Updating CDC Trioplex assay for broad CHIKV genotypes detection

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BACKGROUND

Since 2015, the Virology laboratory at the Institut Pasteur de Dakar (IPD) has conducted real-time molecular diagnostics for suspected arboviruses, including Chikungunya fever (CF) cases, as part of the syndromic sentinel surveillance program of fevers (4S network). During the unprecedented CF outbreak in late 2023, affecting the southern regions of Senegal, specifically Kédougou and Tambacounda in October 2023, 210 cases were confirmed using RT-qPCR. Monitoring of circulating strain in Senegal using genomic approaches highlighted underperformance of CDC trioplex assay to detect WA genotypes. This lack of performance was linked to mismatches on oligos binding regions. Herein we propose to perform global in silico analysis of available CHIKV sequences in Genbank, design and evaluate alternative oligos to overcome lack of performance against WA genotype.

METHODS

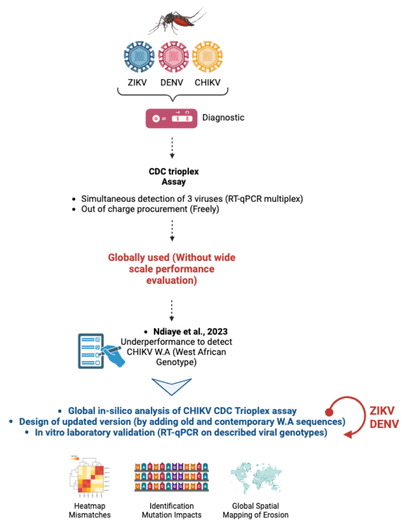

Figure 1.Overal Workflow

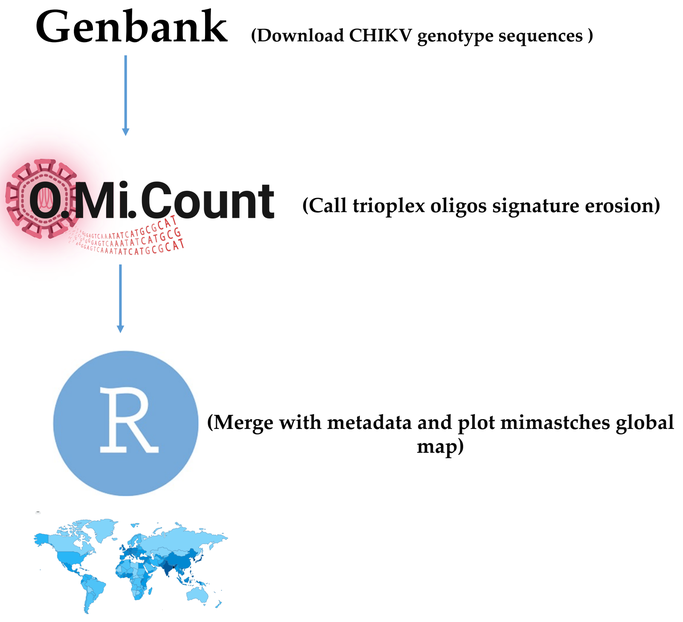

Figure 2. Description In Silico analysis

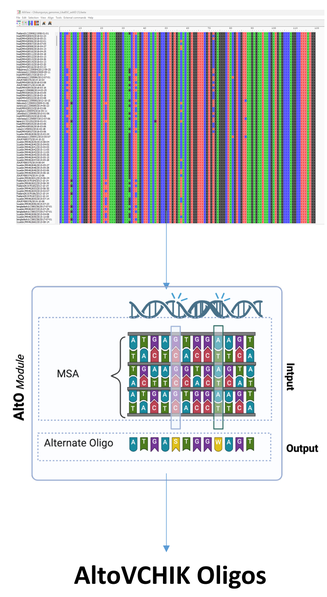

Figure 3. Alternative Oligo Design with developed AltoModule (In house tool) within same native CHIKV trioplex assay oligos binding regions

