawaii, USA	Sabin AB, *et al.* 1945.
	TH-Sman	ATCC VR-1586	JQ922547.1	Tissue culture adapted	Hammon WM, *et al.* 1960.
DENV2	New Guinea C	ATCC VR-1584	KM204118	Isolated in New Guinea from febrile patient sera	Sabin AB, 1952.
DENV3	H87	ATCC VR-1256_FD	KU050695.1	Isolated in Philippines and grown in sucking mouse	Hammon WM, *et al.* 1960.
DENV4	H241	ATCC VR-1490	AY947539.1	Tissue culture adapted	Hammon WM, *et al.* 1960.
WNV	B956	ATCC VR-1267	AY532665.1	Uganda isolate	Smithburn KC, *et al.* 1940.
	385-99	ATCC VR-1507	AY842931.3	New York isolate	Gould EA, *et al.* 1990.
ZIKA	MR766	ATCC VR-84	MW143022.1	Uganda strain from sentinel monkey	Dick GWA, *et al.* 1952.
	PRVABC59	ATCC VR-1843	MK713748.1	CDC isolate from Puerto Rico	Lanciotti RS, *et al.* 2016.
JEV	Nakayama	ATCC VR-74	EF571853.1	Clinical isolate from fatal pediatric infection in Japan	Park JY, *et al.* 2024.
SLE	MSI-7	EVAG 001v-EVA128	DQ359217.1	Isolated from Culex mosquito in Texas, USA	Grard G, *et al.* 2007.
TBE	Hypr	EVAG 008v-EVA285	U39292.1	Isolated from Czech Republic	Traavik T, 1978.
	Ljublijana	EVAG 007V-03086	JQ654701.1	Isolated from Slovenia	Golovljova I, *et al.* 2004.

*Based on references as of November 2024.

Virus-specific Assay Overview (1)*

Virus	Assay	Paper Title	Reference
YFV	Plaque Assay	In vitro potency assay for yellow fever vaccines: comparison of three Vero cell lines sources	Fournier-Caruana J, *et al.* 2000.
	RT-PCR Assay	Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR	Drosten C, *et al.* 2002.
	Replicon System Assay	Construction and applications of yellow fever virus replicons.	Jones CT, *et al.* 2005.
	Replication Based HTS Screen	Generation of a reporter yellow fever virus for high throughput antiviral assays.	Sanchez-Velazquez R, *et al.* 2020.
	Resistance Selection	A Novel Benzodiazepine Compound Inhibits Yellow Fever Virus Infection by Specifically Targeting NS4B Protein	Guo F, *et al.* 2016.
DENV	Plaque Assay	Dengue virus: isolation, propagation, quantification, and storage.	Medina F, *et al.* 2012.
	RT-PCR Assay	Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus.	Shu PY, *et al.* 2003.
	Replicon System Assay	Development of Dengue virus type 2 replicons capable of prolonged expression in host cells	Pang X, *et al.* 2001.
	Replication Based HTS Screen	Development and characterization of a stable luciferase dengue virus for high-throughput screening	Zou G, *et al.* 2011.
	Resistance Selection	Novel dengue virus-specific NS2B/NS3 protease inhibitor, BP2109, discovered by a high-throughput screening assay	Yang CC, *et al.* 2011.
ZIKV	Plaque Assay	Production, Titration and Imaging of Zika Virus in Mammalian Cells	Freppel W, *et al.* 2018.
	RT-PCR Assay	Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes	Faye O, *et al.* 2013.
	Replicon System Assay	Zika Virus Replicons for Drug Discovery	Xie X, *et al.* 2016.
	Replication Based HTS Screen	Development of a replicon cell line-based high throughput antiviral assay for screening inhibitors of Zika virus	Li JQ, *et al.* 2018.
	Resistance Selection	The Compound SBI-0090799 Inhibits Zika Virus Infection by Blocking De Novo Formation of the Membranous Replication Compartment.	Riva L, *et al.* 2021.
WNV	Plaque Assay	Quantification of West Nile Virus by Plaque-Forming Assay.	Neupane B, *et al.* 2023.
	RT-PCR Assay	Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus	Shu PY, *et al.* 2003.
	Replicon System Assay	An infectious clone of the West Nile flavivirus	Yamshchikov VF, *et al.* 2001.
	Replication Based HTS Screen	Potential high-throughput assay for screening inhibitors of West Nile virus replication	Lo MK, *et al.* 2003.
	Resistance Selection	A single-amino acid substitution in West Nile virus 2K peptide between NS4A and NS4B confers resistance to lycorine, a flavivirus inhibitor.	Zou G, *et al.* 2009.

