Gyrolab-Based *In Vitro* Immunoassay for Potency and Quality Control of Chikungunya VLP Vaccine:

A Sensitive Alternative to Animal Models

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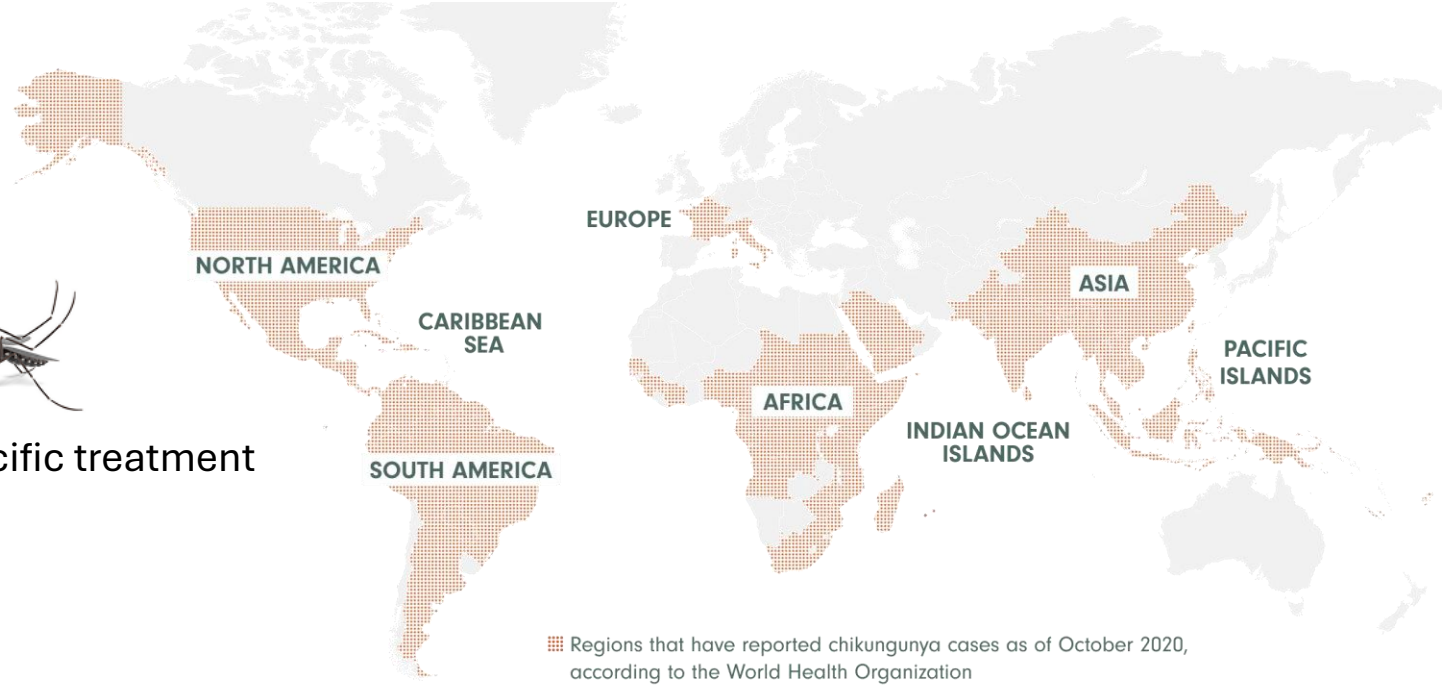
Chikungunya

Disease Overview

mosquito-borne disease

severe joint pain, fever, and headache with no specific treatment



Causative Agent

chikungunya virus (CHIKV)

Geographic Distribution

Latin America, the Caribbean, Asia, Africa, parts of Europe (popular travel destinations)

