



Improvement of molecular and serological tools for the detection of Venezuelan Equine Encephalitis Virus (VEEV)

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European Union Reference Laboratory for Equine Diseases
– WNV and Exotic equine encephalitis virus

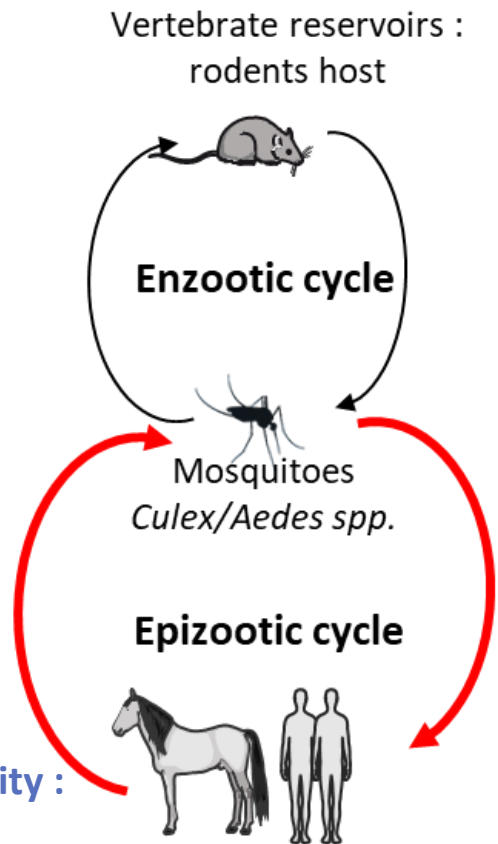
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Venezuelan Equine Encephalitis virus (VEEV)

Family: *Togaviridae*

Genus: *Alphavirus*



Equine mortality :
40-90%

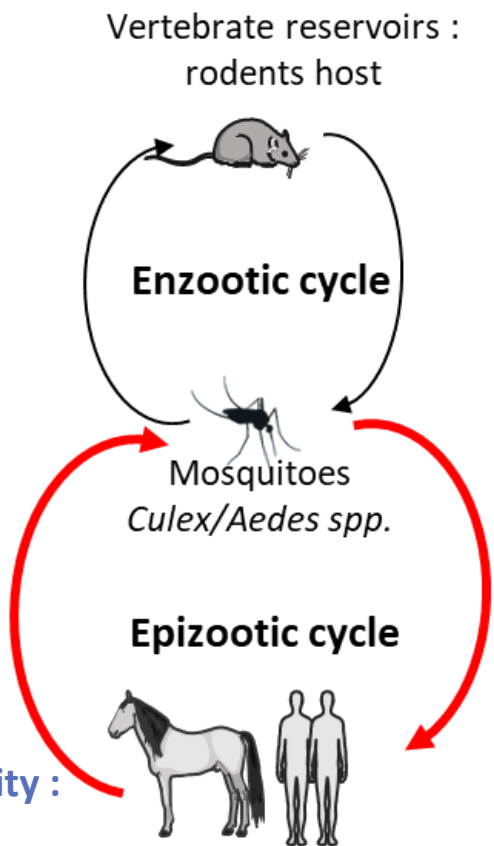
Horses / humans amplifying host

Venezuelan Encephalitis Equine virus (VEEV)