*Based on references as of November 2024.

Virus-specific Assay Overview (2)*

Virus	Assay	Paper Title	Reference
JEV	Plaque Assay	Comparison of plaque reduction and focus reduction neutralization tests for the measurement of neutralizing antibody titers against Japanese encephalitis virus	Park Y, *et al.* 2022.
	RT-PCR Assay	TaqMan reverse transcription polymerase chain reaction for the detection of Japanese encephalitis virus	Yang DK, *et al.* 2004.
	Replicon System Assay	Engineering the Japanese encephalitis virus RNA genome for the expression of foreign genes of various sizes: implications for packaging capacity and RNA replication efficiency	Yun SI, *et al.* 2007.
	Replication Based HTS Screen	Generation and characterization of Japanese encephalitis virus expressing GFP reporter gene for high throughput drug screening	Zhang ZR, *et al.* 2020.
	Resistance Selection	Screening of FDA-Approved Drugs for Inhibitors of Japanese Encephalitis Virus Infection	Wang S, *et al.* 2017.
SLEV	Plaque Assay	A focus assay method for Japanese encephalitis virus using complement and anti-virus serum	Kimura-Kuroda J, *et al.* 1985.
	RT-PCR Assay	Detection of flaviviruses by reverse-transcriptase polymerase chain reaction	Eldadah ZA, *et al.* 1991.
	Replicon System Assay	--	
	Replication Based HTS Screen	--	
	Resistance Selection	Mutagen resistance and mutation restriction of St. Louis encephalitis virus	Griesemer SB, *et al.* 2017.
TBEV	Plaque Assay	Detection of tick-borne encephalitis virus by sample transfer, plaque assay and strand-specific reverse transcriptase polymerase chain reaction: what do we detect?	Kreil TR, *et al.* 1997.
	RT-PCR Assay	Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick-borne encephalitis virus (TBEV) RNA	Schwaiger M, *et al.* 2003.
	Replicon System Assay	Sub-genomic replicons of Tick-borne encephalitis virus	Hayasaka D, *et al.* 2004.
	Replication Based HTS Screen	Development and characterization of recombinant tick-borne encephalitis virus expressing mCherry reporter protein: A new tool for high-throughput screening of antiviral compounds, and neutralizing antibody assays	Haviernik J, *et al.* 2021.
	Resistance Selection	--	

*Based on references as of November 2024.

In Vivo Preclinical Efficacy Models

In Vivo Preclinical Efficacy Models: DENV, JEF, TBEV

Virus	Animal/ Strain	Animal Age	Common Virus Strain	Inoculation Route	Inoculum Size	Type (Duration)	Fidelity to Human Disease	Model Use (Endpoints)	Exemplary Reference
DENV	Mouse BALB/c	6-12 wk	DENV2 NGC	intracranial	10e2 PFU	Lethal challenge (10-12 days)	Low (Medium for DENV encephalitis)	Modeling of neurological disease (paralysis, survival)	Gualano O, et al. 1998.
	Mouse AG129/ A129	4-12 wk	DENV3 C0360/84	i.p.	10e7 PFU	Lethal challenge (5 days)	Low	Antiviral drug development (survival, BW, scoring, VL)	Sarathy VV, et al. 2015.
			DENV2 D2S10		10e7 PFU	Lethal challenge (12 days)			Orozco S, et al. 2012.
			DENV3 D83-144		10e7 PFU	Non-lethal disease model (12 days)	Medium (Vascular leakage, no brain involvement)	Disease modeling	Sarathy VV, et al. 2018.
	Indian rhesus macaque	Adult	DENV2 16681	s.c.	10e3 PFU	Acute-resolving infection (14-21 days)	Low	Antiviral drug development (VL); PK/PD translation to human	Goethals O, et al. 2023.
JEV	Mouse BALB/c	6-12 wk	GP78	s.c.	10e5 PFU	Lethal challenge (9-12 days)	Low	Antiviral drug development (survival, BW, scoring, VL)	Thounaojam MC, et al. 2014.
			SA14		10e7 PFU	Lethal challenge (9-15 days)			Huang L, et al. 2021.
TBEV	Mouse BALB/c	6-12 wk	Hypr; Neudoerfl	s.c.	10e2 PFU	Lethal challenge (12-24 days)	Low	Analysis of immune responses (survival)	Ruzek D, et al. 2009.
	Mouse C57BL/6								Ruzek D, et al. 2011.

