

Mitigating the Risk of Zika Virus Contamination of Raw Materials & Vaccines by In Vitro Assays

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Introduction

The production of biologicals employs sophisticated laboratory techniques, and potential safety concerns exist regarding manufacturing processes, product and process related impurities, and the structural and biological properties of the products.

Regulatory authorities require freedom from adventitious viruses (AV) in drug manufacture. Traditional *in vitro* assays are useful in providing assurance of freedom from AV, but where novel viruses or isolates occur in nature, the detectability of the viruses by the *in vitro* assay should be assessed.

Risk of Contamination of Raw Materials with ZIKV

Zika virus (ZIKV) is an emerging mosquito borne human pathogen, a member of the *Flaviviridae* virus family and the *Flavivirus* genus. Despite identification in 1947¹ and a reported prevalence in Africa and Asia, for many decades little attention has been paid to its potential presence in the raw materials of human or animal origin.

Introduction of ZIKV in Americas in 2015 revealed its epidemic potential and clinical importance. ZIKV may cause neurological complications in adults: encephalitis, meningo-encephalitis and Guillain-Barré syndrome microencephaly in neonates.

Infection with ZIKV in healthy humans is largely asymptomatic, and therefore virus may enter biopharmaceutical production in raw materials from donors. If present, ZIKV may not be detected unless appropriate testing measures are employed.

Raw materials of human origin should be tested, including materials such as blood fractions (plasma, platelets, convalescent serum) and urine - a source of pharmacologically active substances such as gonadotropins.

Strains of Zika virus tested

ZIKV MR766 (African lineage)
LC002520/MR766/1947/Uganda. Isolated in 1947, Rhesus monkeys, Zika forest, Uganda. ZIKV MR766 obtained: BEI, NIAID, NIH. ZIKV MR766 (GenBank: AY632535, DQ859059, KU963573).

ZIKV PE243 (S. American lineage)
ZIKV/H.sapiens/Brazil/PE243/2015. Isolated from a patient in Recife, Brazil in 2015. ZIKV PE243 Acc. No. (GenBank: KX197192).

Methods

In vitro AV assay

For *in vitro* assays, according to regulatory guidance¹⁰⁻¹², detector cells were inoculated with Zika virus at 1000, 100, 10 & 1 TCID₅₀, and assayed, with blind passage at day 14.