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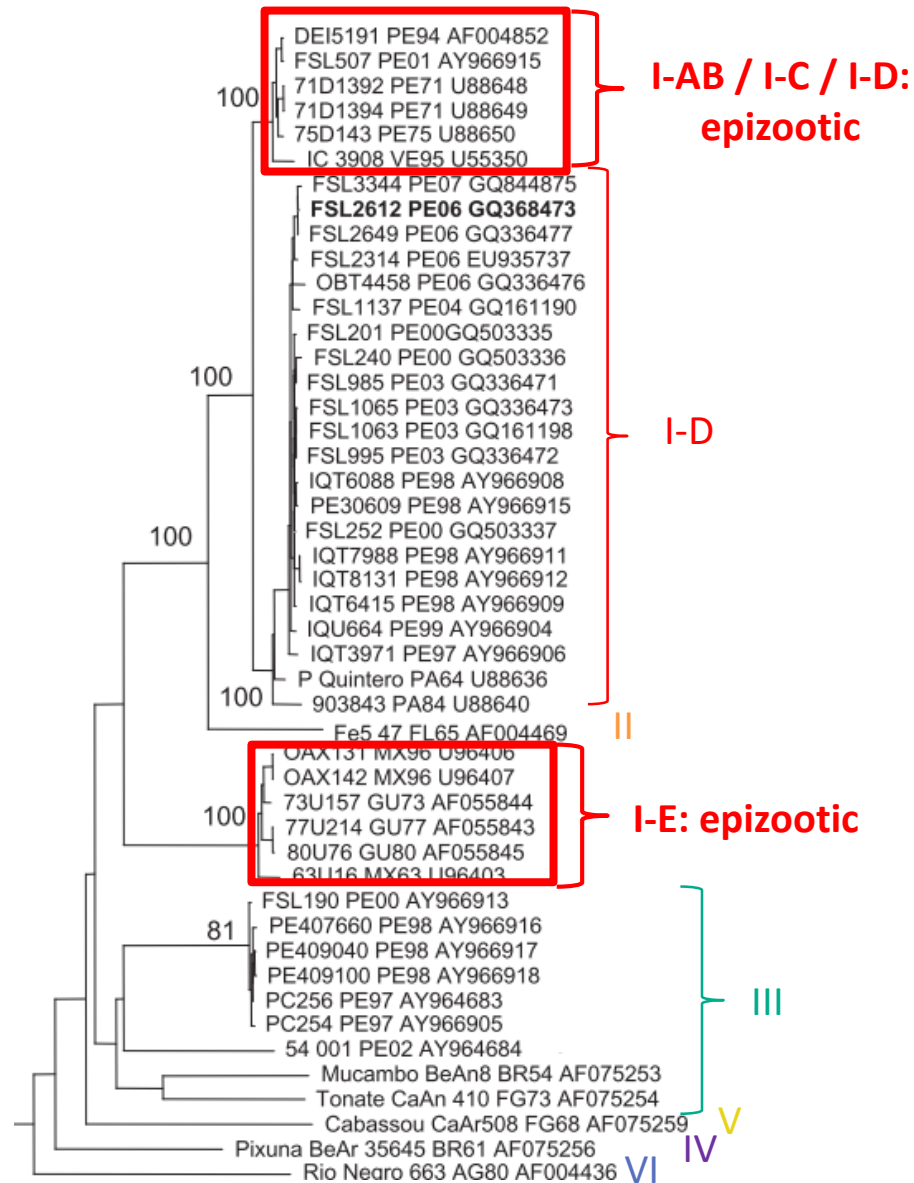
Genus: *Alphavirus*

6 subtypes (I to VI)