Pathogenesis

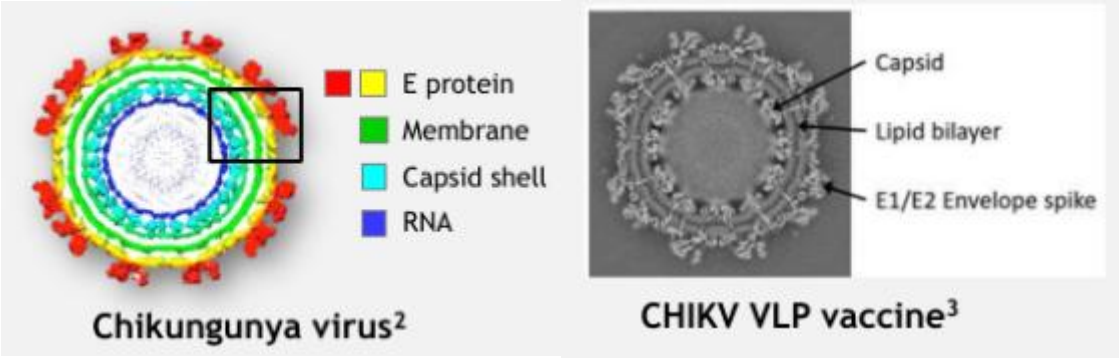
replication in dermal cells

spread to lymphatic system and bloodstream

replication on lymphatic system, spleen, peripheral joints, muscles, tendons (severe case brain and liver)

https://vimkunya.com/

Vimkunya™



Product Type

virus-like particle (VLP), recombinant, aluminium-adsorbed vaccine

Structure of CHIKV-VLP

contains NO viral genetic material, but capsid and envelope proteins E1 and E2 (Senegal strain 37997)

80 spikes of 3 × E1-E2 heterodimers

Route of Administration and Dosage

single intramuscular injection, 0.8 mL pre-filled syringe, shelf-life 36 months

Mechanism of Action

development of protective antibody response

Ownership

Bavarian Nordic acquired VIMKUNYA™ from Emergent Biosolutions in May 2023

Registration Status

registered in the USA, EU, UK

2. Adapted from Sun S, et al. ELife. 2013;2:e00435; 3. Adapted from Basore, K, et al. Cell. 2019;177(7):1725-1737

CHIKV *in vitro* Assay

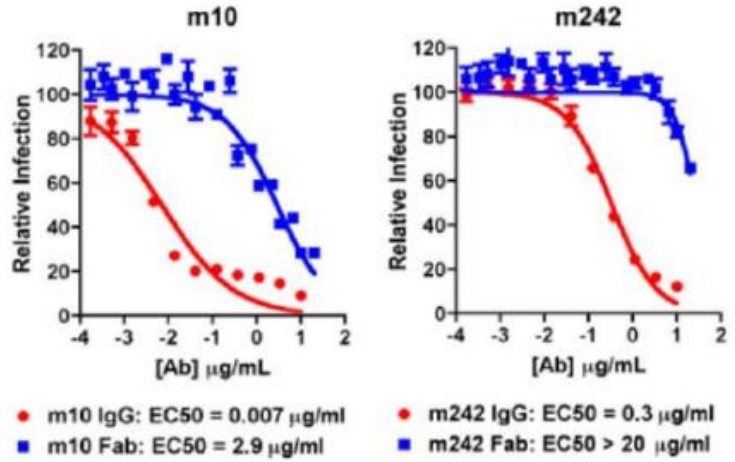
Principle

automated sandwich *in vitro* immunoassay using microfluidics in nanoliter-scale

2 neutralizing monoclonal antibodies (Sun et al. 2013)