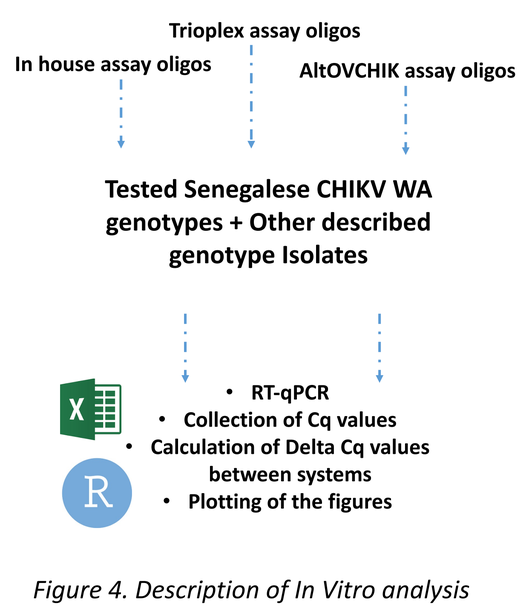

Figure 4. Description of In Vitro analysis

RESULTS & DISCUSSION

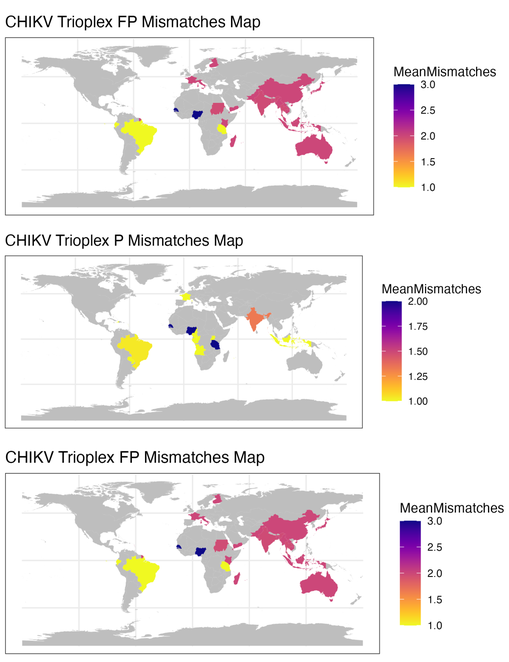

Figure 5: Native trioplex Oligonucleotide mismatches Chloropleth

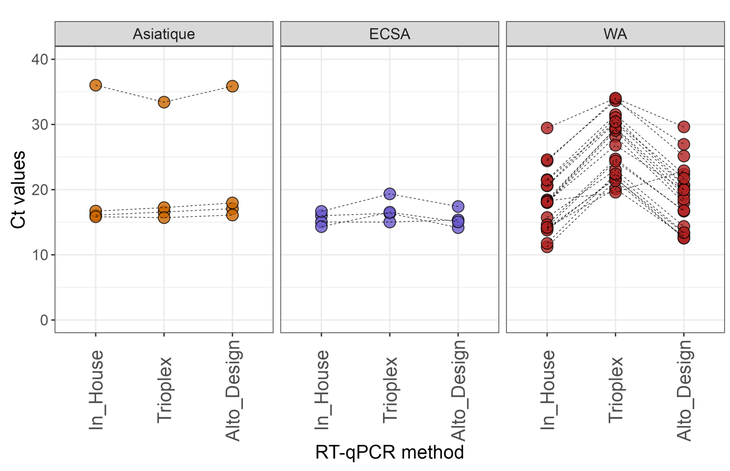

Figure 6: In house, Native Trioplex & AltoVCHIK Ct values using described CHIKV genotypes strains

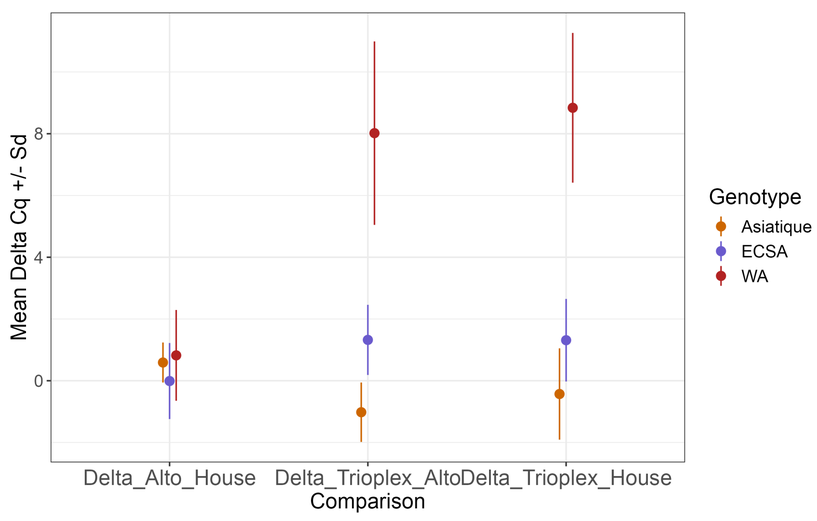

Figure 7: Genotype based delta Ct comparison of assays using described CHIKV genotypes

- The trioplex assay exhibited a high mismatch rate for **CHIKV West African Genotype**
- Using newly developed Bioinformatic module namely **AltoModule** we design updated CHIKV Trioplex **panGenotype** assay oligos (AltoVCHIK)
- AltoVCHIK oligos **improve panGenotype detection efficiency** of CDC Trioplex CHIKV assay for **all genotypes principally WA genotype**
- AltoVCHIK system exhibit low delta Ct when compared to In house assay (which exhibited high sensitivity) while allowing multiplexing with VZIK and VDE

CONCLUSION

These results emphasize the necessity of continuous monitoring of assay performance and conducting genomic surveillance during outbreaks. It highlight the critical importance of improvement of molecular diagnostic tools (by using new genomic data) to prevent genome target failure, which could result in false negatives.

References

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