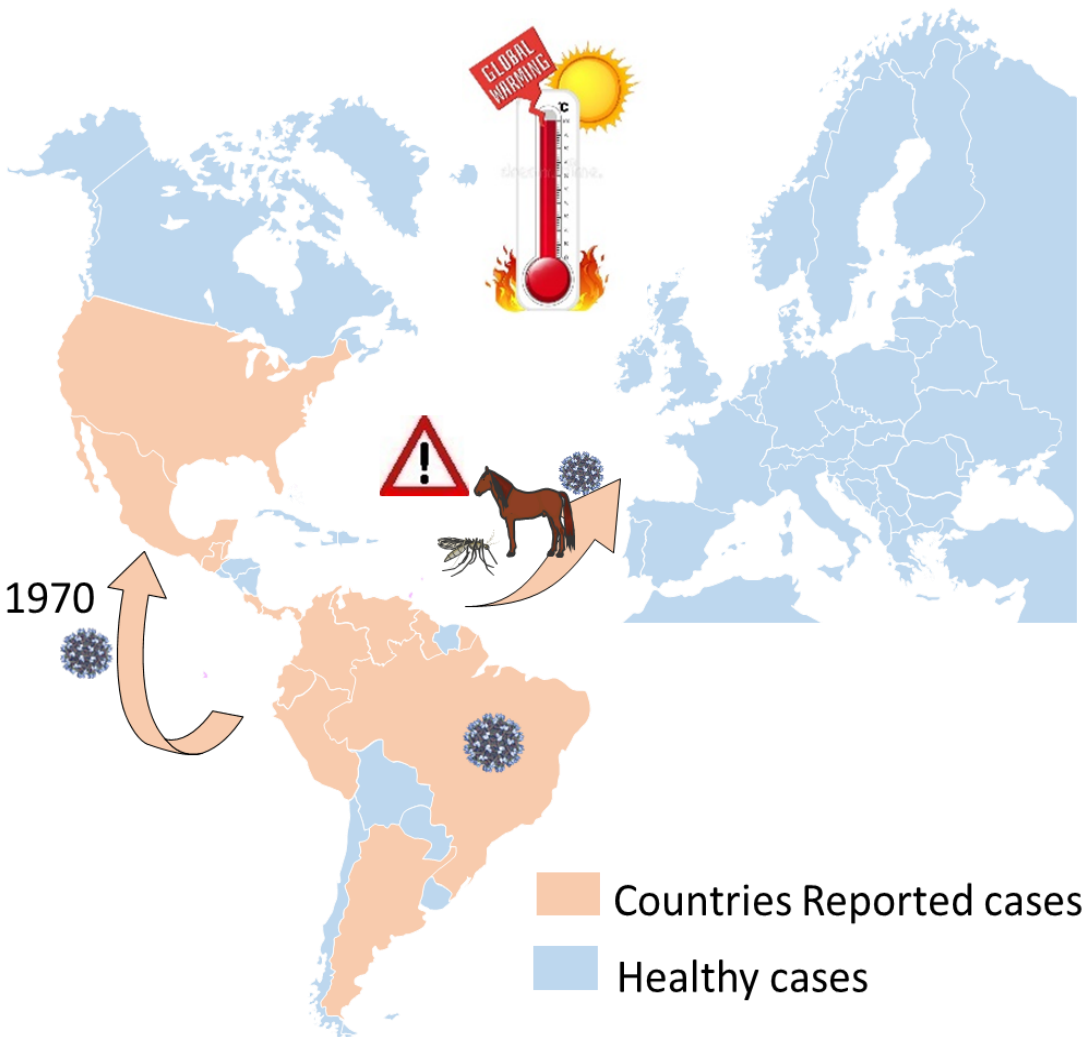


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Horses / humans amplifying host



Geographical distribution



- North / West of South America
- 1970: VEEV emergence in Central America and USA
- **BIOWEAPON**: Easy aerosolization and low extremely infectious dose



- **Risk of emergence in Europe**
- Classified in categories D and E (Animal Health Law 2021)

→ European Commission request systematic control of imported horses

European Commission mandates the **European Union Reference Laboratory for Equine Diseases**

