Viral RNA shedding during asymptomatic and symptomatic ZIKV mono-infection and ZIKV/CHIKV co-infection in Brazilian cases: implications in clinical outcomes.

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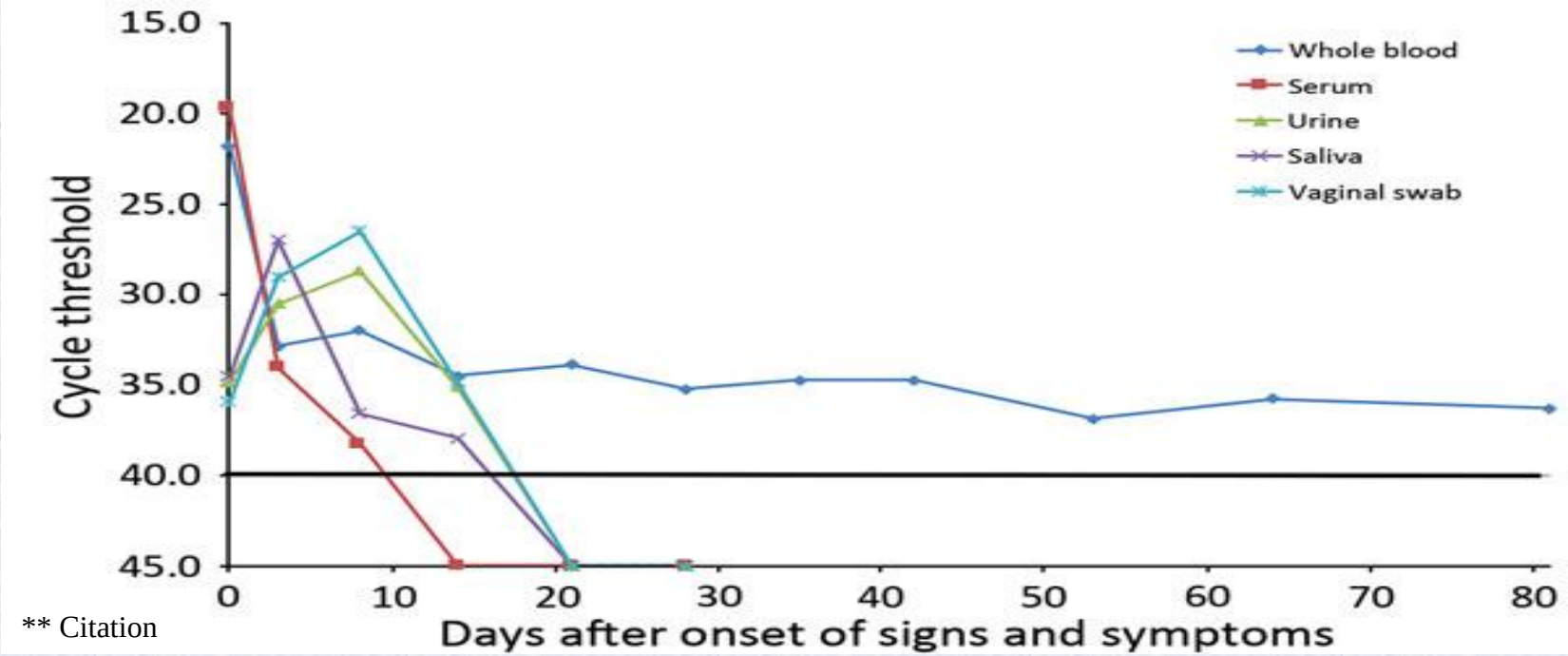
Background

- ZIKV infection clinical presentations range from absence of symptoms or mild self-limited disease lasting one week in both general population and pregnant women. However, severe forms of disease like Guillain-Barré syndrome and microcephaly also present as manifestations of ZIKV infection.
- In the present, the most reliable tool to confirm ZIKV infection is RNA detection by molecular-assays.
- Zika virus RNA may be detected in serum for approximately 4-7 days following onset of symptoms but may be detected longer in a pregnant woman.

Background

- Long periods of RNA shedding in blood, urine and saliva permit delayed diagnosis of infection, but its correlation with disease evolvement is not known.