In Vivo Preclinical Efficacy Models: WNV, YFV, ZIKV

Virus	Animal/ Strain	Animal Age	Common Virus Strain	Inoculation Route	Inoculum Size	Type (Duration)	Fidelity to Human Disease	Model Use (Endpoints)	Exemplary Reference
WNV	Mouse BALB/c	6-12 wk	NY99; NY2000	s.c.	10e4 PFU	Lethal challenge (12 days)	Low	Antiviral drug development (survival, BW, scoring, VL)	Srivastava R, et al. 2015.
	Mouse C57BL/6				10e2-10e6 PFU	Acute-resolving infection		Analysis of immune response	Diamond MS, et al. 2003.
YFV	Mouse BALB/c	5-8 wk	17D	intracranial	10e3-10e5 PFU	Lethal challenge (9-12 days)	Low	Antiviral drug development (survival, BW, paralysis, VL)	Barrett ADT, et al. 1986.
	Mouse AG129/ A129	4-12 wk	Clinical isolates	i.p.	10e3 PFU	Lethal challenge (7-12 days)			De Freitas CS, et al. 2019.
			17D		10e4 PFU	Disease model (7-20 days)	Medium (liver pathology)	Disease modelling	Lemos FO, et al. 2020.
	Syrian Hamster	Adult	Jimenez	i.p.	10e2 PFU	Lethal challenge (12-21 days)	Low	Antiviral drug development (survival, BW, paralysis, VL)	Julander JG, et al. 2022.
	Indian rhesus macaque	Adult	Dakar HD 1297	s.c.	1.5x10e3 PFU	Lethal challenge (5-10 days)	Medium	Antiviral drug development (survival, VL)	Ricciardi MJ, et al. 2023.
ZIKV	Mouse BALB/c	1 day	Clinical isolates	i.p.	10e5 PFU	Disease model (14-28 days)	Medium (neurological symptoms)	Modelling of neurological disease (paralysis, survival)	Rengifo AC, et al. 2023.
	Mouse AG129/ A129	4-12 wk	MR766		10e2 PFU	Lethal challenge (6-10 days)	Low	Antiviral drug development (survival, BW, scoring, VL)	Miao J, et al. 2022.
			PRVABC59		10e5 PFU	Lethal challenge (6-8 days)			Collette NM, et al. 2020.
	Indian rhesus macaque	adult		i.m.	10e5-10e6 PFU	Disease model (14-28 days)	Medium	Antiviral drug development (VL in blood and CSF); PK/PD; treatment timing	Lim SY, et al. 2020.

Molecular Diagnostics for Orthoflaviviruses

Virus	Singleplex PCR	Multiplex PCR					
		Ceretest	Jant Tropical panel	NeoDX Tropical panel	3B Bio Tropical panel	Roche CHIKV/DENV COBAS	Univ. of Florida
CHIKV		+	+	-	-	+	-
DENV	CDC DENV 1-4 PCR (standard), Roche COBAS LightMix assay; CERTEST assay	+	-	+	+	+	+ (I, II, III, IV)
JEV	Limited data; mostly serological diagnosis	-	-	-	-	-	-
MYV	-	+	+	-	-	-	-
POWV	-	-	-	-	-	-	-
SLE	-	-	-	-	-	-	+
TBE	CDC Arboviral testing service; commercial assays seem to lack sensitivity	-	-	-	-	-	+ (RS-SE & CE-E groups)
WNV	Roche Cobas system assay	+	+	+	+	-	+
YFV	RealStar Assay from Altona	+	+	-	-	-	-
ZIKV	Roche Cobas system assay	+	+	+	+	-	-