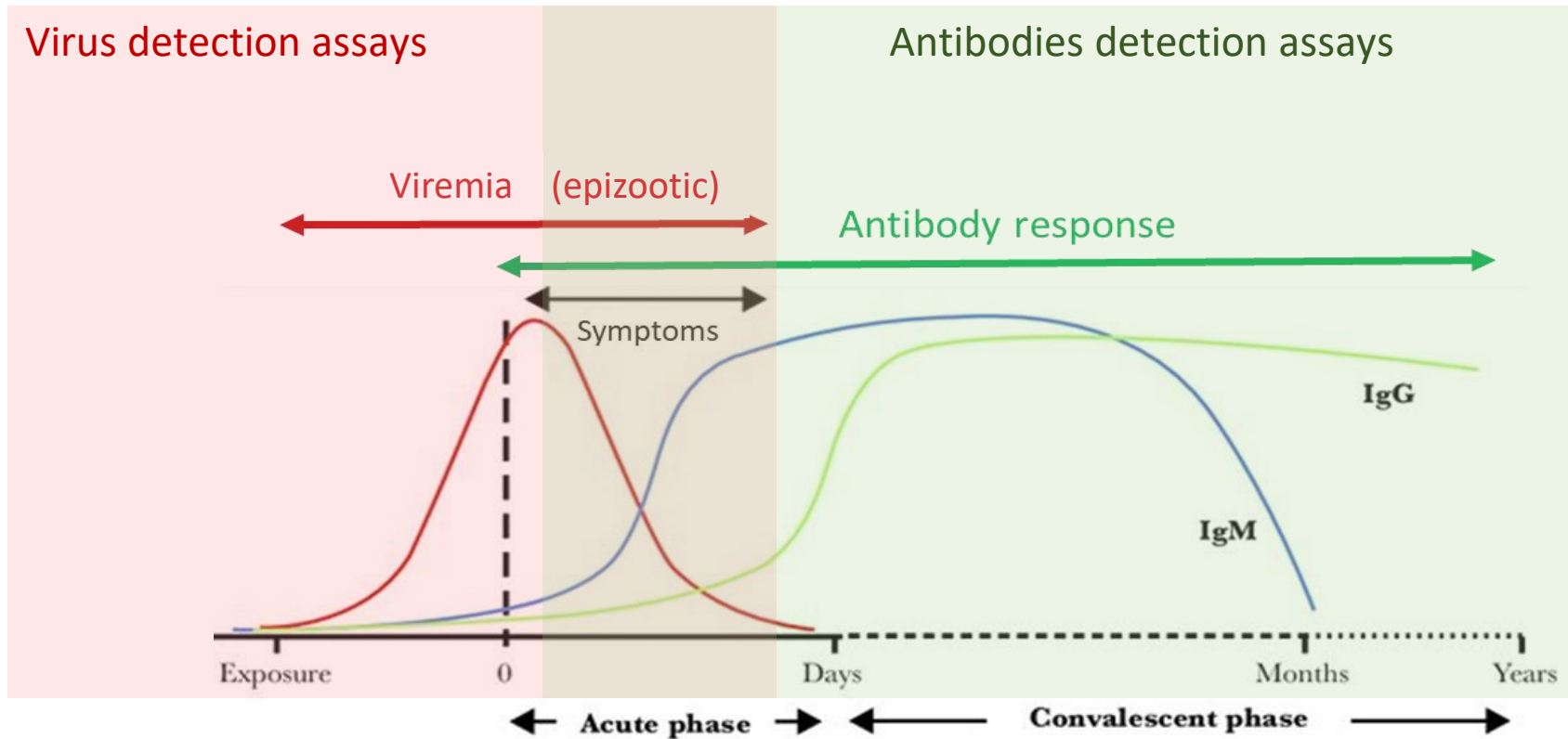


Development of sensitive and specific VEE virus serological and molecular detection tools

Diagnosis

Diagnostic tools in laboratory

→ to be adapted according to the kinetics of infection



Adapted from *Goncalves A et al. 2017*

→ Short viraemia (5-6 days)

→ Long antibody response

Test methods available for the diagnosis

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification²						
RT-PCR	–	++	n/a	+++	–	–
Isolation in tissue culture	–	++	n/a	+++	–	–
Detection of immune response						
IgM capture ELISA	–	+	n/a	++	–	–
Plaque reduction neutralisation	+++	+	n/a	++	+++	+++
Hemagglutination inhibition (paired samples)	+	++	n/a	++	++	++
Complement fixation (paired samples)	–	+	n/a	++	–	–

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.

RT-PCR = reverse-transcription polymerase chain reaction;

IgM ELISA = immunoglobulin M enzyme-linked immunosorbent assay.

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Diagnostic tools in the EURL



Samples

Whole blood (EDTA)
Tissues
Cerebrospinal fluid

Molecular Tools / Techniques

Virus genome detection by RT-qPCR
↓
Virus isolation

Direct diagnostic

RT-qPCR VEEV/ β -actin
(in optimization)