M10-18 biotinylated capture mouse mAb

M242-5 Alexa Fluor 647 conjugated detection mAb



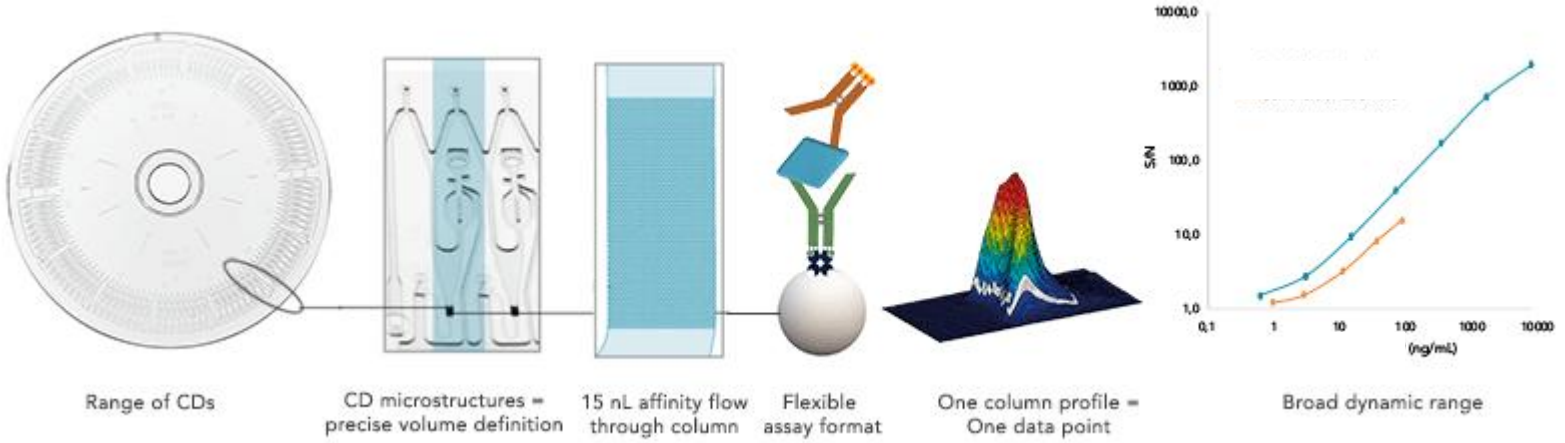
Purpose

quantification of intact epitopes relative to total protein

calculation of *in vitro* potency and confirmation of identity

release testing and stability testing by monitoring the structural integrity of CHIKV VLP

$$\textit{in vitro}\ \text{potency} = \frac{\text{immunoreactivity}}{\text{total protein concentration}}$$



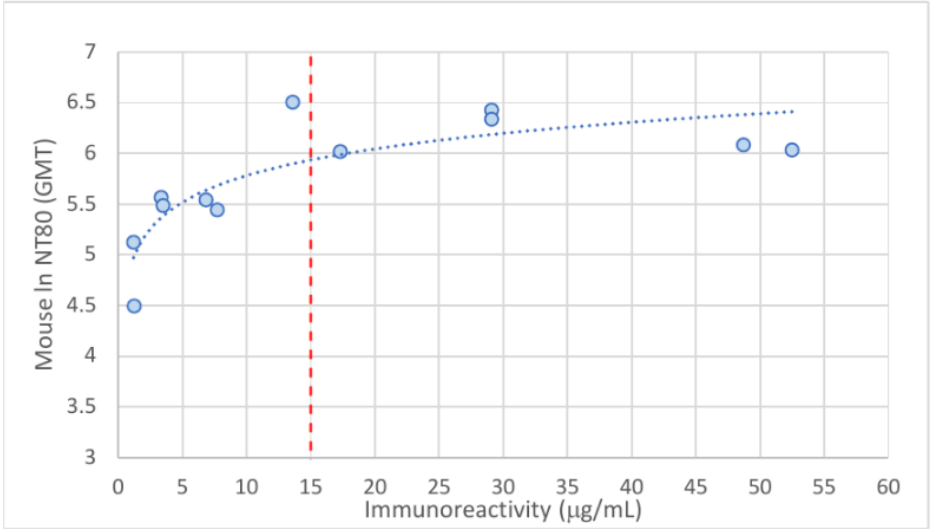
Sun S, et al. ELife. 2013;2:e00435; https://www.gyrosproteintechnologies.com/

Ability of CHIKV *in vitro* Assay to Identify Vaccine Lots of Different Potencies

Study Setup

vaccine formulated in 6 groups with decreasing total protein concentrations (2 – 56 µg/mL final VLP conc.)

immunoreactivity (µg/mL) vs neutralizing antiserum titers (*in vivo* NT80)



Conclusion

decreasing concentrations of vaccine produced correspondingly lower immunoreactivity results

immunoreactivity results corresponded well with neutralizing titers in vaccinated mice > confirming ability to detect sub-potent vaccine lots