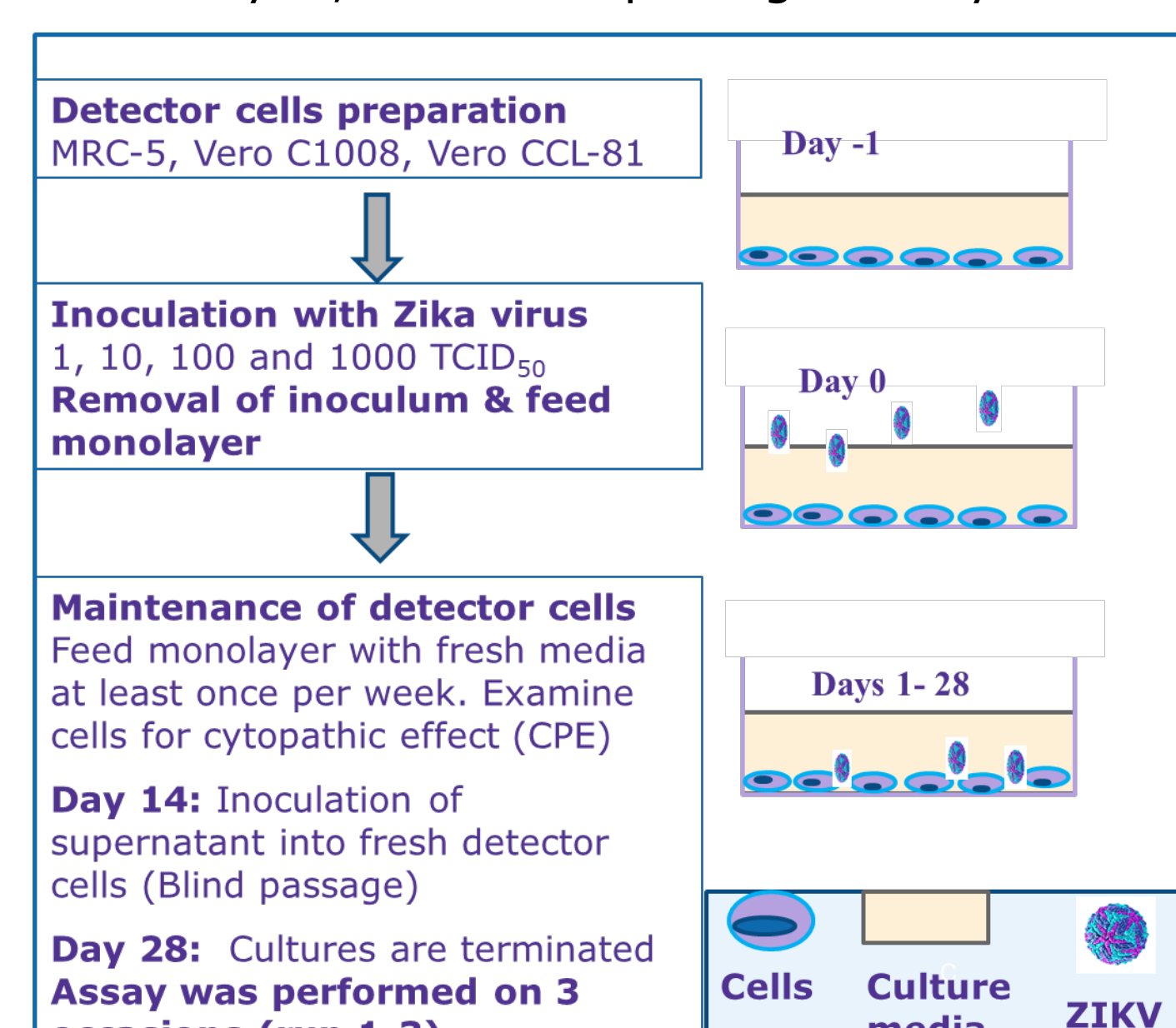


Figure 2. *In vitro* assay schematic

References

- Dick, GWA. *et al.* J. Trans. Roy Soc. 46, (1952).
- Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome, 10 Dec, 2015. ECDC 1-14 (2015).
- Musso, D, *et al.* Potential Sexual Transmission of Zika Virus. 21, 2013-2015 (2015)
- Lanteri, MC. *et al.* Zika virus: a new threat to the safety of the blood supply with worldwide impact and implications. *Transfusion* 56, 1907-1914 (2016).
- Nogueira ML, *et al.* Zika Virus Infection and Solid Organ Transplantation: A New Challenge. *Am J Transpl.* 17: 791-79, (2017).
- Besnard, M. *et al.* Evidence of perinatal transmission of zika virus, French Polynesia, December 2013 and February 2014. *Eurosurveillance* 19, 8-11 (2014).
- Musso, D, *et al.* Detection of Zika virus in saliva. *J. Clin. Virol.* 68, 53-55 (2015).
- Mansuy, JM, *et al.* Zika virus: High infectious viral load in semen, a new sexually transmitted pathogen? *Lancet Infect. Dis.* 16, 405 (2016).
- Zhang, FC, *et al.* Excretion of infectious Zika virus in urine. *Lancet Infect. Dis.* 16, 641-642 (2016).
- Ph. Eur. Chapter 2.6.16, "Test for Extraneous Agents in Viral Vaccines for Human Use";
- Ph. Eur. Chapter 5.2.3 "Cell Substrates for the Production of Vaccines for Human Use"
- Ph. Eur. Chapter 5.2.4 "Cell Cultures for the Production of Veterinary Vaccines"
- Zmurko, J, *et al.* Mitigating the risk of Zika virus contamination of raw materials and cell lines in the manufacture of biologicals. *J Gen Virol* 99; 219-229 (2018).