** Citation

- Also, the role of ZIKV co-infections remains unclear as a risk factor for the development of severe forms of the disease.

**Murray KO, Gorchakov R, Carlson AR, Berry R, Lai L, Natrajan M, et al. Prolonged detection of Zika virus in vaginal secretions and whole blood. Emerg Infect Dis. 2017 Jan [*date cited*]. http://dx.doi.org/10.3201/eid2301.161394 DOI: 10.3201/eid2301.161394

Aims

- To study ZIKV RNA shedding kinetics in asymptomatic and symptomatic individuals during ZIKV mono-infection and co-infection.
- To determine the role of RNA shedding in biological specimens with clinical disease course and outcomes.

Patients, Material and Methods

From 37 out of 163 individuals attending Infectious Diseases Clinics (HUCFF/UFRJ) enrolled in a longitudinal cohort for arboviral infections. Individuals with fever and/or rash and/or arthralgia/arthritis with history of exposure were cosidered confirmed cases after laboratorial diagnosis. Both confirmed cases and households living with confirmed cases in the same house were invited to participate in the study.

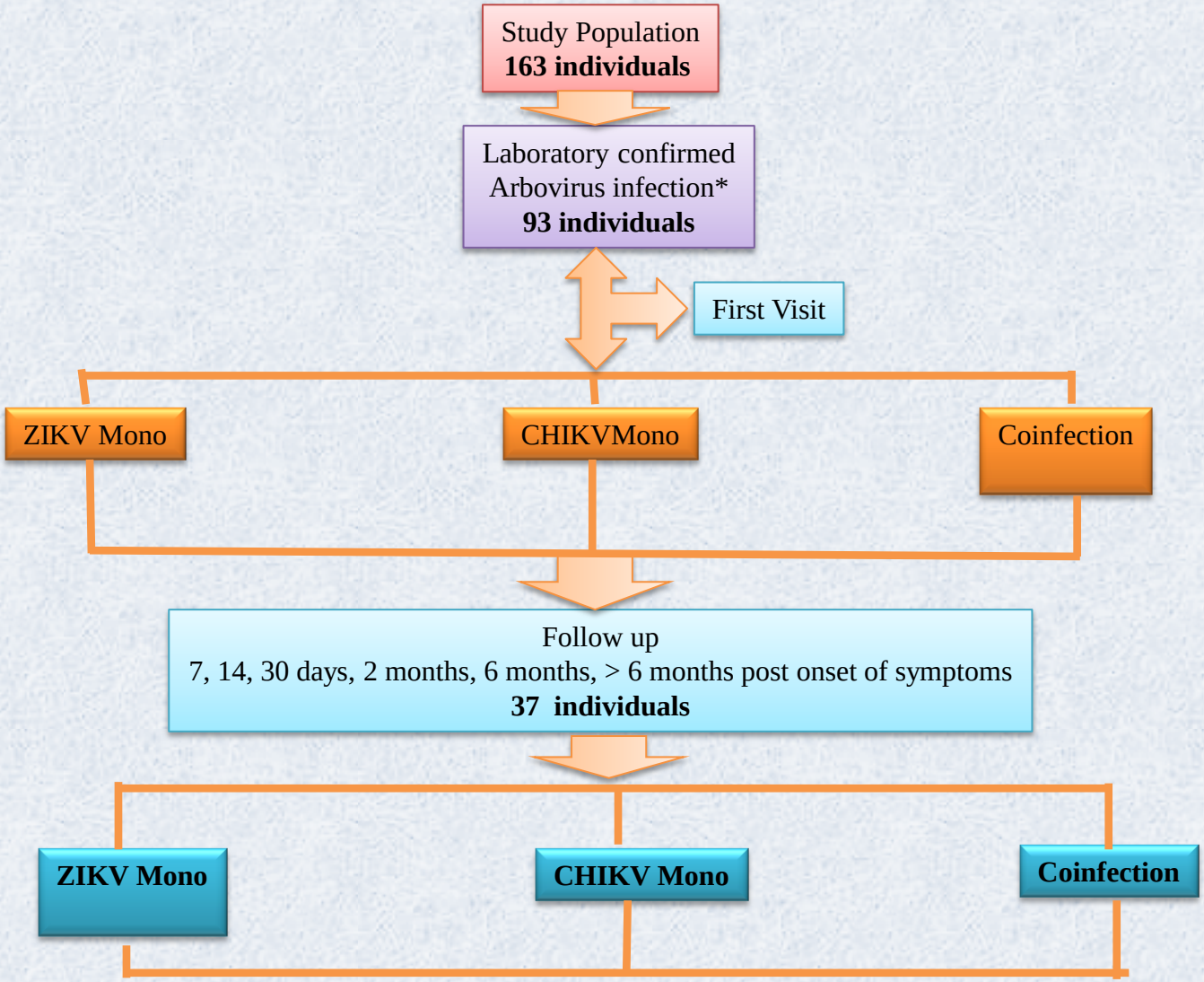
Clinical samples: Serum, urine, breast milk, cerebrospinal fluid, synovial fluid and tissue samples were tested by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) for detection of ZIKV and Chikungunya virus.