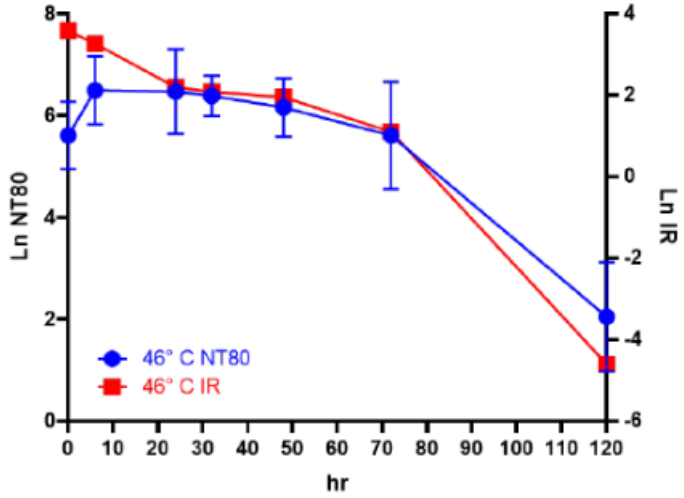
analytical sensitivity of the *in vitro* assay was greater than that of the *in vivo* mouse model

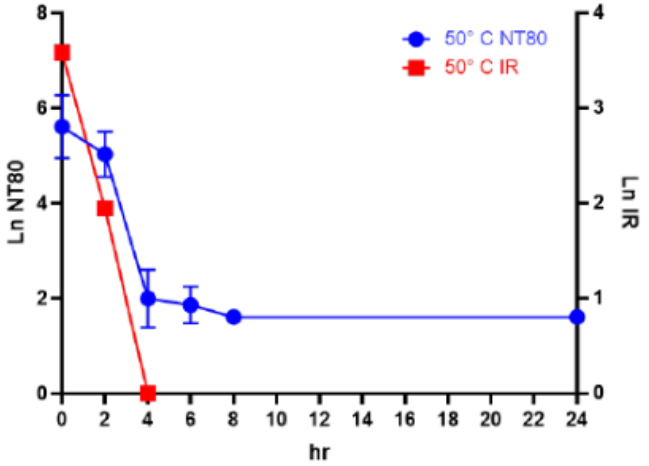
Relationship of Immunoreactivity with Mouse Serum Neutralizing Titers - Aged CHIKV VLP

Study Setup

CHIKV-VLP at 46°C for 5 days and 50°C up to 1 day

immunoreactivity (µg/mL) vs neutralizing antiserum titers (*in vivo* NT80)





Conclusion

slow non-linear decline of immunoreactivity at 46°C

little change within 24 hours for mouse NT80 thereafter levels began to decline at a rate that corresponded well with immunoreactivity

both immunoreactivity and neutralizing titers declined rapidly to near and below detection limits n less than 4h at 50°C

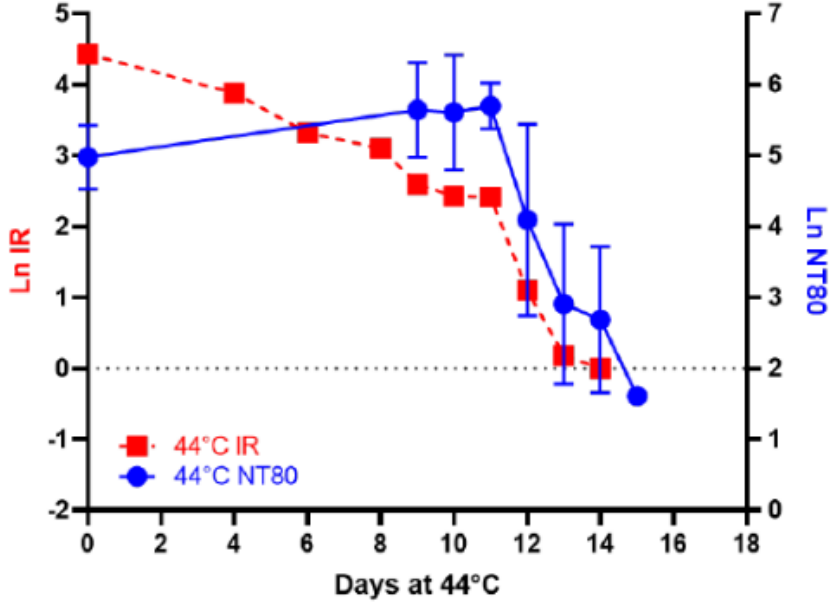
good correlation between both methods and better sensitivity of CHIKV assay