Results and Discussion

In-vitro detection of Zika virus by in vitro assay

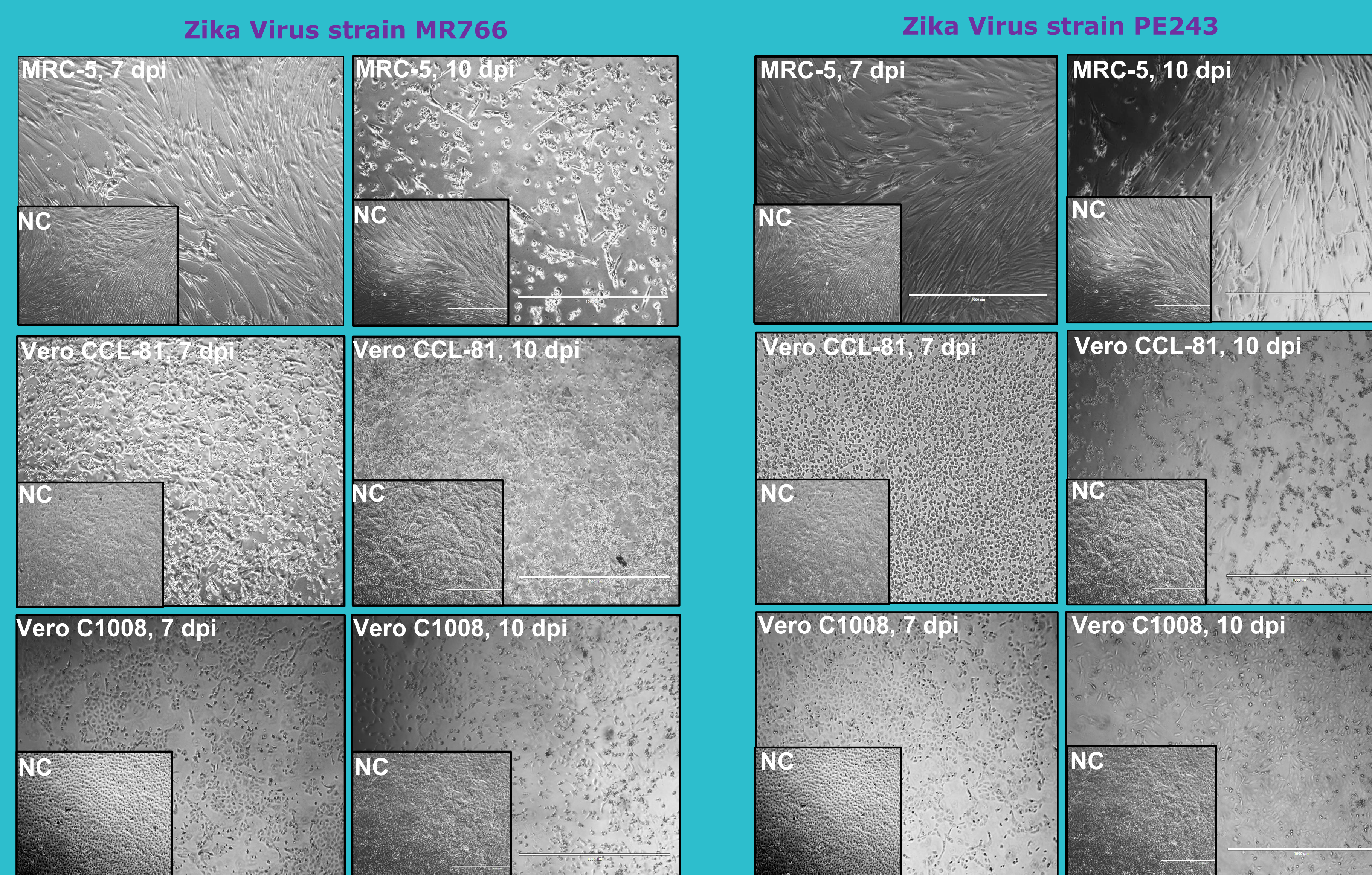


Figure 3. Zika virus is detectable with MRC-5, Vero CCL-81 & C1008 and causes CPE