Collaborations & Partnerships

INTREPID ALLIANCE

Building Networks Through Collaborations & Partnerships

- Tackling orthoflaviviruses will require an integrated approach of antivirals, vaccines, diagnostic, anti-repellents, and community engagement.
- Public-private partnerships, combining big pharma, CROs, biotechs, and academia can be instrumental to achieve this goal.
- Preclinical CROs may help with access to viruses, cells, or other reagents.
  - May also serve as collaborative or fee-for-service partner.
- Clinical CROs may help with strategies for the IND-enabling (or similar) workstream.
- Academia can contribute to deepen scientific insights, high-throughput screenings, and hit to lead projects.
- Big pharma can support late optimization and required preclinical, (non)-GLP toxicity studies.
- A clinical trial network can assist with first-in-human studies, whereas dedicated clinical trial sites in endemic areas can contribute to establishing clinical proof of concept for antivirals against orthoflaviviruses.

▶ *The next slide includes an example listing of CROs and academic institutions with partnership models aligned with orthoflavivirus antiviral drug development needs.*

Examples of Contract Research Organizations & Academic Institutions with Partnership Models

Institution	Screening Capabilities	Website
A*STAR Infectious Diseases Labs	Full range, vector borne & respiratory viruses, and AMR	https://www.a-star.edu.sg/idlabs
Cerba Research (DDL/Viroclinics)	Respiratory viruses, animal models, others possible, CRO	https://cerbaresearch.com
Evotec	Mostly respiratory work and hepatitis, others possible, CRO	https://www.evotec.com
Fiocruz	Full range, public institute	https://fiocruz.br/en
KU Leuven/REGA Institute	Full range, academic	https://rega.kuleuven.be
Scripps Research/ Calibr	Screening, limited containment	https://calibr.scripps.edu
Southern Research	Full range, CRO	https://southernresearch.org
UICC/RIGA	Lots of respiratory, but others possible, academic	https://www.uicc.org/membership/institute-microbiology-virology
UTMB/GNL NEIDL/Boston University	Full range, including BL4, national laboratory	https://www.utmb.edu/gnl https://www.bu.edu/neidl

Appendix

Glossary

- **ADE**: antibody-dependent enhancement
- **ADME**: absorption, distribution, metabolism, and excretion
- **AI/ML**: artificial intelligence/machine learning
- **BHK-21**: Baby Hamster Kidney-21
- **CPE**: cytopathic effect
- **CRO**: contract research organization
- **Cryo-EM**: cryogenic electron microscopy
- **CTA**: clinical trial application
- **CV**: coefficient of variation
- **DENV**: dengue virus
- **DMSO**: dimethyl sulfoxide
- **DSF**: differential scanning fluorimetry
- **DNA**: deoxyribonucleic acid
- **ELISA**: enzyme-linked immunosorbent assay
- **E-protein**: envelope protein
- **ER**: endoplasmic reticulum
- **FFU**: focus forming unit
- **FRET**: fluorescence resonance energy transfer
- **GFP**: green fluorescent protein
- **HTS**: high-throughput screening
- **IND**: investigational new drug
- **JEV**: Japanese encephalitis virus
- **MIAOU**: Minimal Information About an Organoid and its Use
- **MOI**: multiplicity of infection

Glossary (cont'd)

- **PK**: pharmacokinetics
- **PRNT**: plaque reduction neutralization test
- **qRT-PCR**: quantitative real-time reverse transcription polymerase chain reaction
- **RdRp**: RNA-dependent RNA polymerase
- **RNA**: ribonucleic acid
- **SLEV:** St. Louis encephalitis virus
- **SPR**: surface plasmon resonance
- **Target-ID**: target identification
- **TBEV**: tick-born encephalitis virus
- **TGN**: trans-Golgi network
- **TLR3**: Toll-like receptor 3
- **WHO**: World Health Organization
- **WNV**: West Nile virus
- **YFV**: Yellow fever virus
- **ZIKV**: Zika virus

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