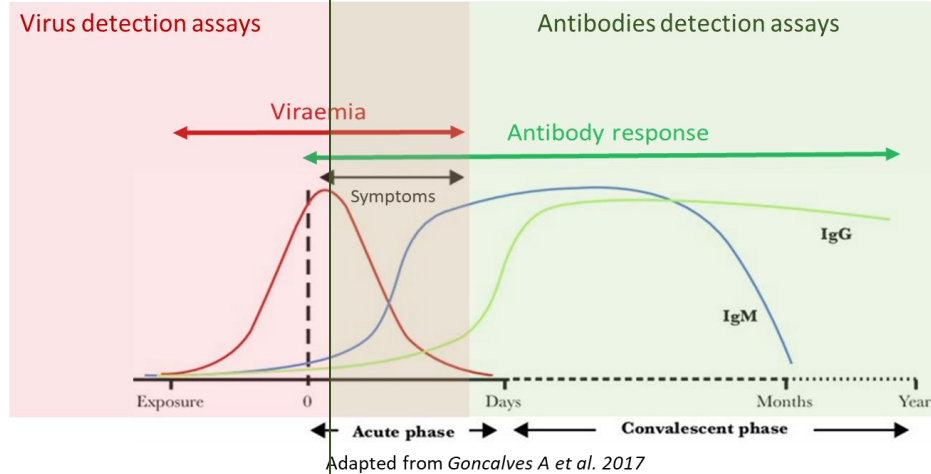
Samples

Serum (+++)
Plasma (+/-)

Serological Tools / Techniques

Virus Neutralization Test

Primary infection → Serial samples



Indirect diagnostic

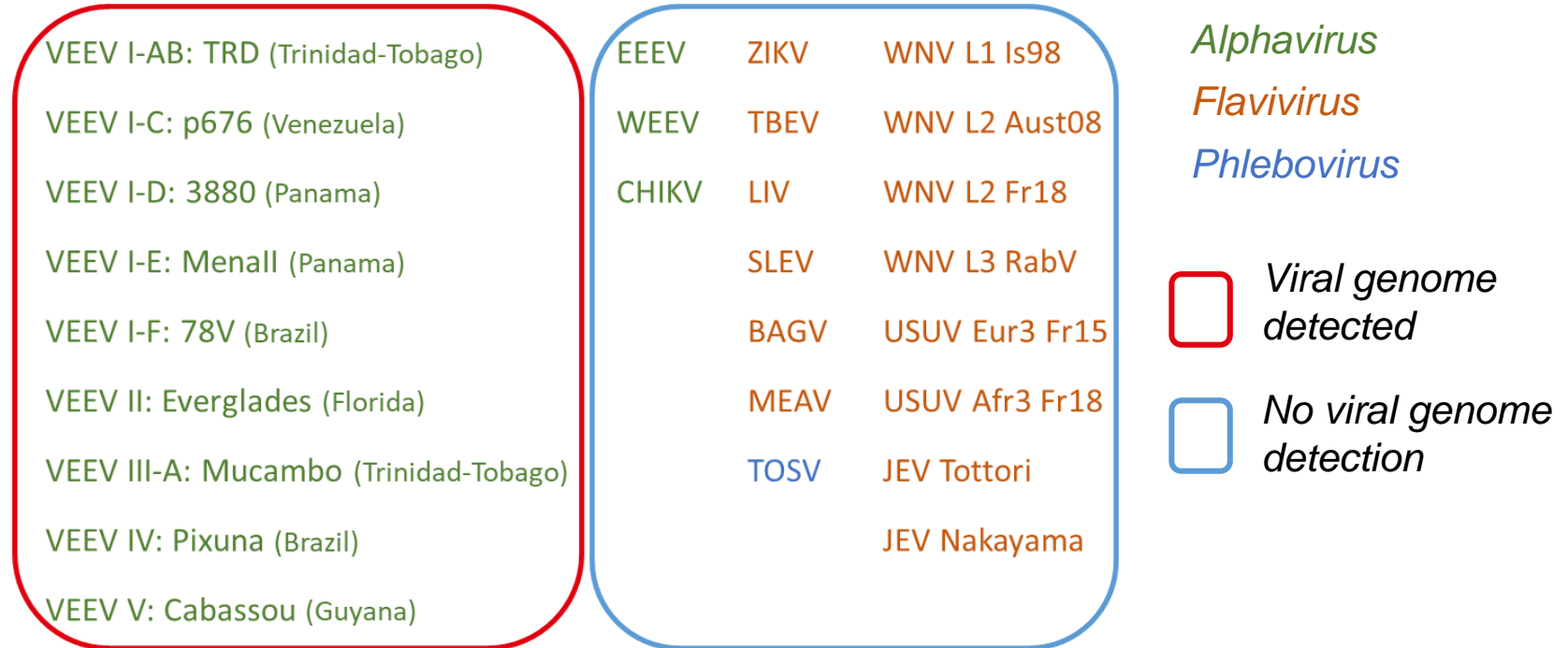
VNT
(in development for used in BSL2)