Molecular Diagnosis :
Viral RNA was extracted from 250 μL of clinical samples using TRIzol LS Reagent (Ambion™, Life Technology, USA) according to the manufacturer's recommendations. cDNA synthesis and real-time RT-PCR were performed by GoTaq 1-Step Probe RT-qPCR System (Promega, USA). The real-time RT-PCR protocol included specific primers and a probe set previously described [1,2]. The results were expressed as Ct values, and Ct up to 38.5 cycles were considered as positive [1].

Virus Isolation : Biological samples showing amplification by real-time RT-PCR underwent culturing for viral isolation in cells of different lineage such as *Macaca mulatta* kidney (LLC-MK2), African green monkey kidney (MA-104) and kidney epithelial cells extracted from an African green monkey (VERO).

[1] Lanciotti RS et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 2008; 14(8):1232–9.
[2]Lanciotti RS et al. Chikungunya virus in US travelers returning from India, 2006. Emerg Infect Dis. 2007; 13(5):764–767.

Experimental Design



* ZIKV and/or CHIKV RNA reactive

Results

Table 1 – Demographic and Clinical characteristics of the study population

Patient Data	ZIKV Mono	CHIKV Mono	Coinfection
Total (%)	8/37	9/37	20/37
Gender Male: Female	2:6	4:5	11:9
Age (years-old)	40.1 ± 20.1	43±15.3	50.3±12.7
Symptoms:			
Fever	5/8 (62.5%)	7/9(77.9%)	17/20(85%)
Rash1	8/8(100%)	6/9(66.7%)	16/20 (80%)
Rash2	0/8	0/8	6/20 (30%)
Arthralgy/Arthritis < 2 w	8/8 (100%)	4/9 (44.4%)	6/20 (30%)
Arthralgy/Arthritis > 2 w– 3 mo	0/8	1/9 (11.1%)	8/20 (40%)
Arthralgy/Arthritis >3 mo	0/8	3/9 (33.3%)	6/20 (30%)
Pruritus	4/8 (50%)	3/9 (33.3%)	11/20 (55%)
Atypical	0/8	1/9 (11.1%)	1/20 (5%)
Hospitalization	0/8	1/9 (11.1%)	3/20 (15%)

Duration of symptomatic disease and viral clearance: a possible correlation?