Sensitivity of *in vitro* assay for detection of ZIKV

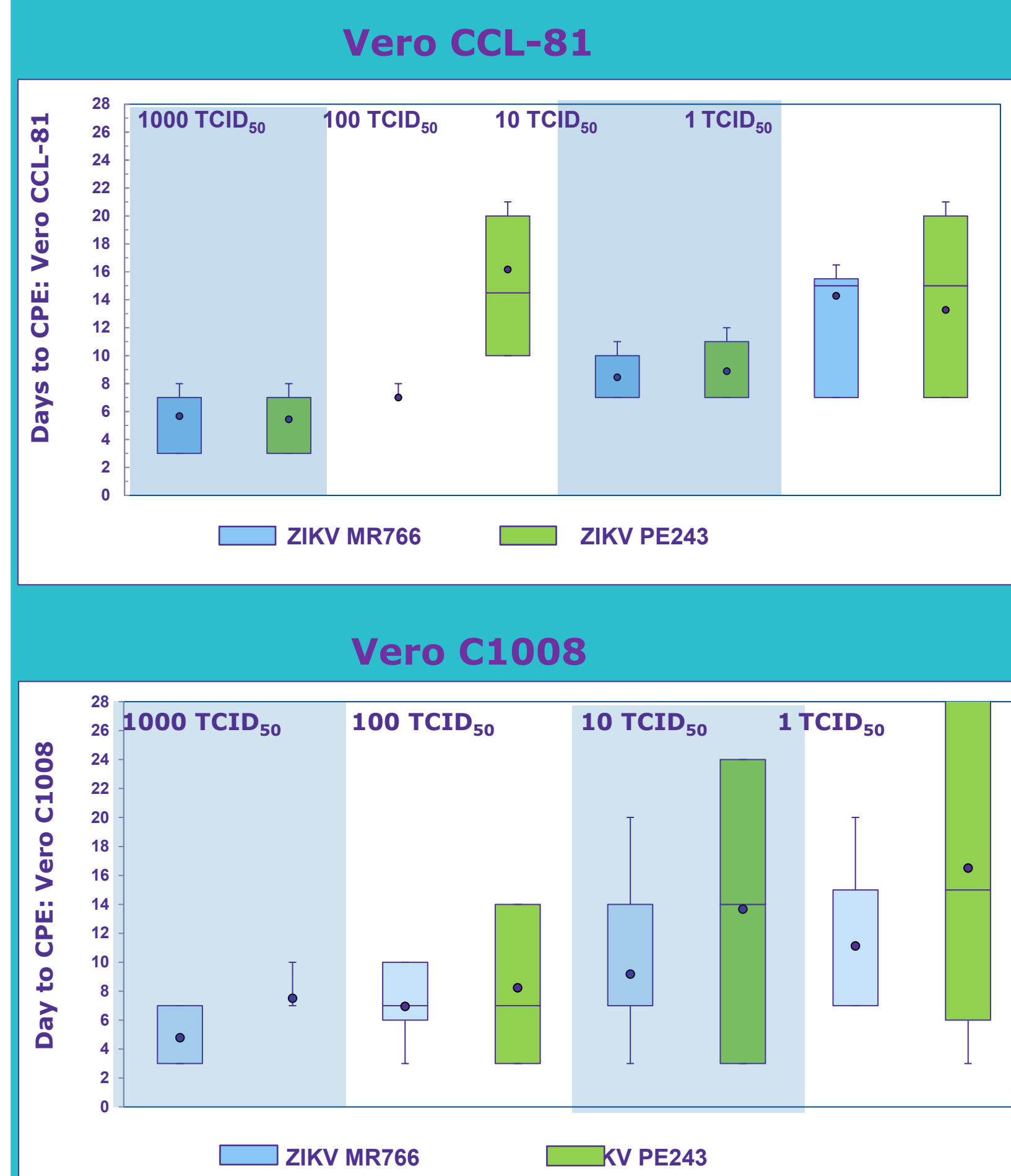


Figure 4. Day of CPE appearance on Vero (CCL-81 & C1008) detector cells inoculated with 1000, 100, 10 & 1 TCID₅₀ ZIKV. Data represent inoculations performed in 18 wells of detector cells seeded in 6-well plates, on 3 separate occasions.

Table 1. Percentage (%) of wells with detector cells inoculated with ZIKV showing cytopathic effect. Detector cells (MRC-5, Vero CCL-81 and Vero C1008) were inoculated with 1000, 100, 10 and 1 TCID₅₀ ZIKV (MR766 and PE243). Data represent inoculations performed in 18 wells of detector cells seeded in 6-well plates, on 3 separate occasions.

Detector cells	MRC-5		Vero CCL-81		Vero C1008							
	MR766	PE243	MR766	PE243	MR766	PE243						
Inoculum (TCID ₅₀)	14 d*	28d**	14d	28d	14d	28d						
	1000	100%	100%	67%	67%	100%	100%	100%	100%	100%	100%	
	100	100%	100%	50%	56%	100%	100%	100%	100%	100%	100%	
	10	61%	100%	33%	50%	94%	100%	100%	94%	100%	67%	100%
1	33%	50%	0%	0%	44%	75%	39%	100%	56%	100%	50%	50%

* = 14 day *in vitro* assay; ** = 28 day *in vitro* assay

In vitro adventitious virus assay robustly detected ZIKV

Vero cells (C1008 and CCL-81) detected the presence down to 1 TCID₅₀ of ZIKV (strain MR766 and PE243) during 28 day *in vitro* assay (Table 1). The first onset of CPE was observed as early as 3 days post infection (Figure 4) in the form of retarded cell growth, extensive cell rounding and lysis (Figure 3).

MRC-5 cell line detected the presence down to 1 TCID₅₀ ZIKV MR766 and 10 TCID₅₀ ZIKV PE243 (Table 1) by reporting the appearance of CPE (Figure 3) during 28 day *in vitro* assay.