ELISA
(in development)

Hemagglutination inhibition
(reference method)

I. Improvement of the VEEV RT-qPCR method

Optimization of the sensitivity of VEEV RT-qPCR method



□ Specificity

Detection of the 9 VEEV strains

Absence of detection for other alphaviruses and flaviviruses

→ **Specificity: 100%**

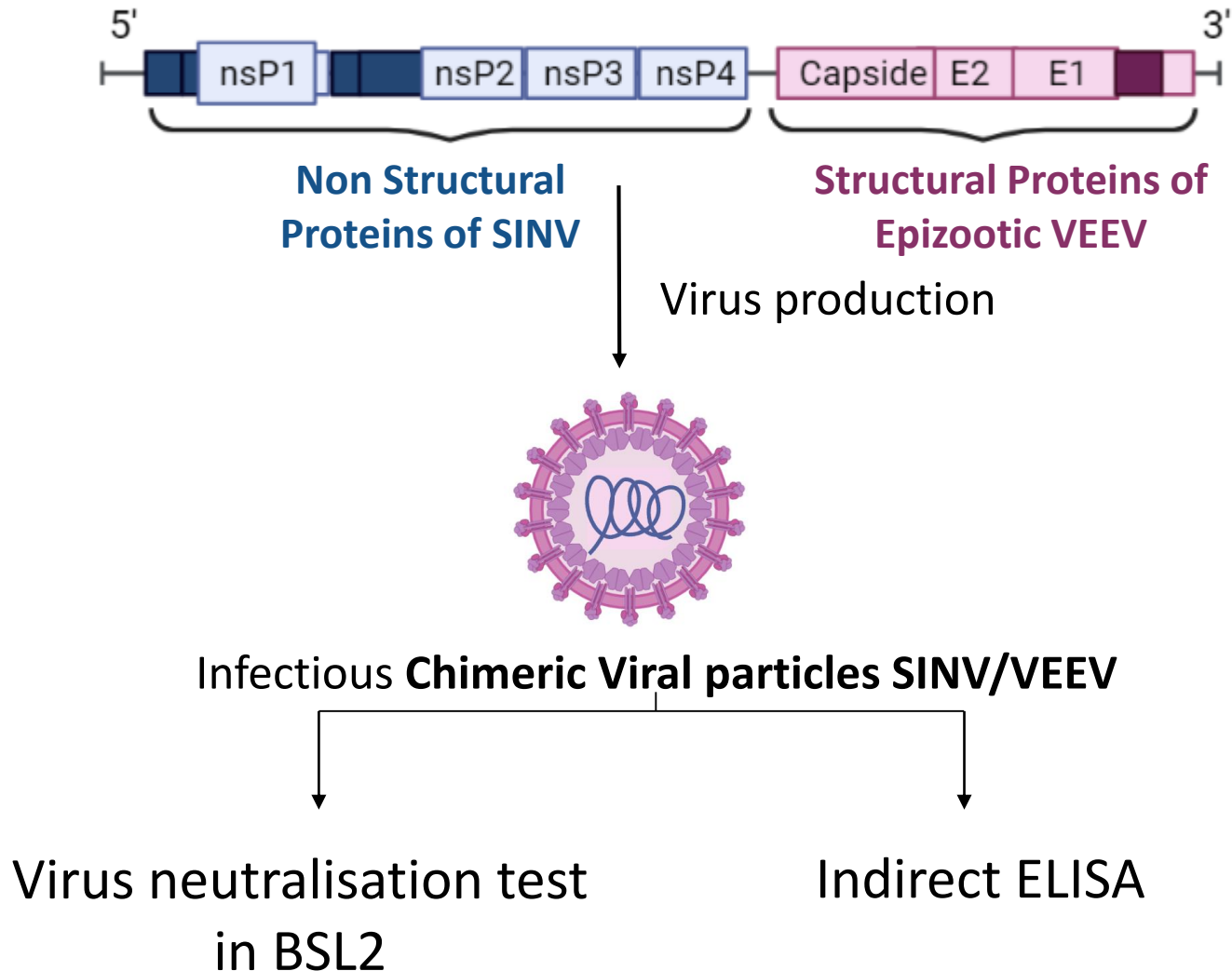
□ Sensitivity

Evaluation of the Detection Limit (homemade standard RNA)

→ **Sensitivity: 50 genome copies/ μ l**

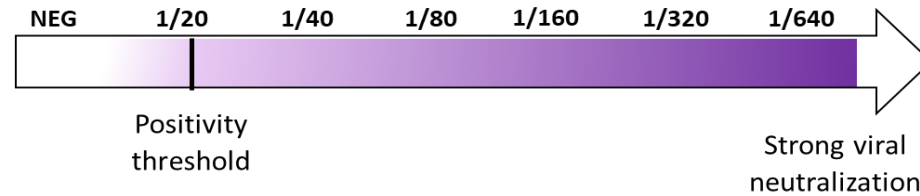
II. Development of serological tools

II.1. The use of Chimeric virus Sindbis/VEEV to perform VNT in BSL 2



II.2. VNT in BSL2 using chimeric SINV/VEEV

Range of neutralizing antibody titers



Sensitivity	Serum infected horse	Virus			
		Parental VEEV I-AB	SINV/VEEV/ I-AB	SINV/VEEV/ I-D	SINV/VEEV/ I-E