Relationship of Immunoreactivity with Mouse Serum Neutralizing Titers - Aged CHIKV VLP

Study Setup

unadjuvanted CHIKV-VLP at 44°C for 18 days

immunoreactivity (IR) vs immunogenicity in mice (neutralizing titer NT80)



Conclusion

immunoreactivity decreased at relatively linear rate

mouse serum neutralization titers began to decline at a rate that corresponded well with immunoreactivity from day 11 (below 10 µg/mL)

immunoreactivity assay is highly sensitive to alterations in the CHIKV VLP and can detect such changes well before they become apparent in mouse model

Effect of Temperature on CHIKV-VLP size distribution, immunoreactivity and NT80

Study Setup

unadjuvanted CHIKV-VLP at 50°C and 70°C

immunoreactivity (µg/mL) vs immunogenicity in mice (NT80)

	Sample Treatments			
Tests	**Control**	**50°C, 0.5h**	**50°C, 2h**	**70°C, 0.5h**
µBCA (µg/ml)	126	112	110	116
DLS particle size (nm)	72	130	386	1943
DLS polydispersity index	0.14	0.14	0.41	0.54
Gyrolab (µg/ml)	103	71	50	3

Conclusion

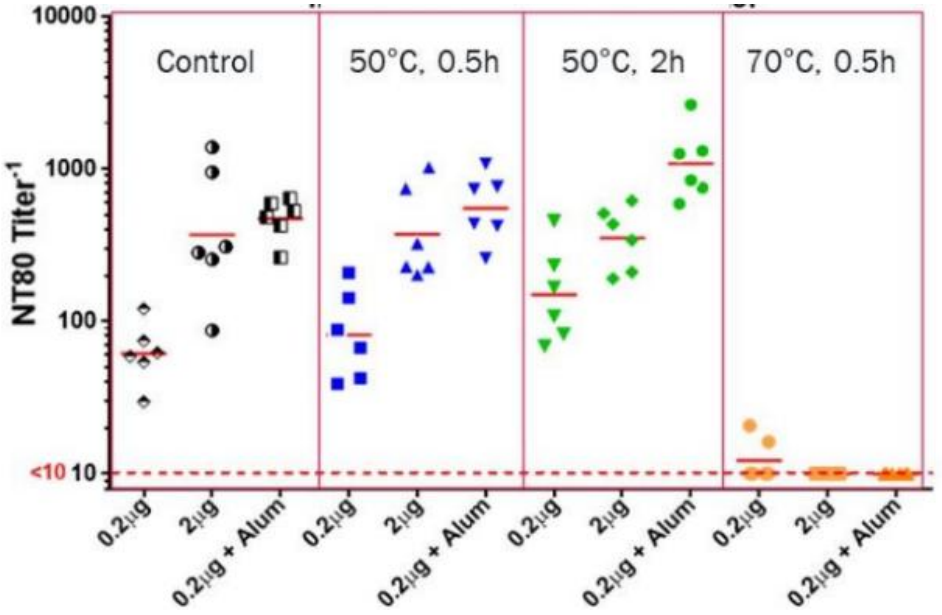
total protein concentration not affected

Immunoreactivity decreased by 50% at 50°C, 2h

immunoreactivity affected due to increased particle size due to aggregation

neutralizing titers abolished only at 70°C, 0.5h

CHIKV assay is more sensitive to small changes to the CHIKV-VLP, well before the changes impact its ability to elicit neutralizing antibodies