Summary

- Zika virus poses a risk of contamination in Substances of Human Origin; plasma, platelets or convalescent serum, urine and stem cells.
- We demonstrate robust detection and report of Zika virus using an *in vitro* assay for detection of adventitious viruses where human diploid fibroblast MRC-5 and secondary African green monkey kidney cells (Vero) were used.
- We demonstrate robust detection at 1 TCID₅₀ of two isolates of Zika virus (MR766 and PE243) in 28 day *in vitro* assay using Vero cells.
- The general *in vitro* adventitious virus test with CPE end point testing is sufficient and robust detection of Zika virus in raw materials or final products where the origin of raw materials or the history of a product indicates a risk for ZIKV contamination

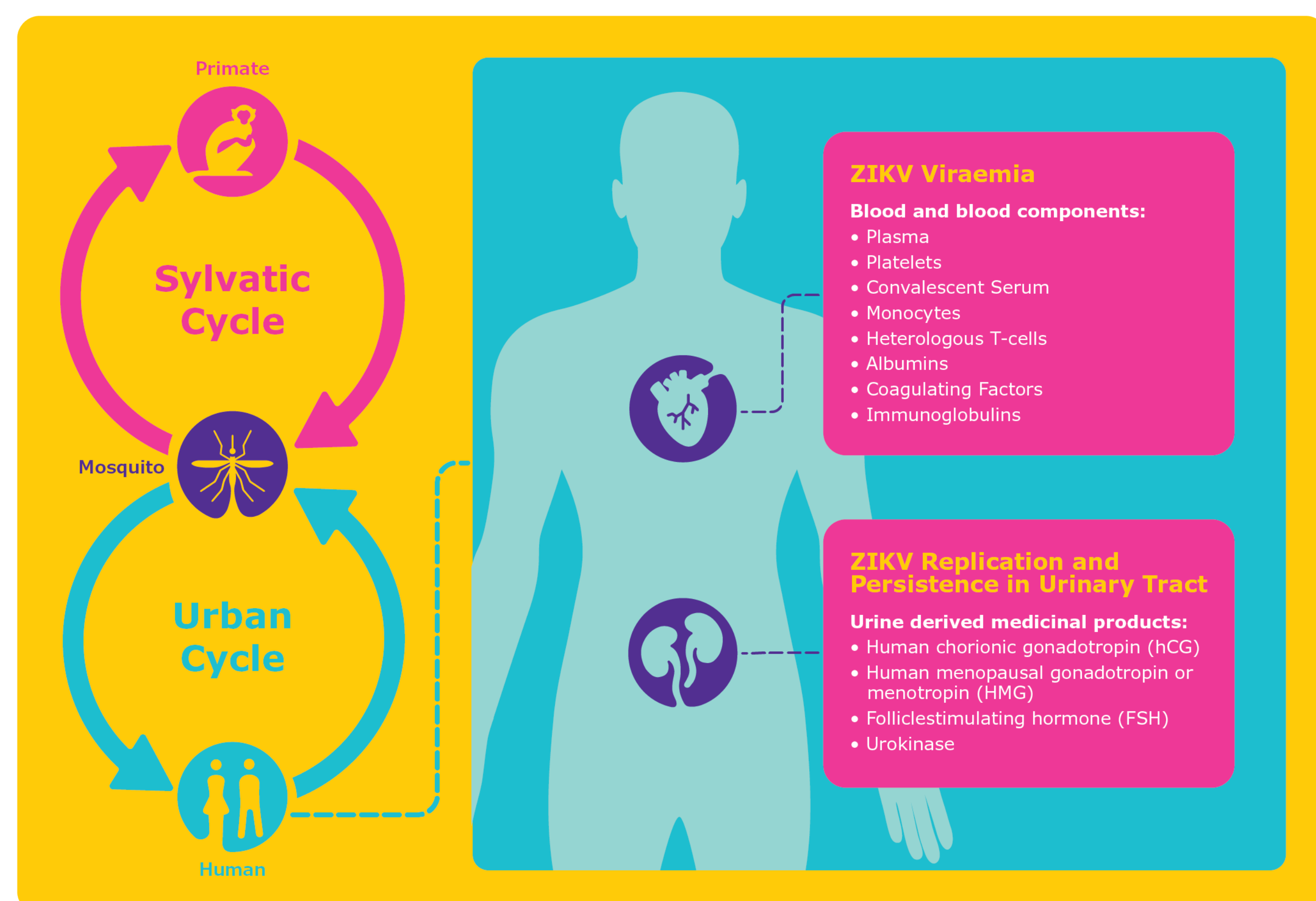


Figure 1. Zika virus contamination and transmission. Routes of ZIKV transmission include sexual transmission³, blood transfusion⁴, organ transplantation⁵ and perinatal transplacental transmission⁶. Infectious viral particles of Zika are detectable in saliva⁷, semen⁸ and urine⁹ of infected humans and therefore may contaminate Substances of Human Origin.

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