# Point-of-Care Multiplex Detection and Differentiation of Chikungunya and Dengue Viruses in Whole Blood

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## ABSTRACT

Background: Dengue (DENV) and Chikungunya (CHIKV) viral infections are emerging and re-emerging with an expanded geographical distribution due to urbanization, increased human travel, and climate changes. These two infections also show a substantial overlap in clinical presentation and are co-prevalent in many countries. A recent infection can only be confirmed by detection of the virus in whole blood because positive results in serological assays may indicate prior infection. As antibodies develop in response to the infection, the viral load in the blood decreases. Thus to meet the need for sensitive detection and accurate differentiation of CHIKV and DENV in whole blood we designed a multiplex real time reverse transcription polymerase chain reaction assay for portable thermocycler T-COR 8™. The objective of the study was to develop an assay that may be used in both point-of-care (POC) and laboratory settings.

Methods: For optimization of this assay we spiked cell culture derived viruses purchased from ZeptoMetrix Corporation in normal human blood samples. This multiplex assay included reagents to amplify DENV, CHIKV, and an internal control (IC) to monitor the reaction inhibition. DENV assay was designed as PAN assay that can detect all four serotypes of DENV. Conditions to dry down the multiplex assay were optimized to make the assay amenable for use in low resource settings. A simple sample collection device and a cartridge with dried down reagents were also developed for field use.

Results: Dried down DENV assay was previously found to be 98.77% sensitive and 100 % specific on Cepheid SmartCycler using set of 81 confirmed positive and 25 negative samples. SF9, L929, Hela, SL29, Vero 76, c6/36 and BHK-2 cell line DNA extracts were not cross reactive. Individual DENV and CHIKV assays showed a wide dynamic range with 4 logs of linearity using spiked samples. Comparison of individual with multiplex assay showed similar sensitivity. Multiplex format is being further optimized and validated for portable T-COR 8 system. Detailed results from this study will be described in this presentation.

Conclusions: The data from this study shows that presence of DENV and CHIKV can be detected in blood samples effectively in centralized labs, on nursing cart or at the patient bed side using our novel multiplex assay and POC platform T-COR 8.

| # | Assay | Target | Fluorescent dye | Quencher |
|---|---|---|---|---|
| 1 | Chikungunya (CHIKV) | NP 3 region | DFO/ATTO 550 | BHQ |
| 2 | Dengue (DENV) | 3' UTR conserved region | FAM | BHQ |
| 3 | Internal Control (IC) | Synthetic Oligo | Cy 5/ATTO 647 | BHQ |

**Table1:** Chikungunya and Dengue Multiplex Assay Design

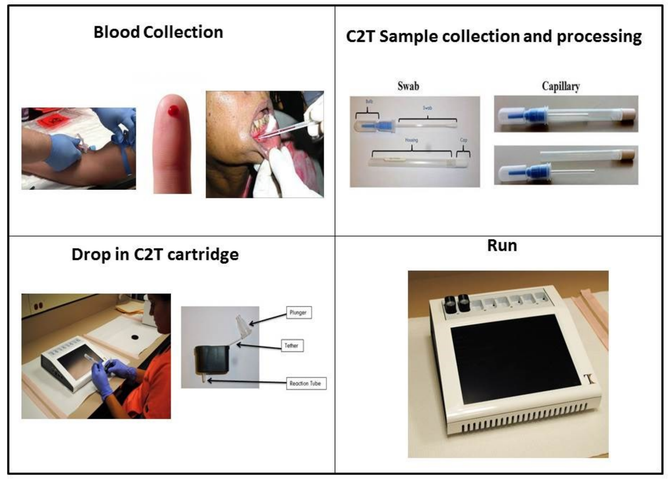


**Figure 1:** Sample-to-Answer schema for testing whole blood samples using T–COR 8 and C2T

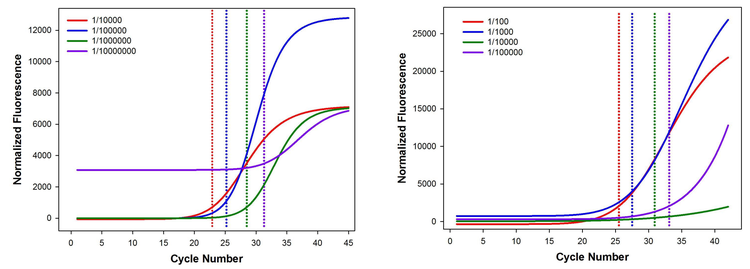


**Figure 2:** Amplification curves for single plex a) CHIKV and b) DENV assays

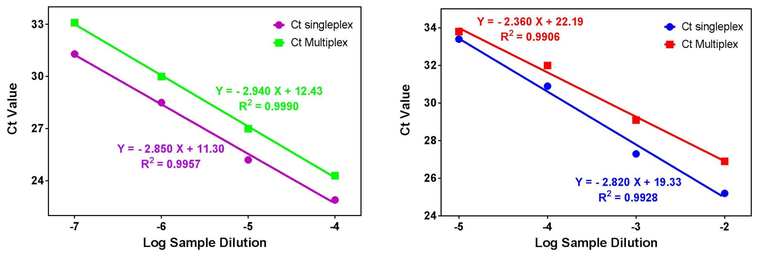


**Figure 3:** Comparison of single-plex and multiplex detection assays for a)CHIKV b) DENV

| Sample | Ct DENV | Ct CHIKV |
|---|---|---|
| NTC | ND | ND |
| CHIKV (PC) | | 24 |
| DENV 1 | 26.3 | ND |
| DENV 2 | 22.7 | ND |
| DENV 3 | 25.6 | ND |
| DENV 4 | 23.6 | ND |