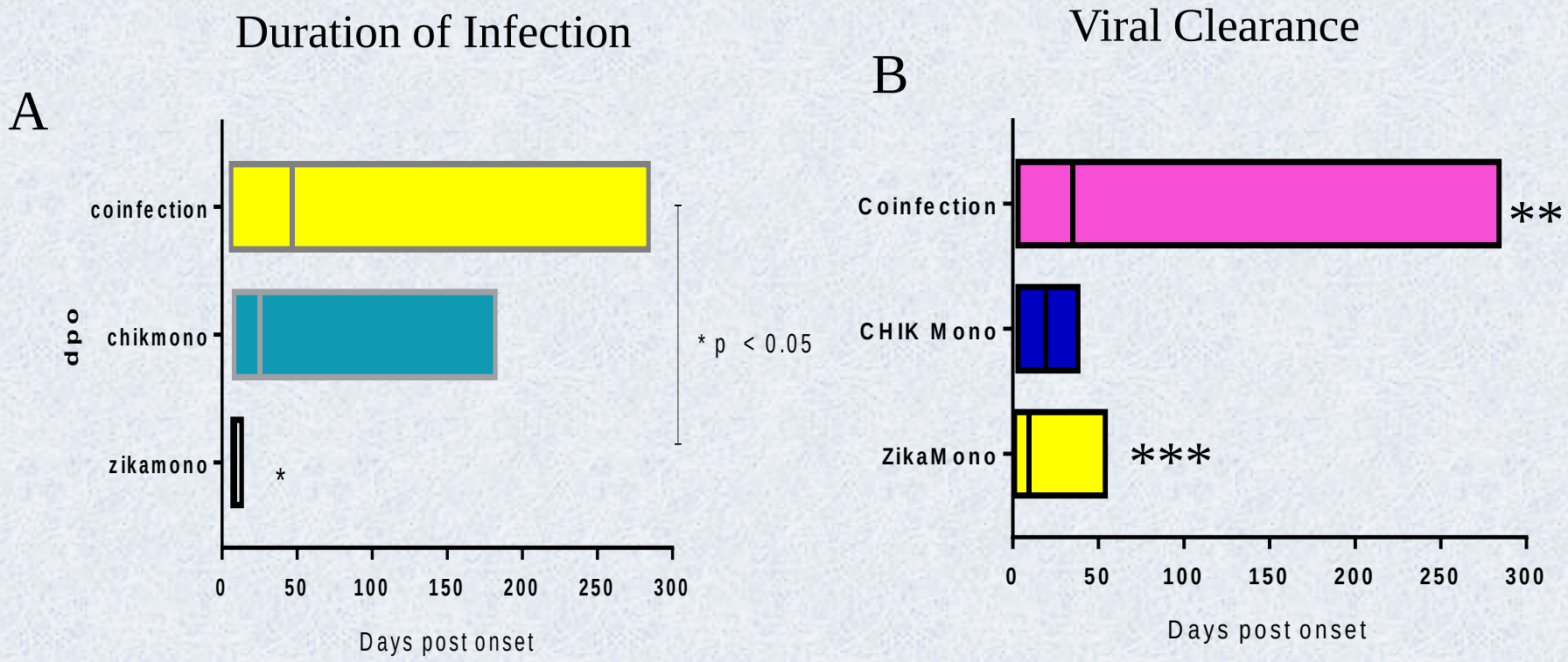
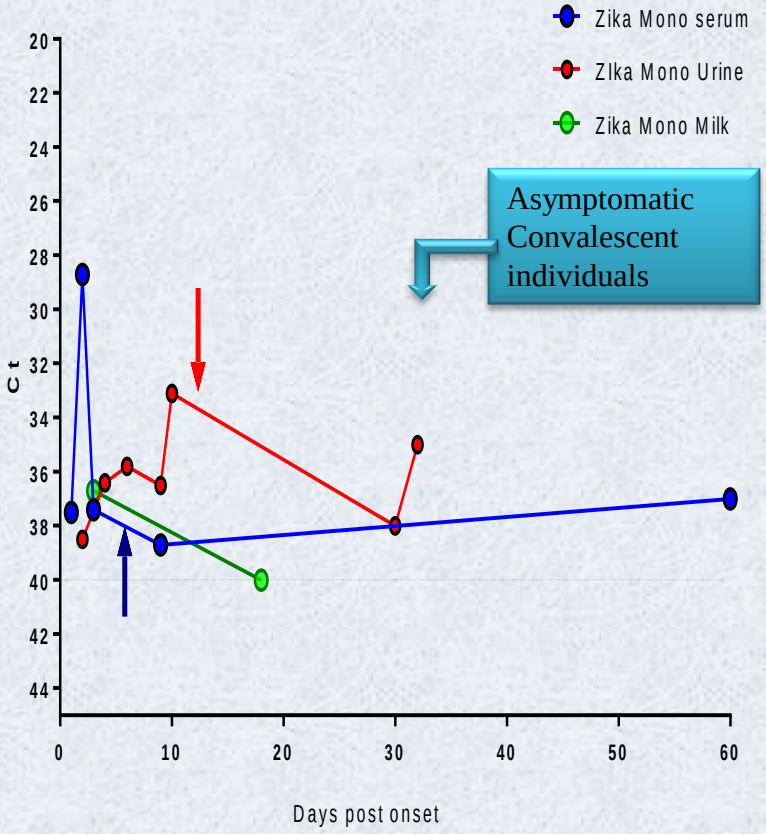