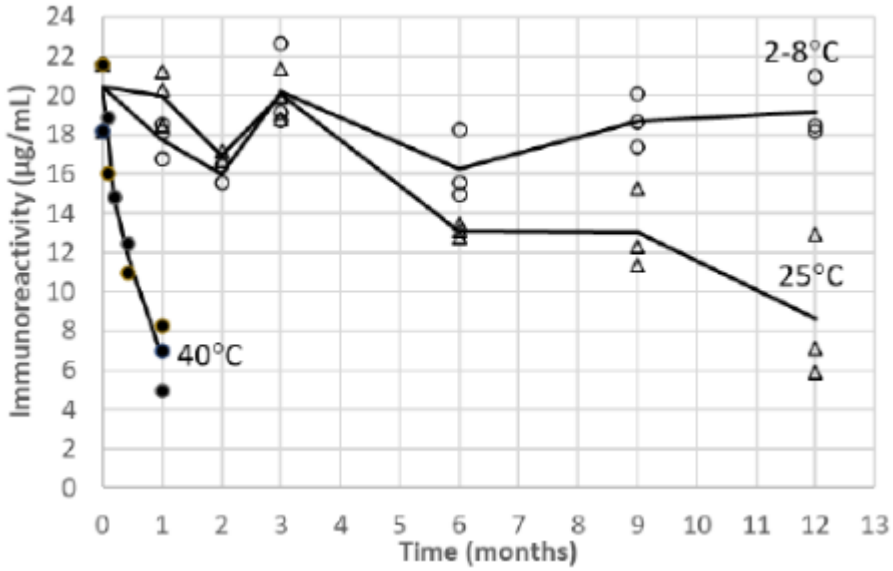


Stability Study

Study Setup

adjuvanted CHIKV stored at 2-8°C, 25°C, 40°C

immunoreactivity vs immunogenicity in mice (neutralizing titer NT80)



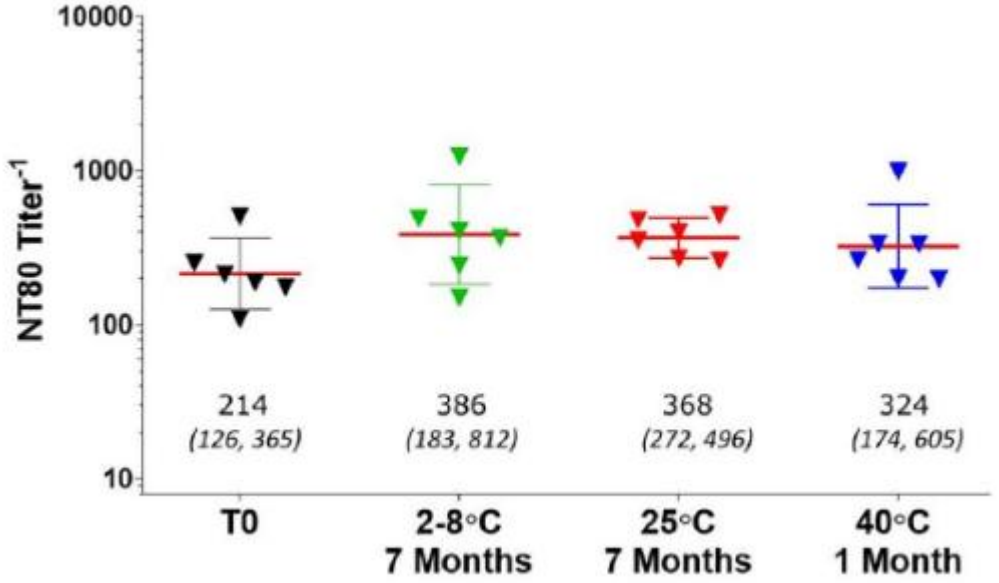
Conclusion

adjuvanted CHIKV stored at 2-8°C stable for 12 months

decline to 40% of immunoreactivity at 25°C after 12 months

decline to 30% of immunoreactivity at 40°C after 1 month

no difference in immunogenicity observed in mice



Regulatory Pathway

Q1 2017

Pre-IND meeting (USA)

Q1 2018

IND submission (USA)

Q2 2018

Fast Track designation granted (USA)

Q3 2020

Breakthrough Therapy Designation granted (USA)

Pre-BLA meeting (USA)

and

Pre-MAA meeting (EU)

Q2 2024

BLA submission (USA)

Rolling review application

and

MAA-submission (EU)

accelarated assessment

Approvals

Q1 2025 FDA (USA)

Q1 2025 EMA (EU)

Q2 2025 Positive ACIP Vote

Q2 2025 MHRA (UK)

Conclusions

CHIKV assay was successfully approved as potency assay

- mAb well characterized
- good correlation between the *in vivo* and *in vitro* assay
- more sensitive that *in vivo* assay
- stability indicating

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Thank you!