| Sample | Ct DENV | Ct CHIKV |
|---|---|---|
| Yellow Fever | ND | ND |
| West Nile Virus | ND | ND |
| Murray Valley Encephalitis | ND | ND |
| JE | ND | ND |
| SLE | ND | ND |

**Table 2:** Multiplex assay a) inclusivity testing for all four serotypes of DENV b) Specificity testing (PC=positive control; ND=Ct not determined meaning no amplification)

| Assay | | T-COR 8 (Portable) | | | Traditional Bench-Top | | |
|---|---|---|---|---|---|---|---|
| | Dilution in Blood | Average Ct DENV ± SD | Average Ct CHIKV ± SD | Average Ct IC ± SD | Average Ct DENV ± SD | Average Ct CHIKV ± SD | Average Ct IC ± SD |
| DENV | 1/10 | 23.9 ± 1.0 | ND | 27.7* ± 0 | 24.3 ± 1.1 | ND | ND |
| | 1/100 | 26.9 ± 0.9 | ND | 28.7 ± 0.6 | 27.6 ± 1.6 | ND | 32.8 ± 3.9 |
| | 1/1000 | 29.7 ± 0.7 | ND | 29.9 ± 0.3 | 31.1 ± 1.4 | ND | 29.3 ± 1.1 |
| | 1/10000 | 32.0 ± 0.7 | ND | 30.5 ± 0.3 | 33.2 ± 1.3 | ND | 29.8 ± 1.8 |
| | 1/100000 | 33.8 ± 0.3 | ND | 30.8 ± 0.2 | 34.7 ± 1.5 | ND | 29.6 ± 1.1 |
| CHIKV | Neat | ND | 28.1 ± 1.4 | 30.1 ± 0.6 | ND | 28.2 ± 0.5 | 29.2 ± 1.2 |
| | 1/5 | ND | 30.3 ± 0.5 | 30.6 ± 0.2 | ND | 28.2 ± 2.6 | 29.2 ± 1.1 |
| | 1/10 | ND | 30.9 ± 0.6 | 30.9 ± 0.2 | ND | 31.3 ± 1.0 | 29.4 ± 1.3 |
| | 1/50 | ND | 31.1 ± 0.8 | 31.1 ± 0.1 | ND | 33.9 ± 1.3 | 29.6 ± 1.5 |
| | 1/100 | ND | 31.8 ± 0.7 | 31.1 ± 0.3 | ND | ND | 29.5 ± 1.4 |
| NTC | N/A | ND | ND | 30.8 ± 0.2 | ND | ND | 29.5 ± 1.4 |

**Table 3:** Average Ct values from 6 replicates each (*Ct was determined for only 1 out 6 replicates; ND = not determined, N/A = not applicable)

## RESULTS AND CONCLUSIONS

1)Reagents that detect both the DENV and CHIKV RNA simultaneously in a single sample were developed for multiplex assay (Figure 2 and 3).

2)Internal control to monitor for the presence of potential PCR-inhibitory factors from matrix was included in the multiplex assay (Table 1).

3)Dried-down assay reagents were optimized to get a room temperature-stable format, to allow end users to avoid costly cold temperature storage and increase the portability of the total detection system.

4)Specificity testing of the assay was performed using a panel of other arboviruses (Table 2).

5)A simplified sample prep method is being optimized for point-of-care use for whole blood samples (Table 3).

6)Whole blood samples were spiked with different levels of virus concentration and processed using the collect-to-test (C2T™) blood collection processing device. Single use nucleic acid amplification cartridge with dried down reagents and T-COR 8 portable thermocycler were used for rRT-PCR reactions. A schema for this integrated system is shown in Figure 1.

7)For amplification of RNA the integrated system was compared with traditional thermocycler (Table 3).

## REFERENCES

1)Wu Sj, Pal S et al (2008) A dry-format field-deployable quantitative reverse transcriptase polymerase chain reaction assay for diagnosis of dengue infection. Am J Trop. Med. Hyg., 79 (4), 505-510.

2)Callahan JD, Wu Sj, et al (2001) Development and Evaluation of Serotype- and Group-Specific Fluorogenic Reverse Transcriptase PCR (TaqMan) Assays for Dengue Virus. J Clin Microbiol., 39 (11), 4119–412

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