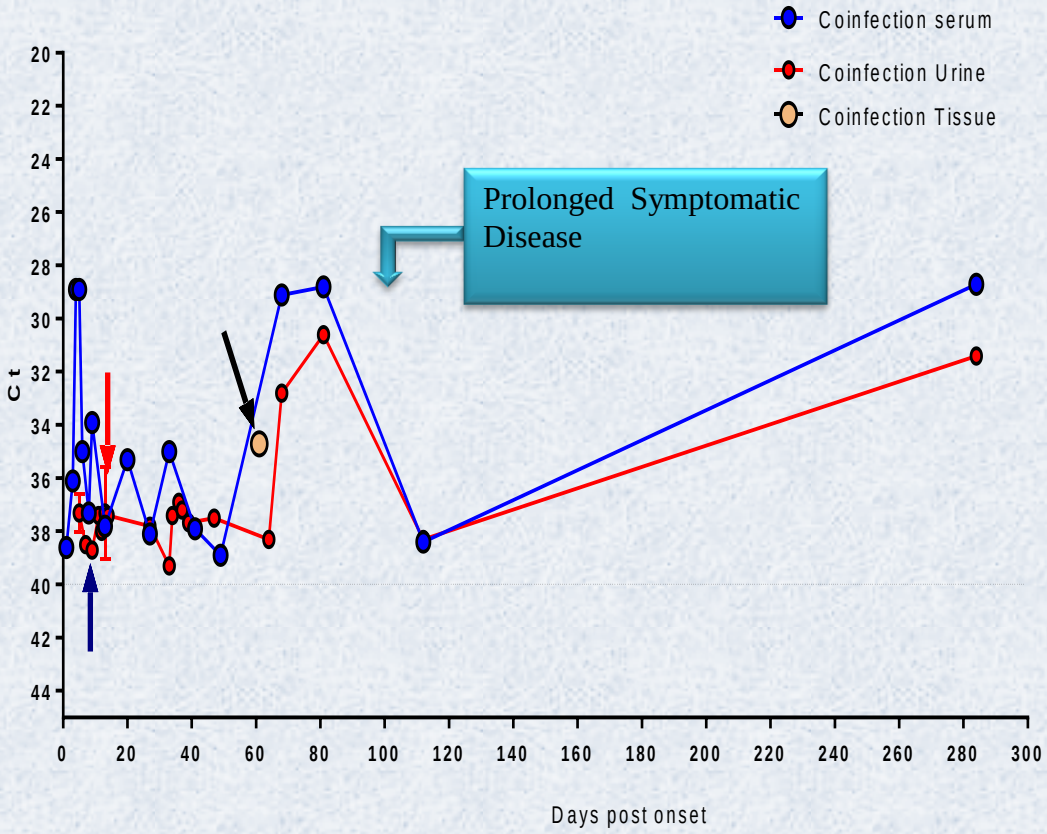