Sensitivity	18	Light Purple	Dark Purple	Dark Purple	Light Purple
	17	Light Purple	Dark Purple	Light Purple	Light Purple
	20	Light Purple	Dark Purple	Dark Purple	Dark Purple
	22	Light Purple	Dark Purple	Light Purple	Light Purple
	23	Light Purple	White	White	White
Cross-reactivity	POS EEEV/WEEV	White	White	White	White

- ✓ Equal and Higher sensitivity of chimeric viruses
→ **SINV/VEEV virus recognized by neutralizing antibodies**
- ✓ Absence of cross-reactivity with other alphaviruses (EEEV / WEEV)

II.3. VNT in BSL2 using chimeric SINV/VEEV: perspectives

- Implementation of VNT in BSL2
 - No constraints of manipulation
 - Make it available to EU members

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➤ Implementation of VNT in BSL2

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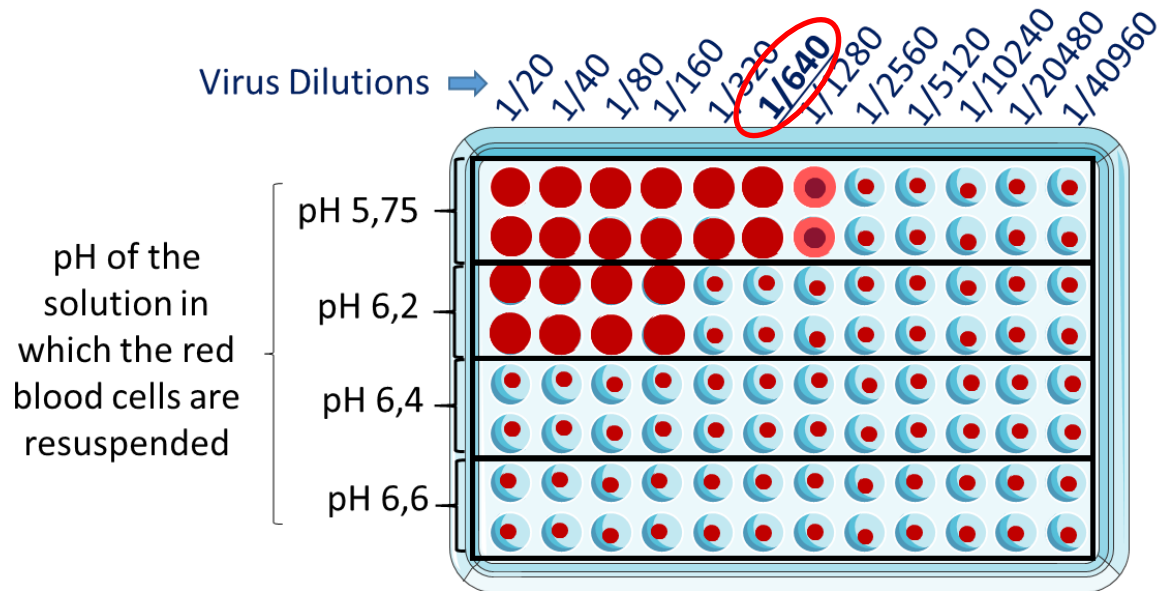
➤ Indirect homemade ELISA

- Based on total inactivated particles



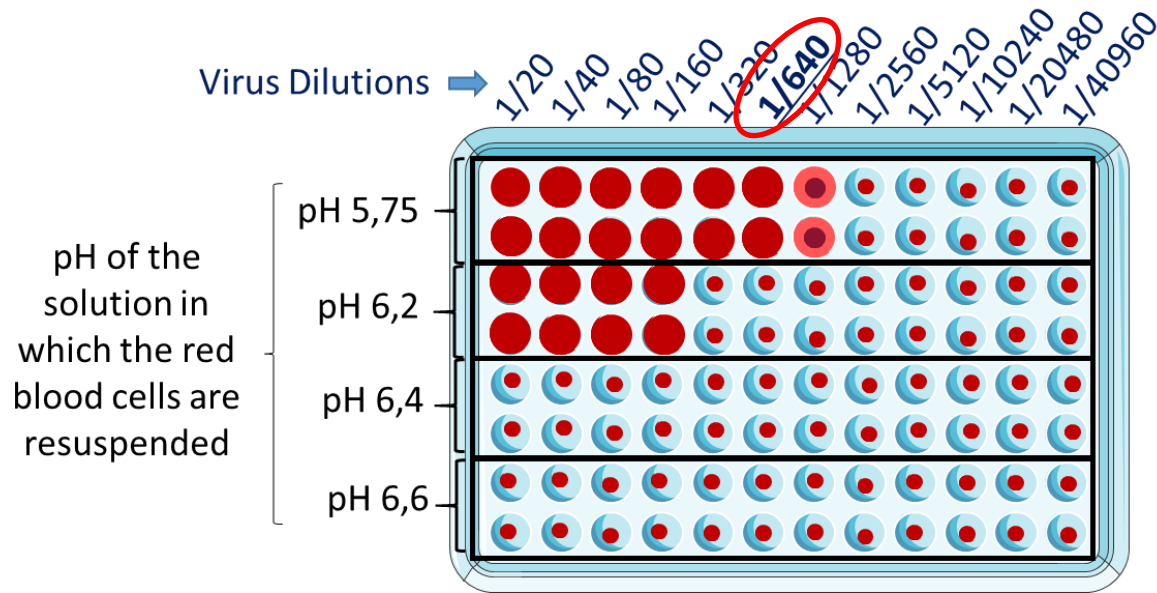
**Waiting for serum
from Ecuador
(collaboration)**

II.4. Hemagglutination inhibition assay (WOAH* ref method)



- Antigens concentration ✓
- Goose red blood cells ✓
- Development of conditions ✓

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* World Organisation for Animal Health

Conclusion / Perspectives



➔ Improved VEEV RT-qPCR available

- RT-qPCR: repeatability, replicability, robustness, COFRAC accreditation

- VNT
- ELISA
- IHA

We're almost there!



website : <https://eurl-equinediseases.anses.fr/>

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➔ Europe needs to be ready in case of emergence (Olympic Games 2024, Paris, France)

Aknowledgements

Thank you for your attention !

EU-RL Director
Stephan Zientara



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