Figure 1: In A, the duration of the sympoms during Zika monoinfection, CHIK monoinfection and ZIKA/CHIK coinfection is represented. And in B, viral clearance viral was determined based on the last of RNA detection in the serum and/or urine. **20 % of the individuals may remain symptomatic in the las t visit. **All individuals presented no symptoms in the last visit. The comparison between groups was determined by using ANOVA. Differences were considered statistically significant when $p < 0.05$ * .

Persistent RNA Shedding in early and poor clearance groups: Role of Coinfection

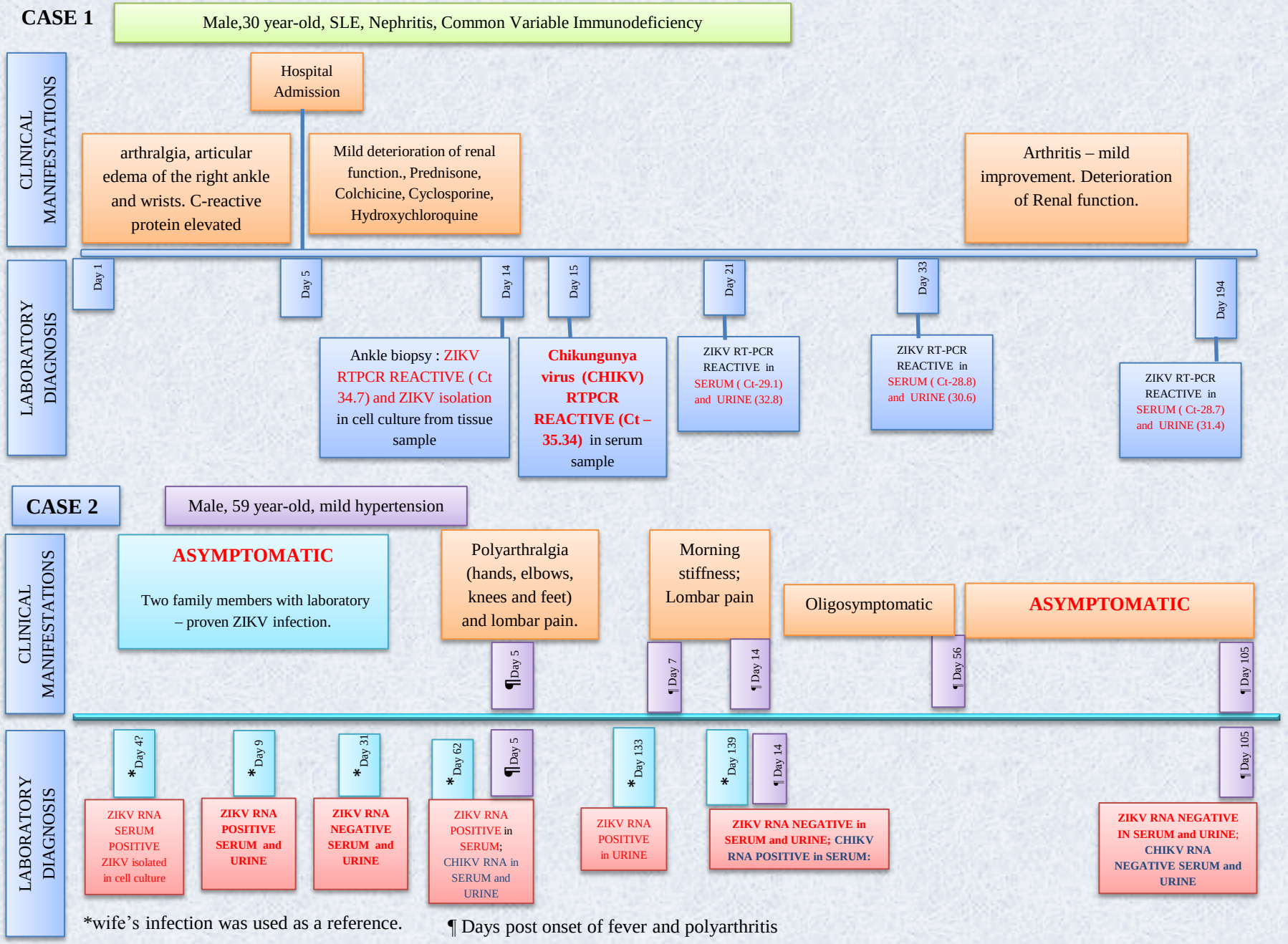




Indicates end of RNA shedding in serum for early clearance group: 10 dpo

Indicates end of RNA shedding in uirne for early clearance group: 14 dpo

Coinfection – associated risk factors and prolonged RNA shedding in symptomatic and asymptomatic individuals



Conclusions:

- Persistent ZIKV RNA shedding can be detected for long periods in blood and urine in ZIKV monoinfected individuals.
- Prolonged ZIKV RNA shedding occurs in asymptomatic individuals with laboratory – proven ZIKV monoinfection.
- Prolonged RNA shedding in individuals infected with ZIKV can be associated to concomitant Arbovirus infections, like Chikungunya, in areas of co-transmission.
- Persistent ZIKV RNA shedding might correlate with extended duration of symptoms and higher morbidity in ZIKV and CHIKV coinfected individuals.
- Coinfection should be considered a risk factor for prolonged ZIKV RNA shedding.

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